J Am Soc Nephrol 10: 647–663, 1999

Physiology and Pathophysiology of Renal Aquaporins[a]

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Abstract. The discovery of aquaporin membrane water channels by Agre and coworkers answered a long-standing biophysical question of how water specifically crosses biologic membranes, and provided insight, at the molecular level, into the fundamental physiology of water balance and the pathophysiology of water balance disorders. Of nine aquaporin isoforms, at least six are known to be present in the kidney at distinct sites along the nephron and collecting duct. Aquaporin-1 (AQP1) is extremely abundant in the proximal tubule and descending thin limb, where it appears to provide the chief route for proximal nephron water reabsorption. AQP2 is abundant in the collecting duct principal cells and is the chief target for vasopressin to regulate collecting duct water reabsorption. Acute regulation involves vasopressin-regulated trafficking of AQP2 between an intracellular reservoir and the apical plasma membrane. In addition, AQP2 is involved in chronic/adaptational regulation of body water balance achieved through regulation of AQP2 expression. Importantly, multiple studies have now identified a critical role of AQP2 in several inherited and acquired water balance disorders. This concerns inherited forms of nephrogenic diabetes insipidus and several, much more common acquired types of nephrogenic diabetes insipidus where AQP2 expression and/or targeting are affected. Conversely, AQP2 expression and targeting appear to be increased in some conditions with water retention such as pregnancy and congestive heart failure. AQP3 and AQP4 are basolateral water channels located in the kidney collecting duct, and AQP6 and AQP7 appear to be expressed at lower abundance at several sites including the proximal tubule. This review focuses mainly on the role of AQP2 in water balance regulation and in the pathophysiology of water balance disorders.

Progress in our understanding of water transport in the kidney has been spurred by the discovery by Agre and associates of a family of water channel proteins, the aquaporins, which provide a pathway for water transport across cell membranes (1). At least six aquaporins (AQP1, -2, -3, -4, -6, and -7) are presently known to be expressed in the kidney (Table 1). Among these, three are expressed in the collecting duct (AQP2, -3, and -4). AQP2 (2), the "vasopressin-regulated water channel," is the apical water channel of collecting duct principal cells and is the chief target for short-term regulation of collecting duct water permeability (3). AQP2 is also regulated through long-term effects that change the total abundance of AQP2 in collecting duct cells (3). Recent studies have identified the role of aquaporins in short-term and long-term regulation of body water balance and have elucidated their critical roles in multiple water balance disorders. Moreover, recent studies of transgenic mice with gene knockouts of renal aquaporins have confirmed essential roles of aquaporins in water balance regulation. These issues will be addressed in this review.

Aquaporin Structure

Aquaporins have six membrane spanning domains (Figure 1), both the amino- and carboxy-termini are cytoplasmic, and have internal tandem repeats that, presumably, are due to an ancient gene duplication (4). The topology is consistent with an obverse symmetry for the two similar N- and C-terminal halves. The tandem repeat structure with two asparagine-proline-alanine (NPA) sequences have been proposed to form tight turn structures that interact in the membrane to form the pathway for translocation of water across the plasma membrane. Of the five loops in AQP1, the B and E loops dip into the lipid bilayer, and it has been proposed that they form "hemichannels" that connect between the leaflets to form a single pathway within a symmetric structure that resembles an "hourglass" (Figure 1) (5). Recently, very detailed studies have further defined the structure and oligomeric organization of AQP1. Fourier transform infrared spectroscopy was used to further characterize the secondary structure of AQP1, and the results revealed that six closely associated alpha helixes span the lipid membrane (6), thus supporting the current model described above. Moreover, the three-dimensional structure of AQP1 was determined at 6 A resolution by cryoelectron microscopy (7). Each AQP1 monomer has six tilted, bilayer-spanning alpha-helixes, which form a right-handed bundle surrounding a central density (7). These studies also confirmed

[a]For the 1998 ASN Gottschalk Symposium on Vasopressin-Regulated Transporters.

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1046-6673/1003-0647$03.00/0
Journal of the American Society of Nephrology

Table 1. Distribution of aquaporins 1 through 9 in kidney and other organs

Group	Species[a]	No. of Amino Acids	Localization in Kidney	Subcellular Distribution[b]	Regulation	Extrarenal Localization
Renal aquaporins						
aquaporin 1	Human	269	Proximal tubules, descending thin limbs	APM/BLM	−	Multiple organs
aquaporin 2	Rat	271	Collecting duct principal cells	APM VES	+++	Testis
aquaporin 3	Rat	292	Collecting duct	BLM	+	Multiple organs
aquaporin 4	Rat	301	Medullary collecting duct	BLM	−	Brain and multiple organs
aquaporin 6	Rat	276	Cortex, medulla ?	?	?	?
aquaporin 7	Rat	269	Cortex ?	APM ?	?	Testis ?
Extrarenal aquaporins						
aquaporin 5	Rat	265	?			Submandibular gland
aquaporin 8	Rat	263	?			Testis, pancreas, liver, colon, heart, placenta
aquaporin 9	Human	295	?			Liver, leukocytes, lung, spleen

[a] Most of the renal aquaporins have been cloned from several species (human, rat, and mouse).
[b] APM, apical plasma membrane; BLM, basolateral plasma membrane; VES, intracellular vesicles.

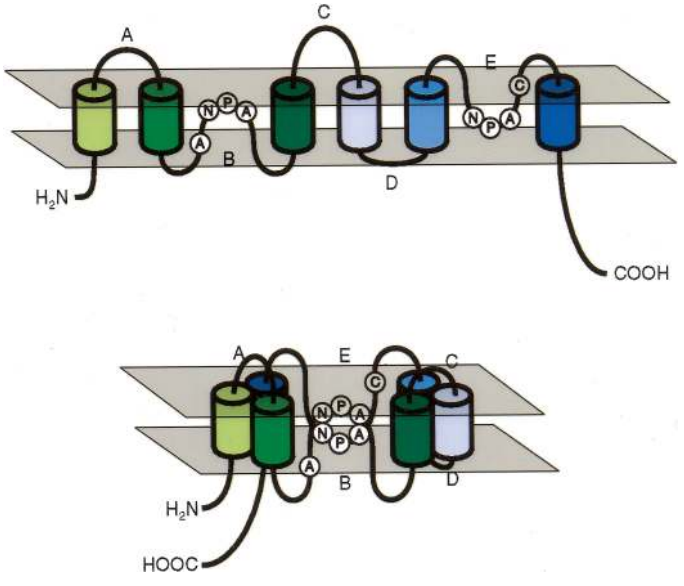

Figure 1. Schematic representation of the structural organization of aquaporin-1 (AQP1) monomers in the membrane.

the organization of the tetrameric complex in the membrane (7). The three-dimensional structure of AQP1 was also reported at 7 A resolution by other investigators (8). Studies by Brown and colleagues using Chinese hamster ovary cells transfected with AQP1 through AQP5 have indicated that AQP2, -3, and -5 may also form tetramers in the membrane (9,10).

Not all aquaporins appear to assemble in the plasma membrane as tetramers. Recently, several studies revealed that AQP4 forms larger multimeric structures in the plasma membrane. It is well established by freeze fracture analyses that glial cells and other cells, later found to express high amounts of AQP4, have a high density of intra-membrane particle square arrays (clusters of intra-membrane particles in a special systematic/geometric organization). The subsequent demonstration that square arrays are absent in cells from transgenic knockout mice lacking AQP4 protein (11) supported the view that AQP4 may form these square arrays. Recently, the presence of AQP4 within these square arrays was established directly using freeze fracture immunogold labeling by Rash and colleagues (12).

Aquaporin Water Channels in Kidney

Absorption of water in the renal tubule depends on the driving force for water reabsorption and the osmotic equilibration of water across the tubular epithelium (3). The driving force is established, at least in part, by active Na^+ transport. Moreover, the generation of a hypertonic medullary interstitium results as a consequence of countercurrent multiplication. This requires active transport and low water permeability in some kidney tubule segments, whereas in other segments there is a need for high water permeability (either constitutive or regulated). A series of studies in the past 8 yr has made it clear that osmotic water transport across the tubule epithelium is chiefly dependent on aquaporin water channels.

At least six aquaporins are expressed in the kidney (Table 1). The archetypical member of the aquaporin family, AQP1 (1), is highly abundant in the proximal tubule (Figure 2) and descending thin limb (Figure 3), and it constitutes almost 3% of total membrane protein in the kidney. Immunocytochemical analysis has documented that AQP1 is highly abundant in both apical and basolateral plasma membranes in proximal tubules and descending thin limbs, consistent with a role for transcel-

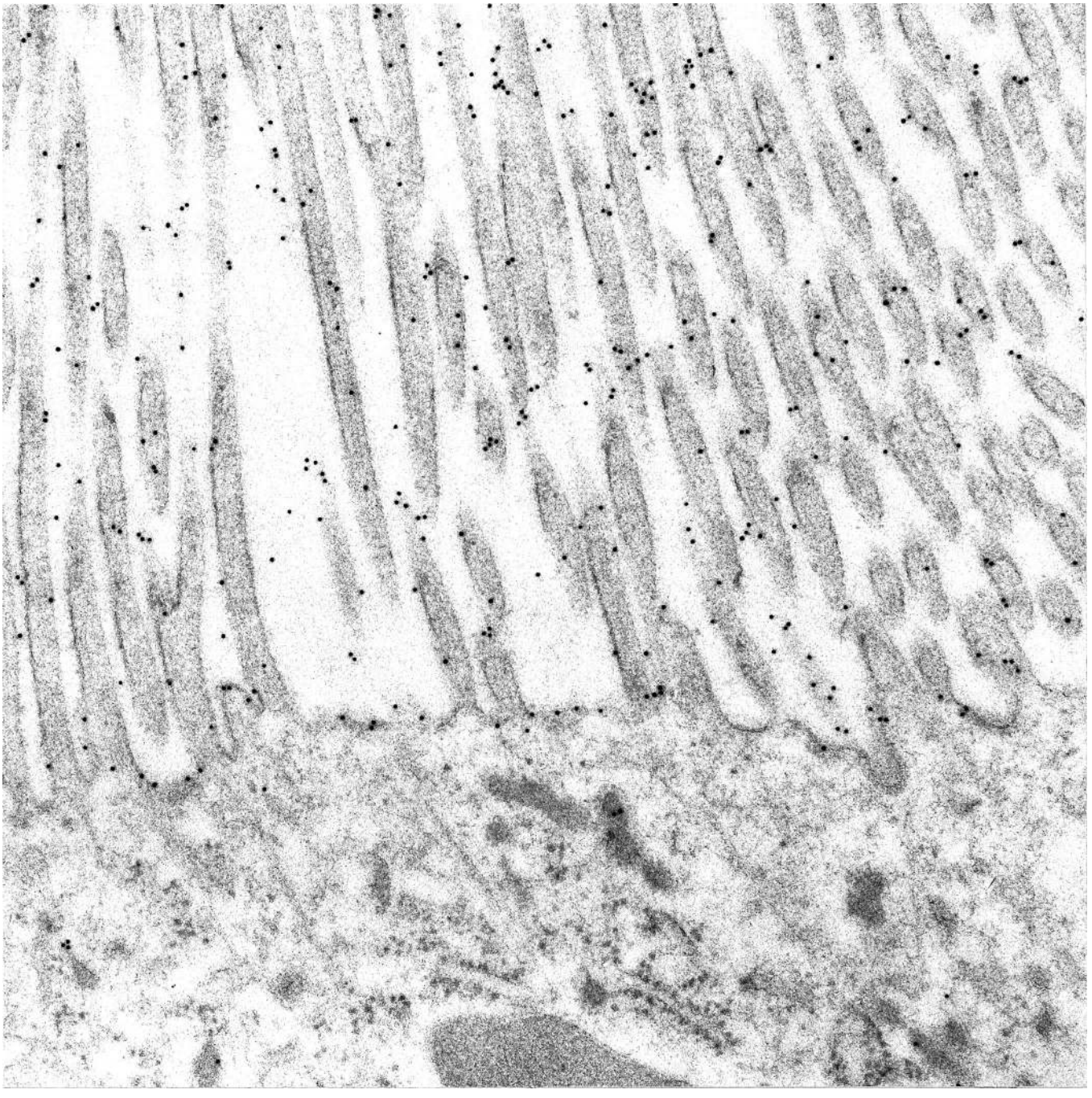

Figure 2. Immunoelectron microscopic localization of AQP1 in segment 3 proximal tubule (cryosubstituted and low-temperature Lowicryl HM20-embedded tissue). AQP1 is extremely abundant in the apical plasma membrane of the brush border. Magnification, ×90,000.

lular water reabsorption in the proximal nephron (13). The critical role of AQP1 in urinary concentration was recently confirmed in transgenic knockout mice lacking AQP1 (14). The AQP1-deficient mice were polyuric and were unable to concentrate urine to more than approximately 1000 mosmol/kg H_2O even in response to water deprivation during which they become rapidly dehydrated. Thus, AQP1 is required for the formation of a concentrated urine. Subsequent studies have demonstrated that the osmotic water permeability of isolated perfused proximal tubules were only 20% of the permeabilities in proximal tubules dissected from kidneys of normal mice (15). Recently, Chou and colleagues (unpublished observations) also demonstrated that the osmotic water permeability of descending thin limb (dissected from kidneys of AQP1-deficient animals) is reduced by 90%. These studies not only indicate a major importance of AQP1 for water reabsorption in the proximal nephron, but also provide strong evidence that the major pathway for water reabsorption in the proximal tubule and descending thin limbs is transcellular (via AQP1) and not paracellular.

AQP2 (2) is abundant in the apical plasma membrane and apical vesicles in the collecting duct principal cells (16) and at

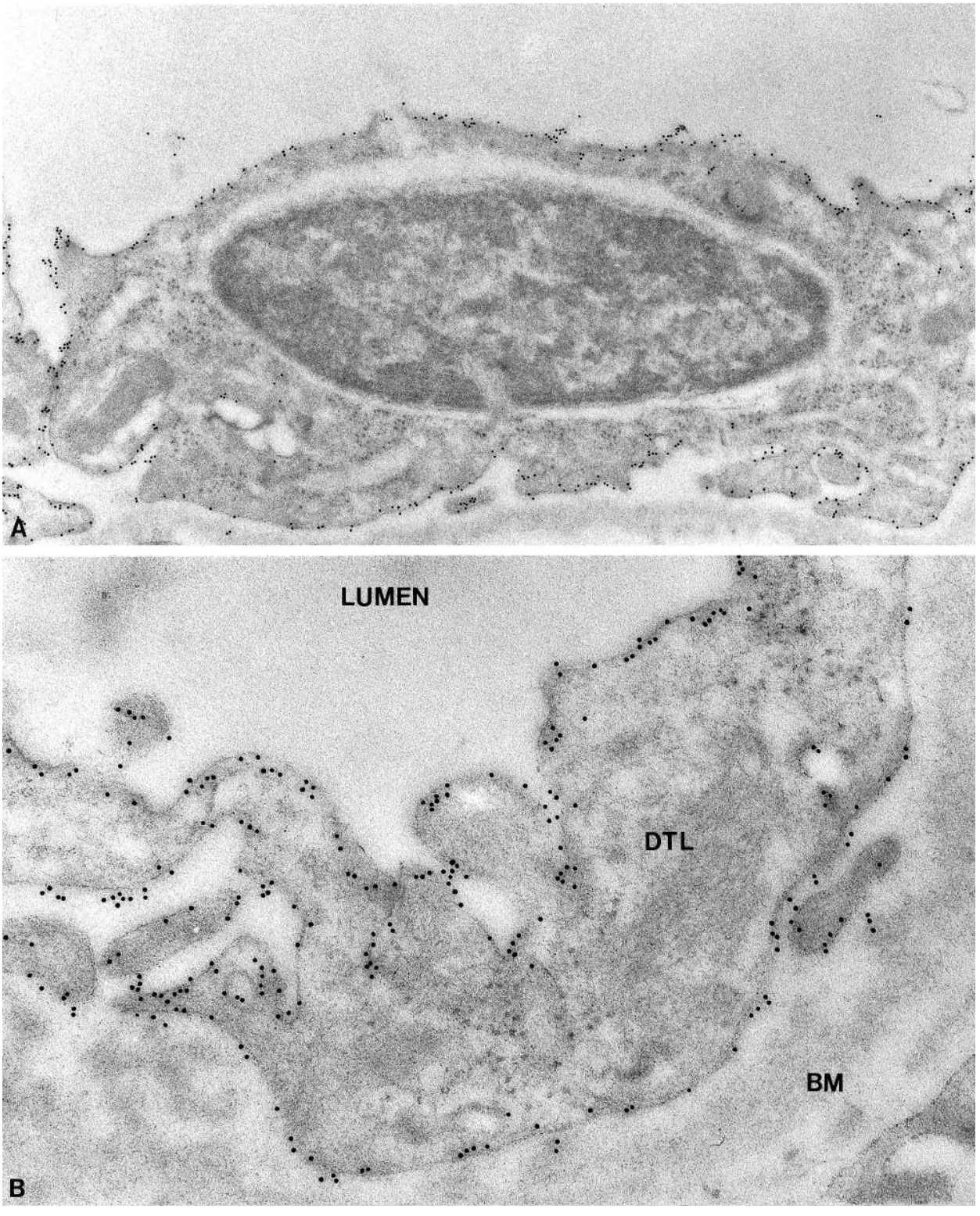

Figure 3. Immunoelectron microscopic localization of AQP1 in descending thin limbs (DTL) of the loop of Henle (cryosubstituted and low-temperature Lowicryl HM20-embedded tissue). AQP1 is extremely abundant in both the apical and basolateral plasma membrane. Lumen and basement membrane (BM) is indicated. Magnification: ×40,000 in A and ×120,000 in B.

lower abundance in connecting tubules (17). AQP2 is the primary target for vasopressin regulation of collecting duct water permeability (3). This conclusion was solidly established in studies showing a direct correlation between AQP2 expression and collecting duct water permeability in rats (18) and in studies demonstrating that humans with mutations in the AQP2 gene (19) or rats with 95% reduction in AQP2 expression (20) have profound nephrogenic diabetes insipidus.

AQP3 and AQP4, which are expressed in cells in multiple organs (for example, see reference (21)), are also present in the collecting duct principal cells and are abundant in the basolateral plasma membranes representing potential exit pathways. Recently, transgenic knockout mice lacking AQP4 showed a mild urinary concentrating defect (14), and studies using isolated perfused collecting ducts from the inner medulla (IMCD) revealed a fourfold reduction in water permeability (22). This indicates that AQP4 is responsible for the majority of basolateral membrane water movement in IMCDs. The lower abundance of AQP4, together with higher abundance of AQP3 in cortical and outer medullary collecting ducts, raises the possibility that AQP3 may play a more significant role in these segments of the collecting duct.

Two additional aquaporin cDNAs have been isolated from kidney. One of these, AQP7 (23), has been suggested to be present in the proximal tubule brush border (Table 1), although this remains to be established. The expression sites for AQP6 also remain to be determined.

Short-Term Regulation of AQP2 and Collecting Duct Water Reabsorption

The final concentration of the urine depends on the medullary osmotic gradient built up by the loop of Henle, and the water permeability of the collecting ducts carrying the urine through the cortex and medulla. Collecting duct water permeability is regulated by vasopressin, and it has been suspected for many years on the basis of indirect biophysical methods that the vasopressin-induced increase in permeability depended on the appearance of specific water channels in the apical plasma membrane of the antidiuretic hormone-responsive cells.

Much of the early work on vasopressin action was done in amphibian skin or bladder, which are functional analogues of the kidney collecting duct. Using freeze fracture electron microscopy, Chevalier *et al.* (24) identified large clusters of particles, arranged in characteristic hexagonal arrays, which appeared in the apical plasma membrane of antidiuretic hormone-responsive cells during hormonal stimulation. The number of these so-called "particle aggregates" in the membrane was subsequently shown to correlate with the increase in water permeability of the epithelium under most circumstances (25,26). In summary, these and subsequent studies in amphibian tissues revealed that: (*1*) membrane turnover was dramatically increased in response to antidiuretic hormone; (*2*) cytoskeletal inhibitors markedly inhibited the water permeability response to vasopressin; (*3*) the intramembrane particle clusters or aggregates were found in intracellular structures in the absence of vasopressin stimulation and could be found in so-called fusion structures after vasopressin stimulation; and (*4*) membrane capacitance measurements revealed an increase in apical plasma membrane area in response to vasopressin stimulation. This led Stetson and colleagues to propose the "membrane shuttle hypothesis" (27), which proposed that water channels were stored in vesicles, and inserted exocytically into the apical plasma membrane in response to vasopressin. However, it proved remarkably difficult to produce definitive evidence for this in the absence of a molecular definition of the water channels or in the absence of good water channel blockers or probes/antibodies to the channels.

The identification of the aquaporins (1) and subsequently AQP2 (2), later shown to be the predominant vasopressin-regulated water channel (for recent review, see reference (28)), made it possible to prepare antibodies and investigate the effects of vasopressin in mammalian collecting ducts directly. As shown in Figure 4, AQP2 is present in the apical and subapical parts of collecting duct principal cells, and immunoelectron microscopy (Figure 5) showed that AQP2 is very abundant both in the apical plasma membrane and in small subapical vesicles (16).

Regulation of the osmotic water permeability of the apical plasma membrane of the collecting duct principal cell, the rate limiting barrier, can, in principle, take place by at least two different mechanisms: either by chemical modification of the channel thereby regulating the water conductance, or by a change in the number of functional water channels in the membrane by vasopressin-regulated trafficking of AQP2. The presence of AQP2 in small vesicles favored the latter hypothesis, and several *in vitro* and *in vivo* studies have now identified the importance of regulated trafficking of AQP2. *In vitro* studies using isolated perfused tubules allowed a direct analysis of both the on-set and off-set responses to vasopressin. In this study, it was demonstrated that changes in AQP2 labeling density of the apical plasma membrane correlated closely with the water permeability in the same tubules (29). *In vivo* studies using normal rats or vasopressin-deficient Brattleboro rats also showed a marked increase in apical plasma membrane labeling of AQP2 in response to vasopressin or desamino-8-D-arginine vasopressin (dDAVP) treatment (30–32). Also, the off-set response has been examined *in vivo* using acute treatment of rats with a vasopressin-V_2 receptor antagonist (33,34) or acute water loading (to reduce endogenous vasopressin levels; see reference (35)). These treatments (both reducing vasopressin action) resulted in a prominent internalization of AQP2 from the apical plasma membrane to small intracellular vesicles, further emphasizing the role of AQP2 trafficking in the regulation of collecting duct water permeability.

With respect to the alternative or additional mode of regulation *viz.* regulation of conductance, two studies have attempted to address this issue. Kuwahara and colleagues demonstrated that protein kinase A (PKA)-induced phosphorylation of AQP2 in *Xenopus* oocytes was only associated with a small (approximately 30%) increase in water permeability (36). This demonstrated that changes in water conductancy of AQP2 by PKA-mediated phosphorylation could not explain the marked changes in collecting duct water permeability in response to vasopressin treatment (three- to 10-fold increase). Consistent with this observation, Lande and coworkers found no change in water permeability in response to PKA treatment of AQP2-bearing vesicles harvested from kidney inner medulla (37). Thus, the major changes in the subcellular distribution of AQP2 in response to vasopressin or vasopressin-receptor antagonist treatment strongly support the view that collecting duct water permeability, and hence water

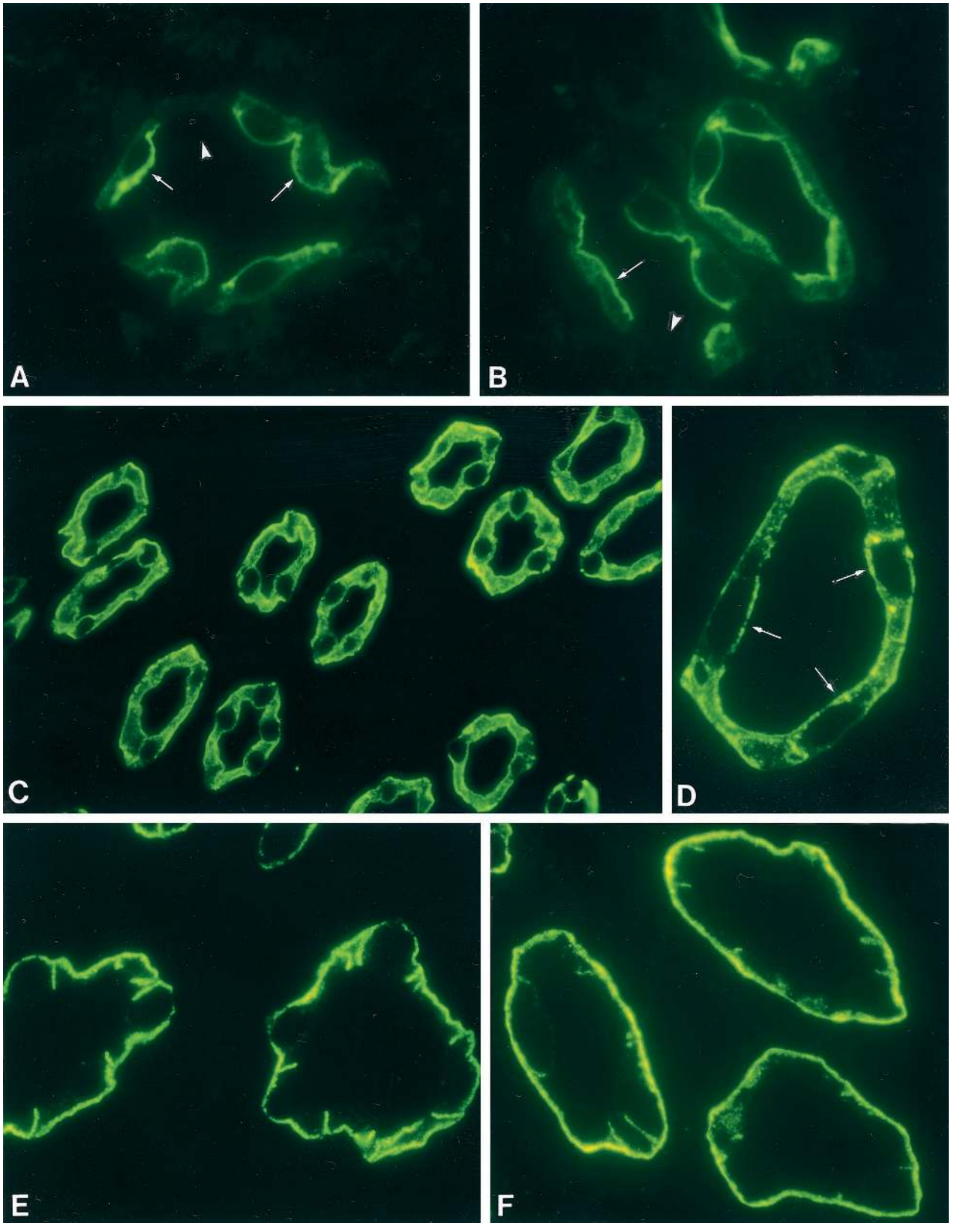

Figure 4. Immunofluorescence microscopy of AQP2 in cortical (A), outer medullary (B), and inner medullary (C and D) collecting duct, and of AQP3 (E) and AQP4 (F) in inner medullary collecting duct. AQP2 is very abundant in the apical plasma membrane domains as well as in subapical domains (arrows in panels A, B, and D), whereas intercalated cells are unlabeled (arrowheads in panels A and B). In the inner medullary collecting duct, AQP2 is also present in the basolateral part of the cell. AQP3 is abundant in both basal and lateral plasma membranes, whereas AQP4 is predominantly expressed in the basal plasma membrane and less prominently in the lateral plasma membranes. Magnification: ×1100 in A, B, and D through F; ×550 in C.

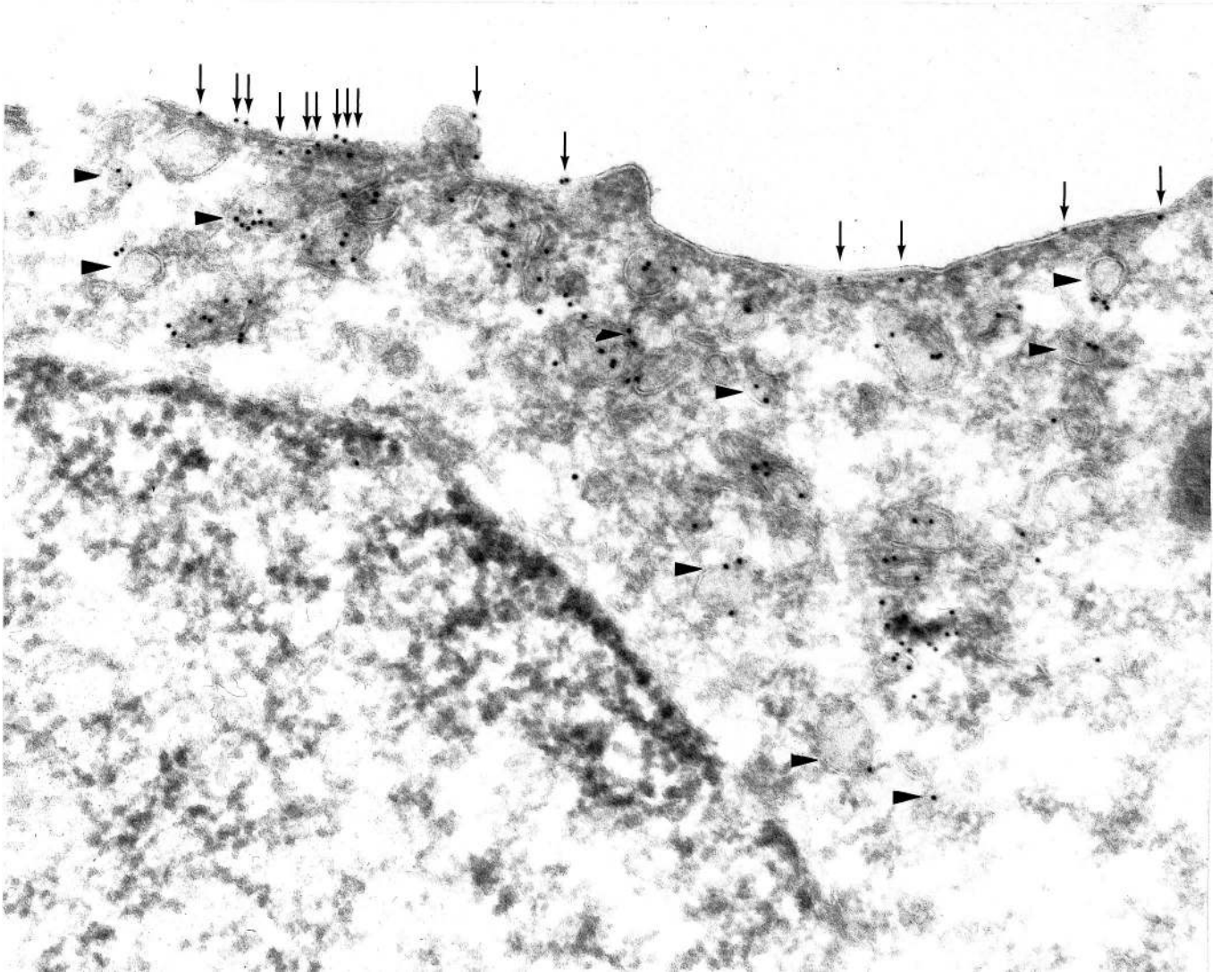

Figure 5. Immunoelectron microscopic labeling of AQP2 in ultrathin cryosection of inner medullary collecting duct. AQP2 is abundant both in the apical plasma membrane (arrows) and in small subapical vesicles (arrowheads). Adapted from reference 16.

balance, is regulated acutely by vasopressin-regulated trafficking of AQP2.

Several groups have now successfully reconstituted the system, using cultured cells transfected with AQP2 or with AQP2 tagged to a marker protein or a fluorescent protein (38–42). Using such cultured cells stably transfected with AQP2, the authors have shown shuttling of AQP2 from vesicles to the plasma membrane, albeit in some cases to the basolateral membrane, as well as retrieval and subsequent trafficking back to the surface upon repeated stimulation. This recycling of AQP2 also occurs in LLC-PK1 cells in the continued presence of cycloheximide preventing *de novo* AQP2 synthesis. Whether repeated trafficking and recycling also occurs in the native tissue remains to be established.

Signal Transduction Pathways Involved in Vasopressin Regulation of AQP2 Trafficking

The signal transduction pathways have been described thoroughly in previous reviews (see reference (43)). cAMP levels in collecting duct principal cells are increased by binding of vasopressin to V_2 receptors (44,45). The synthesis of cAMP by adenylate cyclase is stimulated by a V_2 receptor-coupled heterotrimeric GTP-binding protein, G_s. G_s interconvert between an inactive GDP form and an active GTP form, and the vasopressin-V_2 receptor complex catalyzes the exchange of GTP for bound GDP on the α-subunit of G_s. This causes release of the α-subunit $G_s\alpha$-GTP, which subsequently binds to adenylate cyclase thereby increasing cAMP production. PKA is a multimeric protein that is activated by cAMP and consists in its inactive state of two catalytic subunits and two regulatory subunits. When cAMP binds to the regulatory subunits, these dissociate from the catalytic subunits, resulting in activation of the kinase activity of the catalytic subunit.

Early studies demonstrated that PKA induces phosphorylation of various membrane proteins in bovine kidney (46) and that vasopressin treatment of saponin-permeabilized outer medullary collecting duct segments induced phosphorylation of at least two 45- and 66-kD proteins (47). AQP2 contains a

consensus site for PKA phosphorylation (RRQS) in the cytoplasmic COOH terminus at serine 256 (2). Recent studies using ^{32}P labeling or using an antibody specific for phosphorylated AQP2 (see below) showed a very rapid phosphorylation of AQP2 (within 1 min) in response to vasopressin treatment of slices of the kidney papilla (48). This agrees well with the time course of vasopressin-stimulated water permeability of kidney collecting ducts (49). As described above, PKA-induced phosphorylation of AQP2 apparently does not change the water conductancy of AQP2. Importantly, it was recently demonstrated that vasopressin or forskolin treatment failed to induce translocation of AQP2 when serine 256 was substituted by an alanine (S256A) in contrast to a significant regulated trafficking of wild-type AQP2 in LLC-PK1 cells (39). A parallel study by Fushimi and colleagues also demonstrated the lack of cAMP-mediated exocytosis of mutated (S256A) AQP2 transfected into LLC-PK1 cells (50). Thus, these studies indicate a specific role of PKA-induced phosphorylation of AQP2 in the regulation of trafficking. To explore this possibility further, an antibody was designed that exclusively recognizes AQP2, which is phosphorylated at the PKA consensus site (serine 256). In normal rats, phosphorylated AQP2 is present in both intracellular vesicles and in apical plasma membranes, whereas in Brattleboro rats phosphorylated AQP2 is located mainly in intracellular vesicles as shown by immunocytochemistry (51). Moreover, dDAVP treatment of Brattleboro rats caused a marked redistribution of phosphorylated AQP2 to the apical plasma membrane, which is in agreement with an important role of PKA phosphorylation in this trafficking (51). Conversely, treatment with V_2 receptor antagonist induced a marked decrease in expression of phosphorylated AQP2 (51) likely to be due to either reduced PKA activity and/or increased dephosphorylation of AQP2, *e.g.*, by increased phosphatase activity.

Prostaglandin E_2 inhibits vasopressin-induced water permeability by reducing cAMP levels (reviewed in reference (43)). In preliminary studies, Zelenina *et al.* investigated the effect of prostaglandin E_2 on PKA phosphorylation of AQP2 in kidney papilla, and their results suggest that the actions of prostaglandins are associated with retrieval of AQP2 from the plasma membrane, but that this appears to be independent of AQP2 phosphorylation by PKA (52).

Phosphorylation of AQP2 by other kinases, *e.g.*, protein kinase C or casein kinase II, potentially may participate in regulation of AQP2 trafficking. Phosphorylation of other cytoplasmic or vesicular regulatory proteins may also be involved. These issues remain to be investigated directly.

Cellular Processes Underlying the Insertion Process

Since the fundamentals of the shuttle hypothesis have been confirmed, interest has turned to the cellular mechanisms mediating the vasopressin-induced transfer of AQP2 to the apical plasma membrane. The shuttle hypothesis has a number of features whose molecular basis remains poorly understood. First, AQP2 is delivered in a relatively rapid and coordinated manner, and vesicles move from a distribution throughout the cell to the apical region of the cell in response to vasopressin stimulation. Furthermore, AQP2 is delivered specifically to the apical plasma membrane. Finally, AQP2-bearing vesicles fuse with the apical plasma membrane in response to vasopressin, but not to a significant degree in the absence of stimulation (*e.g.*, in vasopressin-deficient Brattleboro rats in which less than 5% of total AQP2 is present in the apical plasma membrane) (31,53). Thus, there must be some kind of a "clamp" preventing fusion in the unstimulated state, and/or a "trigger" when activation occurs.

Role of the Cytoskeleton

The coordinated delivery of AQP2-bearing vesicles to the apical part of the cell appears to depend on the translocation of the vesicles along the cytoskeletal elements. In particular, the microtubular network has been implicated in this process, since chemical disruption of microtubules inhibits the increase in permeability both in the toad bladder (see below) and in the mammalian collecting duct (54,55). Because microtubule-disruptive agents inhibit the development of the hydrosmotic response to arginine vasopressin, but have no effect on the maintenance of an established response, and because they have been reported to slow the development of the response without affecting the final permeability in toad bladders (56), it has been deduced that microtubules appear to be involved in the coordinated delivery of water channels, without being involved in the actual insertion process, or in recycling of water channels. Presumably, the processes in the kidney collecting duct are similar.

Microtubules are polar structures, arising from microtubule organizing centers (MTOCs), at which their minus ends are anchored, and with the plus ends growing away "into" the cell. In fibroblast cells, there is a single MTOC in the perinuclear region, and the plus ends project to the periphery of the cell. However, there is increasing evidence that in polarized epithelia microtubules arise from multiple MTOCs in the apical region, with their plus ends projecting down toward the basolateral membrane (57). If this is the case in collecting duct cells, and there is some evidence that it is (58), then a minus-end directed motor protein such as dynein would be expected to be involved in the movement of vesicles toward the apical plasma membrane. Recently, it has been shown that dynein is present in the kidney of several mammalian species (for references, see reference (59)), and that both dynein, and dynactin, a protein complex believed to mediate the interaction of dynein with vesicles, associate with AQP2-bearing vesicles (59). Furthermore, both vanadate, a rather nonspecific inhibitor of ATPases, and erythro-9(3-(2-hydroxynonyl))adenine, a relatively specific inhibitor of dynein, inhibit the antidiuretic response in toad bladder (60,61). Thus, it seems likely that dynein may drive the microtubule-dependent delivery of AQP2-bearing vesicles toward the apical plasma membrane.

The apical part of the collecting duct principal cells contains a prominent terminal web made up of actin filaments. These also appear to be involved in the hydrosmotic response, since disruption of microfilaments with cytochalasins inhibits the response in the toad bladder (62–64). Cytochalasins can also inhibit an established response (64), and even the offset of the

response (65). From this it has been concluded that microfilaments are probably involved in the final movement of vesicles through the terminal web, their fusion with the plasma membrane, and with the subsequent endocytic retrieval of the water channels (66). Interestingly, vasopressin itself causes actin depolymerization (67), suggesting that reorganization of the terminal web is an important part of the cellular response to vasopressin, a conclusion reached on morphologic grounds by DiBona (68).

Targeting, Docking, and Fusion of AQP2-Bearing Vesicles: Potential Roles of Vesicle-Targeting Proteins

The problem of delivering vesicles to a particular domain and allowing them to fuse when, and only when, a signal arrives is conceptually very similar to the situation in the neuronal synapse. It therefore seemed possible that a molecular apparatus similar to the SNARE system (soluble NSF attachment protein receptors) described there (69) might be present in the collecting duct principal cells. This hypothesis postulates that there are specific proteins on the vesicles (vSNAREs) and the target plasma membrane (tSNAREs) that interact with components of a fusion complex to induce fusion of the vesicles only with the required target membrane. The process is thought to be regulated by other protein components that sense the signal for fusion (*i.e.*, increased calcium in the synapse). Several groups have now shown that vSNAREs such as VAMP-2 are present in the collecting duct principal cells, and colocalize with AQP2 in the same vesicles (70–72). tSNAREs are also present: Syntaxin 4, but not syntaxins 2 or 3, is present in the apical plasma membrane of collecting duct principal cells (73,74). Some soluble components of the fusion complex, including NSF (NEM (n-ethylmaleimide)-sensitive fusion protein) and SNARE, have also been identified in these cells. Thus, it seems likely that the exocytic insertion of AQP2 is indeed controlled by a set of proteins similar to those involved in synaptic transmission, although considerable work remains to be done in isolating and characterizing the components, their regulation, and physiologic function.

Long-Term Regulation of AQP2 and Collecting Duct Water Reabsorption

Vasopressin regulates body water balance via regulation of the water permeability of the collecting duct by two distinct mechanisms, which both involve aquaporin-2 (Figure 6). The short-term mechanism, *i.e.*, the acute vasopressin-induced increase in collecting duct water reabsorption, is dependent on vasopressin-regulated trafficking of AQP2 between intracellular vesicles and the apical plasma membrane (as described above). Long-term regulation of AQP2 involves mechanisms that alter the total abundance of AQP2, thereby modulating the acute response by changing the number of water channels in the cell. Thus, the short-term and long-term mechanisms act in concert. Long-term regulation of collecting duct water permeability is a conditioning effect in collecting duct cells, and several studies have established that this response is due to changes in the total number of AQP2 channels per cell (3). Water restriction for 24 to 48 h or dDAVP treatment for 5 d results in a marked increase in AQP2 protein levels in rat renal inner medulla, an effect that paralleled the large increase in collecting duct water permeability seen in response to water deprivation or long-term dDAVP treatment (16,18). Brattleboro rats (lacking vasopressin) or normal rats treated with V_2 receptor antagonist for a prolonged period (75,76) did not mount this extensive increase in AQP2 expression, suggesting that vasopressin is in part necessary for the adaptive response to long-term restriction of water intake. Conversely, water loading decreases the overall abundance of AQP2. The adaptational changes in AQP2 abundance change the levels of AQP2 available for short-term regulation of trafficking to/from the apical plasma membrane to regulate body water balance.

This long-term increase in AQP2 abundance is ascribed to regulation of the AQP2 gene transcription, which involves a cAMP response element in the AQP2 promoter (77–79). Conversely, downregulation of AQP2 expression, *e.g.*, in response to water loading, is likely to be due to reduced gene transcription and enhanced (or maintained) delivery of AQP2 into a degradative pathway.

Vasopressin-Independent Regulation of AQP2 Expression

The presence of a cAMP-responsive element in the 5′ flanking element region of the AQP2 gene (80) and a PKA phosphorylation site in the protein sequence of AQP2 (2) are consistent with vasopressin-dependent mechanisms. However, some studies have also revealed the presence of vasopressin-independent signal transduction pathways in AQP2 regulation, which may play important physiologic and pathophysiologic roles. First, Marples *et al.* showed that water deprivation increases AQP2 expression more than sustained dDAVP treatment does in lithium-induced nephrogenic diabetes insipidus rats, although both treatments correct lithium-induced polyuria (20). The second piece of evidence is from water-loaded rats with high exogenous plasma levels of dDAVP (81). Indeed, Ecelbarger *et al.* showed that water loading induces a marked decrease in AQP2 expression despite high plasma levels of dDAVP, supporting the view that vasopressin-independent mechanisms may be involved in regulating AQP2 levels (81). The presence of vasopressin-independent mechanisms is also supported by studies using water deprivation or long-term lithium treatment of vasopressin-deficient Brattleboro rats (82). Although AQP2 is almost completely absent in the apical plasma membrane (53), its expression level is high in the Brattleboro rat corresponding to 30 to 60% of that seen in normal Wistar rats (82,83) despite absence of circulating vasopressin. Lithium treatment causes a dramatic reduction in AQP2 expression (80% reduction), which indicates that the vasopressin- independent regulation of AQP2 may also be cAMP-dependent, since lithium is known to affect adenylate cyclase activity. The signaling transduction pathways involved in the altered long-term regulation of AQP2 during vasopressin escape are at present unknown. Thus, the existence and poten-

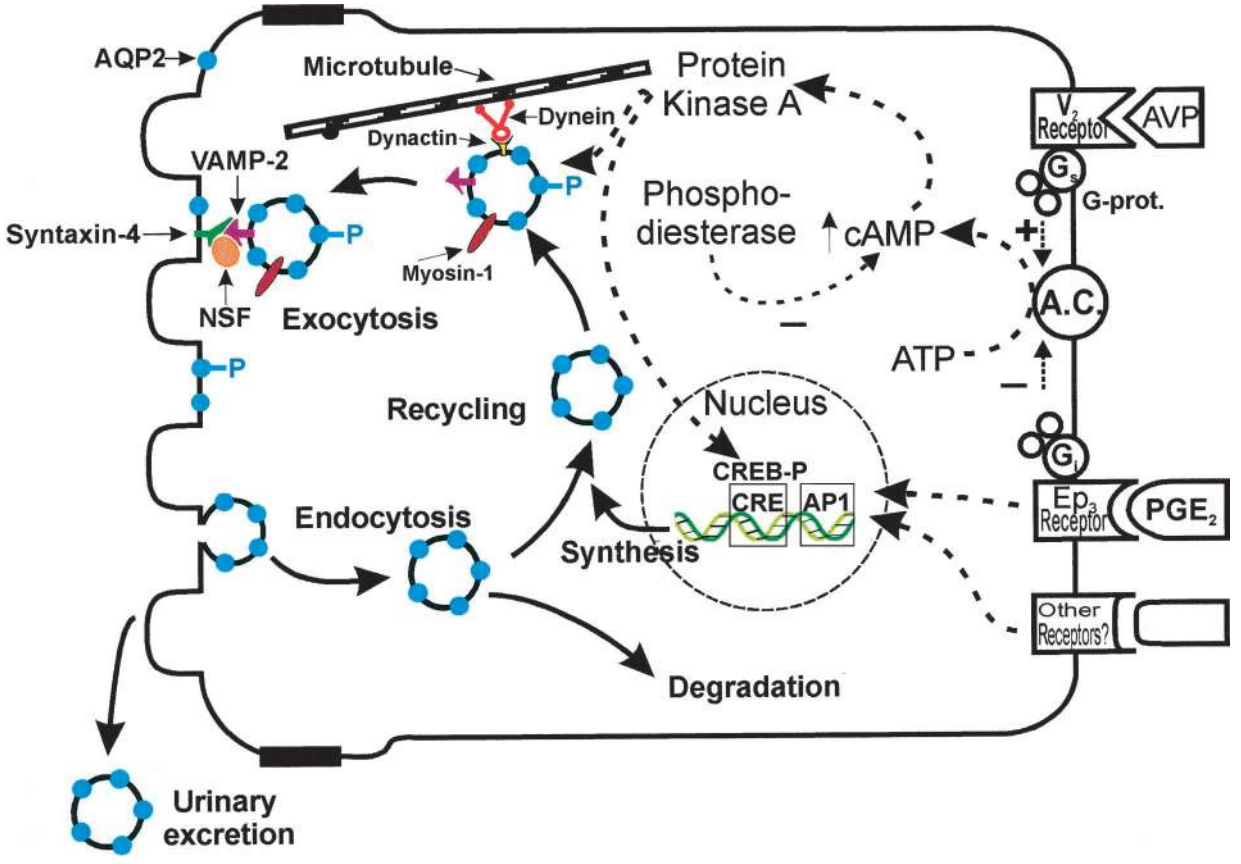

Figure 6. Regulation of AQP2 trafficking and expression in collecting duct principal cells. Vasopressin (AVP) acts on V_2 receptors in the basolateral plasma membrane. Through the GTP-binding protein G_s, adenylyl cyclase (A.C.) is activated, which accelerates the production of cAMP from ATP. Then cAMP binds to the regulatory subunit of protein kinase A (PKA), which activates the catalytic subunit of PKA. PKA phosphorylates AQP2 in intracellular vesicles and possibly other cytosolic or membrane proteins. Microtubule motor proteins (dynein/dynactin) and vesicle targeting receptors (VAMP-2, syntaxin-4, NSF) may participate in the specificity of AQP2 targeting to the apical plasma membrane to increase water permeability. cAMP also participates in long-term regulation of AQP2 by increasing the levels of the catalytic subunit of PKA in the nuclei, which is thought to phosphorylate transcription factors such as CREB-P (cAMP responsive element binding protein) and c-Jun/c-Fos. Binding of these factors is thought to increase gene transcription of AQP2 resulting in synthesis of AQP2 protein, which in turn enters the regulated trafficking system.

tial importance of a vasopressin-independent signaling pathway (20) has gained considerable support.

Not only does AQP2 appear to be regulated on a long-term basis, but several studies have now made it clear that AQP3 abundance (but not AQP4 abundance) is also regulated in conditions associated with altered water intake or changes in vasopressin levels (76,81). Obviously, it also should be emphasized that long-term adaptational changes in response to long-term vasopressin treatment or water deprivation may also induce major changes in the expression and activity of transporters that are responsible for creating the driving force for water reabsorption via aquaporins. These have been dealt with in the review by Knepper in this same symposium.

Pathophysiologic Roles of Aquaporins in Water Balance Disorders

Inherited Central and Nephrogenic Diabetes Insipidus: Role of AQP2

Central diabetes insipidus is a condition characterized by very low or undetectable levels of vasopressin. The massive polyuria can be reversed and urine osmolality substantially increased by exogenous administration of arginine vasopressin. Using vasopressin-deficient Brattleboro rats as a model, it was demonstrated that AQP2 expression levels were markedly lower than in the parent strain of Long-Evans rats (Figure 7) and that there was a low labeling for AQP2 in the apical plasma membrane (18). Moreover, prolonged treatment with either vasopressin or dDAVP results in a marked increase in AQP2 expression and in apical plasma membrane labeling in both inner medulla and cortical collecting ducts (18,32). This strongly supports the view that dysregulation of AQP2 due to absence of vasopressin plays a major role in the development of polyuria.

Inherited forms of nephrogenic diabetes insipidus (NDI) are rare diseases characterized by renal unresponsiveness to vasopressin. The most common form is an X-linked nephrogenic diabetes insipidus due to mutations in the V_2-vasopressin receptor gene (84). Because both AQP2 targeting and expression are regulated tightly by vasopressin, the reduction in V_2 signal transduction due to the mutation in the V_2 receptor is likely to

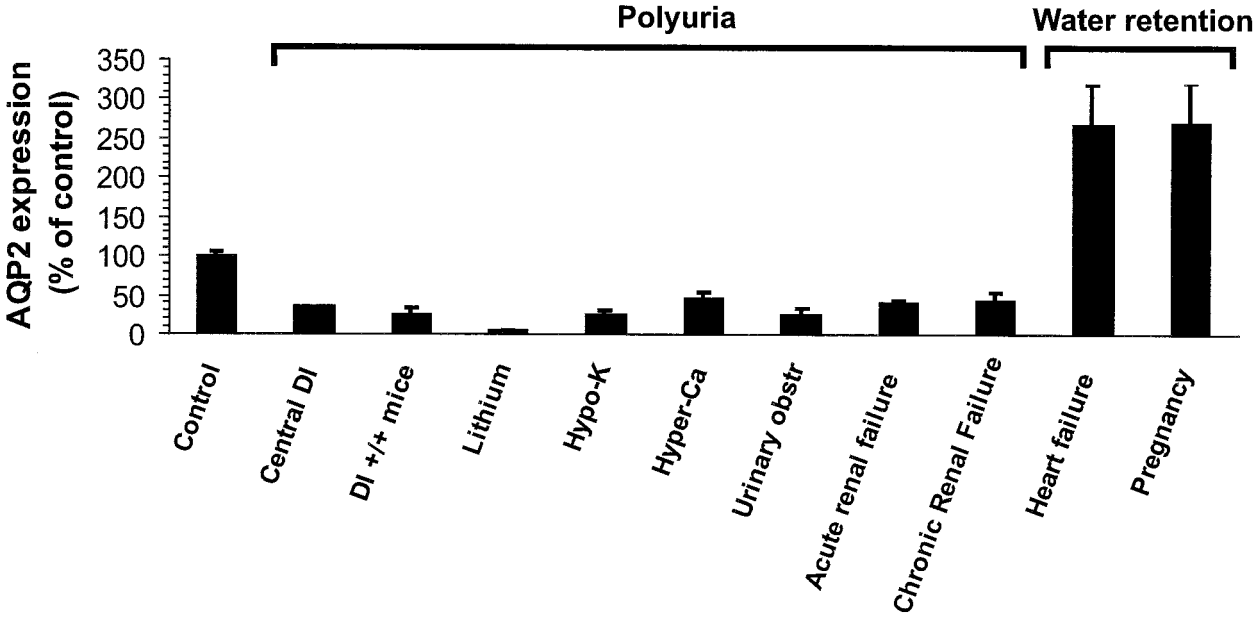

Figure 7. Changes in AQP2 expression observed in association with different water balance disorders. Levels are expressed as a percentage of control levels (leftmost bar). AQP2 expression is reduced, sometimes dramatically, in a wide range of hereditary and acquired forms of diabetes insipidus characterized by different degrees of polyuria. Conversely, congestive heart failure and pregnancy are conditions associated with increased expression of AQP2 levels and excessive water retention.

critically affect AQP2 regulation, which in turn results in the severe polyuria in these patients. Direct evidence for AQP2 playing a critical role in urinary concentration was demonstrated in an elegant study by Deen and colleagues, who found mutated and nonfunctional AQP2 in patients with an extremely rare autosomal recessive disorder caused by mutations in the AQP2 gene (non-X-linked NDI) (19).

Kanno and coworkers demonstrated an increased urinary excretion of AQP2 in response to vasopressin administration in patients with central diabetes insipidus (85), whereas patients with X-linked or non-X-linked nephrogenic diabetes insipidus did not increase urinary levels of AQP2 in response to vasopressin. These initial findings, together with other examinations of urinary AQP2 excretion (86,87), raise the possibility of assessing AQP2 levels in the kidney by the measurement of urinary AQP2 levels. Moreover, it was recently shown that AQP2, but not AQP3, was excreted into urine at a significant level, suggesting that urinary excretion of AQP2 takes place via a selective, apical pathway and not by whole cell shedding (88). It remains to be clarified whether urinary excretion of AQP2 may be helpful diagnostically.

AQP2 Expression Is Reduced in Multiple Forms of Acquired NDI

Although hereditary forms of NDI are rare, a wide range of pathologic conditions and drug treatments can lead to acquired NDI (Table 2). In principal, AQP2 expression might be decreased (and be a causative factor in the polyuria) or increased (in an attempt to compensate for some other defect in the concentrating mechanism) in such conditions. In fact, AQP2 expression is downregulated in a variety of acquired forms of NDI. The most striking example is the effect of lithium. In a rat model, lithium treatment caused about a 95% reduction in AQP2 levels (Figure 7), in parallel with the development of profound polyuria (20). Such a decrease in AQP2 seems almost certain to impair the function of the collecting duct, and hence is probably crucial in the etiology of the polyuria.

AQP2 expression was also reduced in models of two electrolyte disturbances known to cause NDI: chronic hypokalemia (89) and hypercalcemia (90,91). In these cases, neither the polyuria nor the reduction in AQP2 expression (Figure 7) was as marked as after lithium treatment, consistent with the hypothesis that the decrease in AQP2 was at least partly responsible for the diuresis.

Another common cause of a urinary concentrating defect is postobstructive diuresis. In a rat model with bilateral ureteral obstruction (BUO), AQP2 levels were reduced to about one-quarter of control levels (92), and recovered only slowly. After 1 wk, urine output was apparently normal, but the animals still had only half their normal AQP2 levels. Consistent with this finding, the animals were not able to increase their urinary concentration in response to dehydration to the same degree as sham-operated rats (92). A more recent study has shown that these defects in AQP2 and maximal concentrating capacity persist for up to 1 mo (93). Interestingly, defects in AQP1 expression were also found in response to BUO, and this finding may also have important implications for the urinary concentrating defect observed after release of obstruction.

It is well known that elderly patients are often prone to abnormalities in water and salt balance, including an impaired urinary concentrating capacity (94). In addition, there is an age-related reduction in the ability to elevate circulating vaso-

Table 2. Physiologic conditions or water balance disorders associated with altered expression and/or targeting of AQP2[a]

Conditions with reduced AQP2 expression and polyuria
genetic defects
Brattleboro rats (central DI)
DI +/+ severe mice (low cAMP)
AQP2 mutants (human)
V_2 receptor variants (human)[b]
acquired NDI (rat models)
lithium
hypokalemia
hypercalcemia
postobstructive NDI
bilateral
unilateral
low protein diet (urinary concentrating defect with no polyuria)
water loading (compulsive water drinking)
experimental chronic renal failure (5/6 nephrectomy model)
ischemia-induced acute renal failure (polyuric phase in rat model)
age-induced NDI[c]
Conditions with reduced AQP2 expression and altered urinary concentration and dilution without polyuria
nephrotic syndrome models (rat models)
PAN
adriamycin
hepatic cirrhosis (CBL, compensated)
ischemia-induced acute renal failure (oliguric phase in rat model)
Conditions with increased AQP2 expression with expansion of extracellular fluid volume
vasopressin infusion (SIADH)
congestive heart failure
hepatic cirrhosis (CCL_4-induced, noncompensated) ?
pregnancy

[a] AQP2, aquaporin-2; DI, diabetes insipidus; NDI, nephrogenic diabetes insipidus; PAN, puromycin aminonucleoside; CBL, common bile duct ligation; SIADH, syndrome of inappropriate secretion of antidiuretic hormone.

[b] Reduced V_2 receptor density has profound effect on the AQP2 targeting and expression.

[c] Mild increase in urine production rates.

pressin levels in response to dehydration (95). Dysregulation of renal transporters and channels may also participate in this urinary concentrating defect. It was recently demonstrated that 72 h of water deprivation in 4-mo-old rats and in 30-mo-old rats resulted in increased AQP2 expression in only the young animals, whereas AQP2 expression was unchanged in 30-mo-old rats (96). Similar to the age-related changes in urinary concentrating capacity, low protein diet decreases urine concentrating capacity. In rats fed a low (8%) protein diet for 2 wk, Sands *et al.* found that AQP2 expression was significantly reduced in the inner medulla (97). This was associated with a significant reduction in vasopressin-stimulated osmotic water reabsorption in perfused collecting ducts. Thus, downregulation of AQP2 may play some role in the impaired urinary concentrating capacity observed in elderly individuals or in response to a low protein diet.

Several Factors Probably Modulate AQP2 Downregulation in Acquired NDI

As described above, vasopressin levels appear to modulate AQP2 expression, probably via cAMP (80). However, some of the results obtained in studies of acquired forms of NDI suggest that other pathways also exist. In some of the models, AQP2 trafficking (hypokalemia and BUO) to the apical surface appeared to be intact, suggesting that circulating vasopressin levels were at least normal, and that the intracellular signaling cascades leading to trafficking were intact (89,92). Thus, the decreased expression of AQP2 must be due to a signal other than the activity of vasopressin. In the lithium study, normal trafficking appeared to be inhibited, because the fraction of AQP2 in the apical plasma membrane fell (20). However, when dDAVP was infused by minipumps, it was possible to induce efficient delivery of AQP2, with some improvement in the diuresis, but only with a minimal increase in AQP2 expression. In contrast, water deprivation of the lithium-treated animals caused a much greater increase in AQP2 levels, but without overcoming the blockade of the delivery process. Thus, water deprivation was able to increase AQP2 expression via a pathway different from that involved in causing trafficking to the apical surface.

Another approach was taken in another form of experimental acquired NDI, postobstructive polyuria (98). To investigate the mechanisms involved in AQP2 downregulation during BUO or after release of obstruction, the expression of AQP2 was investigated in unilateral ureteral obstruction. When only one ureter is obstructed, the contralateral kidney can compensate with an increased urine output, and can keep the plasma solutes within physiologic ranges. Under these conditions, AQP2 levels in the obstructed kidney were still markedly reduced, while levels in the nonobstructed kidney were only modestly reduced. This is consistent with a potential role of local factors, such as metabolites or raised intrarenal pressure, being responsible for a large part of the decrease in AQP2 expression, while circulating factors, perhaps including decreased vasopressin, also played a part.

Dysregulation of Aquaporins in Complex Experimental Renal Diseases: Nephrotic Syndrome, Acute Renal Failure, and Chronic Renal Failure

Experimental Nephrotic Syndrome in Rats. The nephrotic syndrome is characterized by extracellular volume expansion with excessive renal salt and water reabsorption. The mechanisms of salt and water retention are poorly understood; however, they can be expected to be associated with dysregulation of solute transporters and water channels. In contrast to congestive heart failure and liver cirrhosis, which are associated with extracellular volume expansion and hyponatremia,

rats with nephrotic syndrome do not develop hyponatremia despite extensive extracellular fluid volume expansion. This absence of hyponatremia may reflect an absence of upregulation of AQP2 expression in the collecting duct. Indeed, a marked downregulation of AQP2 and AQP3 expression was demonstrated in rats with puromycin aminonucleoside-induced and adriamycin-induced nephrotic syndrome (99,100). This reduced expression of collecting duct water channels could represent a physiologically appropriate response to extracellular volume expansion. The signal transduction involved in this process is not clear, but circulating vasopressin levels are high in rats with puromycin aminonucleoside-induced nephrotic syndrome. Thus, the marked downregulation of AQP2 in experimental nephrotic syndrome may share similarities with the downregulation of AQP2 in water-loaded dDAVP-treated rats that escape from the action of vasopressin (81,101).

Experimental Acute Renal Failure Induced by Ischemia and Reperfusion Injury in Rat. Renal failure, both acute and chronic, is associated with polyuria and a concentrating defect, although in both cases there is a wide range of glomerular and tubular abnormalities that contribute to the overall renal dysfunction. Ischemia-induced experimental acute renal failure in rats is characterized by structural alterations in the renal tubule, in association with an impairment in urinary concentration. The proximal tubule (S3 segment) and thick ascending limb are known to be the main sites of the ischemic insult, whereas collecting ducts are generally considered to be relatively invulnerable (102,103). Recently, Fernandez-Llama *et al.* demonstrated that collecting duct water channel AQP2, AQP3, and AQP4 levels, as well as proximal nephron water channel AQP1 expression levels, are significantly decreased in response to mild-to-moderate renal ischemia, in association with polyuria (104). This suggests that: (*1*) collecting duct cells are also significantly affected by renal ischemia; and (*2*) the polyuria associated with acute renal injury is due at least in part to reduced expression of collecting duct aquaporins.

Experimental Chronic Renal Failure Induced by 5/6 Nephrectomy in Rat. Patients with advanced chronic renal failure have urine that remains hypotonic to plasma in spite of the administration of supramaximal doses of vasopressin (105,106). This vasopressin-resistant hyposthenuria specifically suggests abnormalities in collecting duct water reabsorption in chronic renal failure patients. Chronic renal failure can be experimentally induced by 5/6 nephrectomy. Micropuncture and microcatheterization studies have demonstrated that the impaired urinary concentrating ability may, at least partly, be caused by impairment of vasopressin-stimulated water reabsorption in the kidney collecting duct (107,108). Fine *et al.* observed that isolated and perfused cortical collecting ducts dissected from remnant kidneys of severely uremic rabbits exhibited a decreased water flux and adenylate cyclase activity in response to vasopressin (109). Importantly, they also showed that 8-bromo-cAMP failed to induce a normal hydrosmotic response in cortical collecting ducts from remnant kidneys. As an extension of these observations, Teitelbaum and McGuinness demonstrated that reverse transcription-PCR of total RNA from the inner medulla of chronic renal failure rat kidneys revealed virtual absence of V_2 receptor mRNA (110,111). Recently, we analyzed whether this collecting duct dysfunction could be associated with dysregulation of collecting duct aquaporins. The results demonstrated that chronic renal failure induced by 5/6 nephrectomy is associated with polyuria and a vasopressin-resistant downregulation of AQP2 and AQP3 (112). Immunocytochemistry and immunoelectron microscopy confirmed a marked reduction in AQP2 and AQP3 levels in the principal cells. This suggests that reduced AQP2 and AQP3 levels may be significant factors involved in the impaired collecting duct water permeability and reduced or impaired vasopressin responsiveness in chronic renal failure.

Congestive Heart Failure

Congestive heart failure is associated with salt and water retention. Two recent studies addressed the question of whether there is dysregulation of aquaporins associated with congestive heart failure, and, if this is true, whether dysregulation of aquaporins might be important for the development of hyponatremia. AQP2 expression was examined in rats with experimentally induced congestive heart failure by left coronary artery ligation. Both studies revealed that congestive heart failure with renal water retention (determined as a significant reduction in plasma Na^+ concentration) was associated with a marked increase in AQP2 mRNA and protein levels (113,114). In addition, there was a redistribution of AQP2 in the collecting duct principal cells with increased targeting to the apical plasma membrane. Thus, congestive heart failure was associated with an increased expression of AQP2 and an increased targeting, which would be consistent with a reduced dilutional capacity and an increased water reabsorption. Importantly, this dysregulation was seen only in rats with severe congestive heart failure (with increased left ventricular end diastolic pressure [LVEDP] and reduced plasma sodium concentrations), but not in rats with compensated heart failure (increased LVEDP but normal serum sodium concentrations) (113). This strongly supports the view that increased AQP2 expression and enhanced delivery to the apical plasma membrane play a significant role in water retention and development of hyponatremia associated with severe heart failure. Both the increased expression and targeting may be ascribed to increased baroreceptor-mediated vasopressin release. Consistent with this finding, rats with congestive heart failure had significantly increased plasma vasopressin levels and administration of the vasopressin-V_2 receptor antagonist OPC 31260 was associated with a significant reduction in AQP2 protein and mRNA levels (114).

Pregnancy is a physiologic condition associated with water retention, which is especially prominent in the last trimester. Recently, it was demonstrated that AQP2 expression levels were increased in pregnant rats (115), suggesting that AQP2 may also play a role in the water retention associated with pregnancy. Again, it should be emphasized that dysregulation of solute transporters is likely to play a key role in conditions associated with hyponatremia.

Cirrhosis

Hepatic cirrhosis is another serious chronic condition associated with pathologic water retention. Similar to congestive heart failure, it has been suggested that increased plasma levels of vasopressin could play an important pathophysiologic role in the impaired ability to excrete water. It was therefore hypothesized that increased AQP2 expression could be a factor in the increased water reabsorption and reduced dilutional ability. However, as will be discussed, the changes in AQP2 expression differ significantly depending on the model of hepatic cirrhosis that is studied. Hepatic cirrhosis induced by chronic intraperitoneal administration of carbon tetrachloride was found to be associated with increased expression of both AQP2 protein and AQP2 mRNA (116,117). In this model, however, the rats had normal serum sodium levels or osmolality, indicating that inappropriate renal retention of water was not a major factor in the generation of ascites. Interestingly, AQP2 mRNA levels correlated with the amount of ascites, suggesting that AQP2 may play a role in the development of water retention. As with congestive heart failure, treatment with the vasopressin V_2 receptor antagonist OPC31260 reversed the increase in AQP2 mRNA levels. In a second model, hepatic cirrhosis (compensated) was induced by ligation of the common bile duct (118). Immunoblotting and semiquantitative densitometry demonstrated a significant reduction in AQP2 expression. This reduction in collecting duct water channels was consistent with functional data demonstrating a reduced effect of vasopressin-V_2 receptor antagonist treatment (118). In yet a third model, using CCl_4 inhalation, hepatic cirrhosis was associated with ascites and hyponatremia (evidence of excess water retention). However, no change in AQP2 expression was observed in this model of hepatic cirrhosis, but AQP1 expression in the cortex was increased (119). The authors concluded that the water retention is likely, in part, to be due to increased reabsorption in the proximal tubule, combined with a failure of the normal "vasopressin escape" phenomenon. Thus, dysregulation of AQP2 may participate in the dynamic changes in water handling in hepatic cirrhosis. However, from these studies it is also evident that cirrhosis is a very complex condition, in which there are multiple disturbances of normal physiology, and that additional studies will be needed to fully clarify the role of aquaporins, and solute transporters, in compensated and decompensated cirrhosis.

Aquaretics

As discussed above, oral administration of the nonpeptide V_2 receptor antagonist OPC31260 effectively downregulates AQP2 expression and targeting to the apical plasma membrane in both congestive heart failure and hepatic cirrhosis induced by intraperitoneal administration of carbon tetrachloride. Similar effects were obtained in the syndrome of inappropriate secretion of antidiuretic hormone (SIADH), providing evidence that nonpeptide V_2 receptor antagonists may be potential aquaretic agents in conditions associated with water retention and hyponatremia. Administration of aquaporin inhibitors would potentially have the same effect in these conditions. At present, the only known aquaporin inhibitors are $HgCl_2$ and other mercurials, which were also constituents in different effective diuretics used several decades ago. Further characterization of the molecular structure of aquaporins may provide insights necessary for future drug development for a variety of water balance disorders.

References

1. Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S: Aquaporin CHIP: The archetypal molecular water channel. *Am J Physiol* 265: F463–F476, 1993
2. Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S: Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 361: 549–552, 1993
3. Knepper MA: Molecular physiology of urinary concentrating mechanism: Regulation of aquaporin water channels by vasopressin. *Am J Physiol* 272: F3–F12, 1997
4. Agre P, Bonhivers M, Borgnia MJ: The aquaporins, blueprints for cellular plumbing systems. *J Biol Chem* 273: 14659–14662, 1998
5. Jung JS, Preston GM, Smith BL, Guggino WB, Agre P: Molecular structure of the water channel through Aquaporin CHIP: The hourglass model. *J Biol Chem* 269: 14648–14654, 1994
6. Cabiaux V, Oberg KA, Pancoska P, Walz T, Agre P, Engel A: Secondary structures comparison of aquaporin-1 and bacteriorhodopsin: A Fourier transform infrared spectroscopy study of two-dimensional membrane crystals. *Biophys J* 73: 406–417, 1997
7. Walz T, Hirai T, Murata K, Heymann JB, Mitsuoka K, Fujiyoshi Y, Smith BL, Agre P, Engel A: The three-dimensional structure of aquaporin-1. *Nature* 387: 624–627, 1997
8. Cheng A, van Hoek AN, Yeager M, Verkman AS, Mitra AK: Three-dimensional organization of a human water channel. *Nature* 387: 627–630, 1997
9. 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
10. Van Hoek AN, Yang B, Kirmiz S, Brown D: Freeze-fracture analysis of plasma membranes of CHO cells stably expressing aquaporins 1–5. *J Membr Biol* 165: 243–254, 1998
11. Verbavatz JM, Ma T, Gobin R, Verkman AS: 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
12. Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S: Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc Natl Acad Sci USA* 95: 11981–11986, 1998
13. Nielsen S, Smith B, Christensen EI, Knepper MA, Agre P: CHIP28 water channels are localized in constitutively water-permeable segments of the nephron. *J Cell Biol* 120: 371–383, 1993
14. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS: Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J Biol Chem* 273: 4296–4299, 1998
15. Schnermann J, Chou CL, Ma T, Traynor T, Knepper MA, Verkman AS: Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci USA* 95: 9660–9664, 1998
16. Nielsen S, DiGiovanni SR, Christensen EI, Knepper MA, Harris HW: Cellular and subcellular immunolocalization of vasopressin- regulated water channel in rat kidney. *Proc Natl Acad Sci USA* 90: 11663–11667, 1993
17. Kishore BK, Mandon B, Oza NB, DiGiovanni SR, Coleman RA,

Ostrowski NL, Wade JB, Knepper MA: Rat renal arcade segment expresses vasopressin-regulated water channel and vasopressin V2 receptor. *J Clin Invest* 97: 2763–2771, 1996
18. DiGiovanni SR, Nielsen S, Christensen EI, Knepper MA: Regulation of collecting duct water channel expression by vasopressin in Brattleboro rat. *Proc Natl Acad Sci USA* 91: 8984–8988, 1994
19. Deen PM, Verdijk MA, Knoers NV: Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 264: 92–95, 1994
20. Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen S: Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest* 95: 1838–1845, 1995
21. Frigeri A, Gropper MA, Turck CW, Verkman AS: Immunolocalization of the mercurial-insensitive water channel and glycerol intrinsic protein in epithelial cell plasma membranes. *Proc Natl Acad Sci USA* 92: 4328–4331, 1995
22. Chou CL, Ma T, Yang B, Knepper MA, Verkman AS: Fourfold reduction of water permeability in inner medullary collecting duct of aquaporin-4 knockout mice. *Am J Physiol* 274: C549–C554, 1998
23. Ishibashi K, Kuwahara M, Gu Y: Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. *J Biol Chem* 272: 20782–20786, 1997
24. Chevalier J, Bourguet J, Hugon JS: Membrane-associated particles: Distribution in frog urinary bladder epithelium at rest and after oxytocin treatment. *Cell Tissue Res* 152: 129–140, 1974
25. Kachadorian WA, Levine SD, Wade JB, Di-Scala VA, Hays RM: Relationship of aggregated intramembranous particles to water permeability in vasopressin-treated toad urinary bladder. *J Clin Invest* 59: 576–581, 1977
26. Humbert F, Montesano R, Grosso A, de-Sousa RC, Orci L: Particle aggregates in plasma and intracellular membranes of toad bladder (granular cell). *Experientia* 33: 1364–1367, 1977
27. Stetson DL, Wade JB, Giebisch G: Morphologic alterations in the rat medullary collecting duct following potassium depletion. *Kidney Int* 17: 45–56, 1980
28. Nielsen S, Frøkiær J, Knepper MA: Renal apuapoins: Key roles in water balance and water balance disorders. *Curr Opin Nephrol Hypertens* 7: 509–516, 1998
29. Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA: Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci USA* 92: 1013–1017, 1995
30. Marples D, Knepper MA, Christensen EI, Nielsen S: Redistribution of aquaporin-2 water channels induced by vasopressin in rat kidney inner medullary collecting duct. *Am J Physiol* 269: C655–C664, 1995
31. Sabolic I, Katsura T, Verbavatz JM, Brown D: The AQP2 water channel: Effect of vasopressin treatment, microtubule disruption, and distribution in neonatal rats. *J Membr Biol* 143: 165–175, 1995
32. Yamamoto T, Sasaki S, Fushimi K, Ishibashi K, Yaoita E, Kawasaki K, Marumo F, Kihara I: Vasopressin increases AQP-CD water channel in apical membrane of collecting duct principal cells in Brattleboro rats. *Am J Physiol* 268: C1546–C1551, 1995
33. Christensen BM, Marples D, Jensen UB, Frøkiær J, Sheikh-Hamad D, Knepper MA, Nielsen S: Acute effects of vasopressin V_2-receptor antagonist on kidney AQP2 expression and subcellular distribution. *Am J Physiol* 275: F285–F297, 1998
34. Hayashi M, Sasaki S, Tsuganezawa H, Monkawa T, Kitajima W, Konishi K, Fushimi K, Marumo F, Saruta T: Expression and distribution of aquaporin of collecting duct are regulated by vasopressin V2 receptor in rat kidney. *J Clin Invest* 94: 1778–1783, 1994
35. Saito T, Ishikawa SE, Sasaki S, Fujita N, Fushimi K, Okada K, Takeuchi K, Sakamoto A, Ookawara S, Kaneko T, Marumo F: Alteration in water channel AQP-2 by removal of AVP stimulation in collecting duct cells of dehydrated rats. *Am J Physiol* 272: F183–F191, 1997
36. Kuwahara M, Fushimi K, Terada Y, Bai L, Marumo F, Sasaki S: cAMP-dependent phosphorylation stimulates water permeability of aquaporin-collecting duct water channel protein expressed in *Xenopus* oocytes. *J Biol Chem* 270: 10384–10387, 1995
37. Lande MB, Jo I, Zeidel ML, Somers M, Harris HW Jr: Phosphorylation of aquaporin/2 does not alter the membrane water permeability of rat papillary water channel-containing vesicles. *J Biol Chem* 271: 5552–5557, 1996
38. Katsura T, Ausiello DA, Brown D: Direct demonstration of aquaporin-2 water channel recycling in stably transfected LLC-PK1 epithelial cells. *Am J Physiol* 270: F548–F553, 1996
39. Katsura T, Gustafson CE, Ausiello DA, Brown D: Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. *Am J Physiol* 272: F817–F822, 1997
40. Katsura T, Verbavatz JM, Farinas J, Ma T, Ausiello D, Brown D: Constitutive and regulated membrane expression of aquaporin 1 and aquaporin 2 water channels in stably transfected LLC-PK1 epithelial cells. *Proc Natl Acad Sci USA* 92: 7212–7216, 1995
41. Deen PM, Rijss JP, Mulders SM, Errington RJ, van Baal J, van Os CH: Aquaporin-2 transfection of Madin-Darby canine kidney cells reconstitutes vasopressin-regulated transcellular osmotic water transport. *J Am Soc Nephrol* 8: 1493–1501, 1997
42. Deen PM, Knoers NV: Physiology and pathophysiology of the aquaporin-2 water channel. *Curr Opin Nephrol Hypertens* 7: 37–42, 1998
43. Knepper MA, Nielsen S, Chou CL, DiGiovanni SR: Mechanism of vasopressin action in the renal collecting duct. *Semin Nephrol* 14: 302–321, 1994
44. Kurokawa K, Massry SG: Interaction between catecholamines and vasopressin on renal medullary cyclic AMP of rat. *Am J Physiol* 225: 825–829, 1973
45. Edwards RM, Jackson BA, Dousa TP: ADH-sensitive cAMP system in papillary collecting duct: Effect of osmolality and PGE_2. *Am J Physiol* 240: F311–F318, 1981
46. Dousa TP, Sands H, Hechter O: Cyclic AMP-dependent reversible phosphorylation of renal medullary plasma membrane protein. *Endocrinology* 91: 757–763, 1972
47. Gapstur SM, Homma S, Dousa TP: cAMP-binding proteins in medullary tubules from rat kidney: Effect of ADH. *Am J Physiol* 255: F292–F300, 1988
48. Nishimoto G, Zelenina M, Li D, Yasui M, Aperia A, Nielsen S, Nairn AC: Arginine vasopressin stimulates phosphorylation of aquaporin-2 in rat renal tissue. *Am J Physiol* 1999, in press
49. Wall SM, Han JS, Chou CL, Knepper MA: Kinetics of urea and water permeability activation by vasopressin in rat terminal IMCD. *Am J Physiol* 262: F989–F998, 1992
50. Fushimi K, Sasaki S, Marumo F: Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J Biol Chem* 272: 14800–14804, 1997
51. Christensen BM, Zelenina M, Aperia A, Nielsen S: Localization of phosphorylated aquaporin-2 (AQP2) in kidney collecting duct

principal cells in response to dDAVP or V_2 receptor antagonist treatment [Abstract]. *J Am Soc Nephrol* 9: 16A, 1998
52. Zelenina M, Christensen BM, Nielsen S, Aperia A: Prostaglandin E2 interaction with arginine vasopressin (AVP): Effects on aquaporin (AQP) 2 abundance in membrane and the state of AQP2 phosphorylation [Abstract]. *J Am Soc Nephrol* 9: 29A, 1998
53. Gronbeck L, Marples D, Nielsen S, Christensen S: Mechanism of antidiuresis caused by bendroflumethiazide in conscious rats with diabetes insipidus. *Br J Pharmacol* 123: 737–745, 1998
54. Phillips ME, Taylor A: Effect of colcemid on the water permeability response to vasopressin in isolated perfused rabbit collecting tubules. *J Physiol Lond* 456: 591–608, 1992
55. Phillips ME, Taylor A: Effect of nocodazole on the water permeability response to vasopressin in rabbit collecting tubules perfused in vitro. *J Physiol Lond* 411: 529–544, 1989
56. Valenti G, Hugon JS, Bourguet J: To what extent is microtubular network involved in antidiuretic response? *Am J Physiol* 255: F1098–F1106, 1988
57. Achler C, Filmer D, Merte C, Drenckhahn D: Role of microtubules in polarized delivery of apical membrane proteins to the brush border of the intestinal epithelium. *J Cell Biol* 109: 179–189, 1989
58. Marples D, Turner M, Taylor A: Increase in microtubule content in response to cAMP in rabbit cortical collecting ducts [Abstract]. *Kidney Int* 47: 662, 1995
59. Marples D, Schroer TA, Ahrens N, Taylor A, Knepper MA, Nielsen S: Dynein and dynactin colocalize with AQP2 water channels in intracellular vesicles from kidney collecting duct. *Am J Physiol* 274: F384–F394, 1998
60. de-Sousa RC, Grosso A: Vanadate blocks cyclic AMP-induced stimulation of sodium and water transport in amphibian epithelia. *Nature* 279: 803–804, 1979
61. Marples D, Barber B, Taylor A: Effect of a dynein inhibitor on vasopressin action in toad urinary bladder. *J Physiol Lond* 490: 767–774, 1996
62. Wade JB, Kachadorian WA: Cytochalasin B inhibition of toad bladder apical membrane responses to ADH. *Am J Physiol* 255: C526–C530, 1988
63. Pearl M, Taylor A: Actin filaments and vasopressin-stimulated water flow in toad urinary bladder. *Am J Physiol* 245: C28–C39, 1983
64. Kachadorian WA, Ellis SJ, Muller J: Possible roles for microtubules and microfilaments in ADH action on toad urinary bladder. *Am J Physiol* 236: F14–F20, 1979
65. Muller J, Kachadorian WA: Aggregate-carrying membranes during ADH stimulation and washout in toad bladder. *Am J Physiol* 247: C90–C98, 1984
66. Pearl M, Taylor A: Role of the cytoskeleton in the control of transcellular water flow by vasopressin in amphibian urinary bladder. *Biol Cell* 55: 163–172, 1985
67. Ding GH, Franki N, Condeelis J, Hays RM: Vasopressin depolymerizes F-actin in toad bladder epithelial cells. *Am J Physiol* 260: C9–C16, 1991
68. DiBona DR: Cytoplasmic involvement in ADH-mediated osmosis across toad urinary bladder. *Am J Physiol* 245: C297–C307, 1983
69. Rothman JE: Mechanisms of intracellular protein transport. *Nature* 372: 55–63, 1994
70. Nielsen S, Marples D, Birn H, Mohtashami M, Dalby NO, Trimble M, Knepper M: Expression of VAMP-2-like protein in kidney collecting duct intracellular vesicles: Co-localization with aquaporin-2 water channels. *J Clin Invest* 96: 1834–1844, 1995
71. Franki N, Macaluso F, Gao Y, Hays RM: Vesicle fusion proteins in rat inner medullary collecting duct and amphibian bladder. *Am J Physiol* 268: 792–797, 1995
72. Jo I, Harris HW, Amedt-Raduege AM, Majewski RR, Hammond TG: Rat kidney papilla contains abundant synaptobrevin protein that participates in the fusion of antidiuretic hormone (ADH) water channel-containing endosomes in vitro. *Proc Natl Acad Sci USA* 92: 1876–1880, 1995
73. Mandon B, Chou CL, Nielsen S, Knepper MA: Syntaxin-4 is localized to the apical plasma membrane of rat renal collecting duct cells: Possible role in aquaporin-2 trafficking. *J Clin Invest* 98: 906–913, 1996
74. Mandon B, Nielsen S, Kishore BK, Knepper MA: Expression of syntaxins in rat kidney. *Am J Physiol* 273: F718–F730, 1997
75. Marples D, Christensen BM, Frøkiær J, Knepper MA, Nielsen S: Dehydration reverses vasopressin antagonist-induced diuresis and aquaporin-2 downregulation in rats. *Am J Physiol* 275: F400–F409, 1998
76. Terris J, Ecelbarger CA, Nielsen S, Knepper MA: Long-term regulation of four renal aquaporins in rats. *Am J Physiol* 271: F414–F422, 1996
77. Yasui M, Zelenin SM, Celsi G, Aperia A: Adenylate cyclase-coupled vasopressin receptor activates AQP2 promoter via a dual effect on CRE and AP1 elements. *Am J Physiol* 272: F443–F450, 1997
78. Hozawa S, Holtzman EJ, Ausiello DA: cAMP motifs regulating transcription in the aquaporin 2 gene. *Am J Physiol* 270: C1695–C1702, 1996
79. Matsumura Y, Uchida S, Rai T, Sasaki S, Marumo F: Transcriptional regulation of aquaporin-2 water channel gene by cAMP. *J Am Soc Nephrol* 8: 861–867, 1997
80. Uchida S, Sasaki S, Fushimi K, Marumo F: Isolation of human aquaporin-CD gene. *J Biol Chem* 269: 23451–23455, 1994
81. Ecelbarger CA, Nielsen S, Olson BR, Murase T, Baker EA, Knepper M, Verbalis JG: Role of renal aquaporins in escape from vasopressin-induced antidiuresis in rat. *J Clin Invest* 99: 1852–1863, 1997
82. Promeneur D, Kwon TH, Christensen BM, Frøkiær J, Knepper MA, Nielsen S: Vasopressin independent regulation of collecting duct AQP2 expression in rat [Abstract]. *J Am Soc Nephrol* 9: 24A, 1998
83. Kishore BK, Terris JM, Knepper MA: Quantitation of aquaporin-2 abundance in microdissected collecting ducts: Axial distribution and control by AVP. *Am J Physiol* 271: F62–F70, 1996
84. Bichet DG: Vasopressin receptors in health and disease. *Kidney Int* 49: 1706–1711, 1996
85. Kanno K, Sasaki S, Hirata Y, Ishikawa S, Fushimi K, Nakanishi S, Bichet DG, Marumo F: Urinary excretion of aquaporin-2 in patients with diabetes insipidus. *N Engl J Med* 332: 1540–1545, 1995
86. Deen PM, van Aubel RA, van Lieburg AF, van Os CH: Urinary content of aquaporin 1 and 2 in nephrogenic diabetes insipidus. *J Am Soc Nephrol* 7: 836–841, 1996
87. Elliot S, Goldsmith P, Knepper M, Haughey M, Olson B: Urinary excretion of aquaporin-2 in humans: A potential marker of collecting duct responsiveness to vasopressin. *J Am Soc Nephrol* 7: 403–409, 1996
88. Frøkiær J, Wen H, Isikay L, Marples D, Knepper MA, Djurhuus JC, Ørskov H, Nielsen S: Aquaporin-2 is excreted in urine by a selective apical pathway different from whole-cell shedding [Abstract]. *J Am Soc Nephrol* 8: 17A, 1998
89. Marples D, Frøkiær J, Dørup J, Knepper MA, Nielsen S: Hy-

pokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *J Clin Invest* 97: 1960–1968, 1996
90. Earm JH, Christensen BM, Frøkiær J, Marples D, Han JS, Knepper MA, Nielsen S: Decreased aquaporin-2 expression and apical plasma membrane delivery in kidney collecting ducts of polyuric hypercalcemic rats. *J Am Soc Nephrol* 9: 2181–2193, 1998
91. Sands JM, Flores FX, Kato A: Vasopressin-elicited water and urea permeabilities are altered in IMCD in hypercalcemic rats. *Am J Physiol* 274: F978–F985, 1998
92. Frøkiær J, Marples D, Knepper MA, Nielsen S: Bilateral ureteral obstruction downregulates expression of vasopressin-sensitive AQP-2 water channel in rat kidney. *Am J Physiol* 270: F657–F668, 1996
93. Frøkiær J, Kwon TH, Wen JG, Isikay L, Marples D, Djurhuus JC, Knepper MA, Nielsen S: Bilateral ureteral obstruction is associated with long-term downregulation of both aquaporin-1 and aquaporin-2 which parallels the impairment in urinary concentrating capacity [Abstract]. *J Am Soc Nephrol* 9: 18A, 1998
94. Lavizzo-Mourey RJ: Dehydration in the elderly: A short review. *J Natl Med Assoc* 79: 1033–1038, 1987
95. Sladek CD, McNeill TH, Gregg CM, Blair ML, Baggs RB: Vasopressin and renin response to dehydration in aged rats. *Neurobiol Aging* 2: 293–302, 1981
96. Swenson KL, Sands JM, Jacobs JD, Sladek CD: Effect of aging on vasopressin and aquaporin responses to dehydration in Fischer 344-brown-Norway F1 rats. *Am J Physiol* 273: R35–R40, 1997
97. Sands JM, Naruse M, Jacobs JD, Wilcox JN, Klein JD: Changes in aquaporin-2 protein contribute to the urine concentrating defect in rats fed a low-protein diet. *J Clin Invest* 97: 2807–2814, 1996
98. Frøkiær J, Christensen BM, Marples D, Djurhuus JC, Jensen UB, Knepper MA, Nielsen S: Downregulation of aquaporin-2 parallels changes in renal water excretion in unilateral ureteral obstruction. *Am J Physiol* 273: F213–F223, 1997
99. Apostol E, Ecelbarger CA, Terris J, Bradford AD, Andrews P, Knepper MA: Reduced renal medullary water channel expression in puromycin aminonucleoside-induced nephrotic syndrome. *J Am Soc Nephrol* 8: 15–24, 1997
100. Fernandez-Llama P, Andrews P, Ecelbarger CA, Nielsen S, Knepper M: Concentrating defect in experimental nephrotic syndrone: Altered expression of aquaporins and thick ascending limb Na+ transporters. *Kidney Int* 54: 170–179, 1998
101. Ecelbarger CA, Chou CL, Lee AJ, DiGiovanni SR, Verbalis JG, Knepper MA: Escape from vasopressin-induced antidiuresis: Role of vasopressin resistance of the collecting duct. *Am J Physiol* 274: F1161–F1166, 1998
102. Venkatachalam MA, Bernard DB, Donohoe JF, Levinsky NG: Ischemic damage and repair in the rat proximal tubule: Differences among the S1, S2, and S3 segments. *Kidney Int* 14: 31–49, 1978
103. Brezis M, Rosen S, Silva P, Epstein FH: Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. *J Clin Invest* 73: 182–190, 1984
104. Fernandez-Llama P, Andrews P, Turner R, Nielsen S, Safirstein RL, Knepper M: Collecting duct aquaporins in non-oliguric ischemic renal injury [Abstract]. *J Am Soc Nephrol* 9: 576A, 1998
105. Holliday MA, Egan TJ, Morris CR, Jarrah AS, Harrah JL: Pitressin-resistant hyposthenuria in chronic renal disease. *Am J Med* 42: 378–387, 1967
106. Tannen RL, Regal EM, Dunn MJ, Schrier RW: Vasopressin-resistant hyposthenuria in advanced chronic renal disease. *N Engl J Med* 280: 1135–1141, 1969
107. Buerkert J, Martin D, Prasad J, Chambless S, Klahr S: Response of deep nephrons and the terminal collecting duct to a reduction in renal mass. *Am J Physiol* 236: F454–F464, 1979
108. Wilson DR, Sonnenberg H: Medullary collecting duct function in the remnant kidney before and after volume expansion. *Kidney Int* 15: 487–501, 1979
109. Fine LG, Schlondorff D, Trizna W, Gilbert RM, Bricker NS: Functional profile of the isolated uremic nephron: Impaired water permeability and adenylate cyclase responsiveness of the cortical collecting tubule to vasopressin. *J Clin Invest* 61: 1519–1527, 1978
110. Teitelbaum I, McGuinness S: Vasopressin resistance in chronic renal failure: Evidence for the role of decreased V2 receptor mRNA. *J Clin Invest* 96: 378–385, 1995
111. Goldfine ID: Interaction of insulin, polypeptide hormones, and growth factors with intracellular membranes. *Biochim Biophys Acta* 650: 53–67, 1981
112. Kwon TH, Frøkiær J, Knepper MA, Nielsen S: Reduced AQP1, -2, and -3 levels in kidneys of rats with CRF induced by surgical reduction in renal mass. *Am J Physiol* 275: F724–F741, 1998
113. Nielsen S, Terris J, Andersen D, Ecelbarger C, Frøkiær J, Jonassen T, Marples D, Knepper MA, Petersen JS: Congestive heart failure in rats is associated with increased expression and targeting of aquaporin-2 water channel in collecting duct. *Proc Natl Acad Sci USA* 94: 5450–5455, 1997
114. Xu DL, Martin PY, Ohara M, St. John J, Pattison T, Meng X, Morris K, Kim JK, Schrier RW: Upregulation of aquaporin-2 water channel expression in chronic heart failure rat. *J Clin Invest* 99: 1500–1505, 1997
115. Ohara M, Martin PY, Xu DL, St. John J, Pattison TA, Kim JK, Schrier RW: Upregulation of aquaporin 2 water channel expression in pregnant rats. *J Clin Invest* 101: 1076–1083, 1998
116. Fujita N, Ishikawa SE, Sasaki S, Fujisawa G, Fushimi K, Marumo F, Saito T: Role of water channel AQP-CD in water retention in SIADH and cirrhotic rats. *Am J Physiol* 269: F926–F931, 1996
117. Asahina Y, Izumi N, Enomoto N, Sasaki S, Fushimi K, Marumo F, Sato C: Increased gene expression of water channel in cirrhotic rat kidneys. *Hepatology* 21: 169–173, 1995
118. Jonassen T, Nielsen S, Christensen S, Petersen JS: Decreased vasopressin-mediated renal water reabsorption in rats with compensated liver cirrhosis. *Am J Physiol* 275: F216–F225, 1998
119. Fernandez-Llama P, Jimenez W, Bosch-Marce M, Arroyo V, Nielsen S, Knepper MA: Dysregulation of aquaporin-1 and aquaporin-2 in the kidneys of cirrhotic rats [Abstract]. *J Am Soc Nephrol* 9: 18a, 1998