Physiology and Pathophysiology of Renal Aquaporins

SØREN NIELSEN,* TAE-HWAN KWON,* BIRGITTE MØNSTER CHRISTENSEN,* DOMINIQUE PROMENEUR,* JØRGEN FRØKIÆR,† and DAVID MARPLES‡
*Department of Cell Biology, Institute of Anatomy, University of Aarhus, and †Institute of Experimental Clinical Research, Aarhus University Hospital, Aarhus, Denmark; and ‡School of Biomedical Sciences, University of Leeds, United Kingdom.

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 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.

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 helices span the lipid membrane (6), thus supporting the current model described above. Moreover, the three-dimensional structure of AQP1 was determined at 6 Å resolution by cryoelectron microscopy (7). Each AQP1 monomer has six tilted, bilayer-spanning alpha-helices, which form a right-handed bundle surrounding a central density (7). These studies also confirmed

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Correspondence to Dr. Søren Nielsen, Department of Cell Biology, Institute of Anatomy, University of Aarhus, DK-8000 Aarhus, Denmark. Phone: +45 8942 3046; Fax: +45 8619 8664; E-mail: SN@ANA.AAU.DK

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the organization of the tetrameric complex in the membrane (7). The three-dimensional structure of AQP1 was also reported at 7 Å 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 transecl-

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Species*</th>
<th>No. of Amino Acids</th>
<th>Localization in Kidney</th>
<th>Subcellular Distribution</th>
<th>Regulation</th>
<th>Extrarenal Localization</th>
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<td></td>
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<tr>
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<td>Human</td>
<td>269</td>
<td>Proximal tubules, descending thin limbs</td>
<td>APM/BLM</td>
<td>-</td>
<td>Multiple organs</td>
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<tr>
<td>aquaporin 2</td>
<td>Rat</td>
<td>271</td>
<td>Collecting duct principal cells</td>
<td>APM</td>
<td>++</td>
<td>Testis</td>
</tr>
<tr>
<td>aquaporin 3</td>
<td>Rat</td>
<td>292</td>
<td>Collecting duct</td>
<td>VES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aquaporin 4</td>
<td>Rat</td>
<td>301</td>
<td>Medullary collecting duct</td>
<td>BLM</td>
<td>+</td>
<td>Multiple organs</td>
</tr>
<tr>
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<td>Rat</td>
<td>276</td>
<td>Cortex, medulla?</td>
<td>BLM</td>
<td>-</td>
<td>Brain and multiple organs</td>
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<td>BLM</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
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<td>Rat</td>
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<td>Cortex?</td>
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<td>?</td>
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<td></td>
<td></td>
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<tr>
<td>aquaporin 9</td>
<td>Human</td>
<td>295</td>
<td>?</td>
<td></td>
<td></td>
<td>Liver, leukocytes, lung, spleen</td>
</tr>
</tbody>
</table>

* 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.

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**Figure 1.** Schematic representation of the structural organization of aquaporin-1 (AQP1) monomers in the membrane.
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 H₂O 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
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.

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.
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-V2 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.
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 V₂ receptors (44,45). The synthesis of cAMP by adenylate cyclase is stimulated by a V₂ receptor-coupled heterotrimeric GTP-binding protein, G₅G₆G₇, which interconvert between an inactive GDP form and an active GTP form, and the vasopressin-V₂ receptor complex catalyzes the exchange of GTP for bound GDP on the α-subunit of G₅. This causes release of the α-subunit G₅α-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 vasoressin treatment of slices of the kidney papilla (48). This agrees well with the time course of vasoressin-stimulated water permeability of kidney collecting ducts (49). As described above, PKA-induced phosphorylation of AQP2 apparently does not change the water conductance of AQP2. Importantly, it was recently demonstrated that vasoressin 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 V2 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 vasoressin-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 vasoressin-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 vasoressin stimulation. Furthermore, AQP2 is delivered specifically to the apical plasma membrane. Finally, AQP2-bearing vesicles fuse with the apical plasma membrane in response to vasoressin, but not to a significant degree in the absence of stimulation (e.g., in vasoressin-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 hydromotic response to arginine vasoressin, 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 hydromotic 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 microfila-
mements are probably involved in the final movement of vesicles
through the terminal web, their fusion with the plasma mem-
brane, 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 attach-
ment 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 vesi-
cles 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 pro-
tein) 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 in-
crease 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
congruent. Long-term regulation of collecting duct water perme-
ability 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
depprivation or long-term dDAVP treatment (16,18). Brattle-
boro rats (lacking vasopressin) or normal rats treated with V2
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 adap-
tational 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). Con-
versely, downregulation of AQP2 expression, e.g., in response
to water loading, is likely to be due to reduced gene transcrip-
tion and enhanced (or maintained) delivery of AQP2 into a
degrative pathway.

**Vasopressin-Independent Regulation of AQP2 Expression**

The presence of a cAMP-responsive element in the 5′ flank-
ing element region of the AQP2 gene (80) and a PKA phos-
phorylation 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 in-
creases AQP2 expression more than sustained dDAVP treat-
ment 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, suggesting 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 va-
opressin. 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-
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 V₂-vasopressin receptor gene (84). Because both AQP2 targeting and expression are regulated tightly by vasopressin, the reduction in V₂ signal transduction due to the mutation in the V₂ receptor is likely to
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

<table>
<thead>
<tr>
<th>Conditions with reduced AQP2 expression and polyuria</th>
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<tbody>
<tr>
<td>genetic defects</td>
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<tr>
<td>Brattleboro rats (central DI)</td>
</tr>
<tr>
<td>DI +/+ severe mice (low cAMP)</td>
</tr>
<tr>
<td>AQP2 mutants (human)</td>
</tr>
<tr>
<td>V2 receptor variants (human) b</td>
</tr>
<tr>
<td>acquired NDI (rat models)</td>
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<tr>
<td>lithium</td>
</tr>
<tr>
<td>hypokalemia</td>
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<tr>
<td>hypercalcaemia</td>
</tr>
<tr>
<td>postobstructive NDI</td>
</tr>
<tr>
<td>bilateral</td>
</tr>
<tr>
<td>unilateral</td>
</tr>
<tr>
<td>low protein diet (urinary concentrating defect with no polyuria)</td>
</tr>
<tr>
<td>water loading (compulsive water drinking)</td>
</tr>
<tr>
<td>experimental chronic renal failure (5/6 nephrectomy model)</td>
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<tr>
<td>ischemia-induced acute renal failure (polyuric phase in rat model)</td>
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<td>age-induced NDF</td>
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<tr>
<th>Conditions with increased AQP2 expression with expansion of extracellular fluid volume</th>
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<tr>
<td>vasopressin infusion (SIADH)</td>
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<tr>
<td>congestive heart failure</td>
</tr>
<tr>
<td>hepatic cirrhosis (CCL4-induced, noncompensated) ?</td>
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<td>pregnancy</td>
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<table>
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<tr>
<th>Conditions with altered expression and dilution without polyuria</th>
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<tbody>
<tr>
<td>nephrotic syndrome models (rat models)</td>
</tr>
<tr>
<td>PAN</td>
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<tr>
<td>adriamycin</td>
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<tr>
<td>hepatic cirrhosis (CBL, compensated)</td>
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<tr>
<td>ischemia-induced acute renal failure (oliguric phase in rat model)</td>
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<td>pregnancy</td>
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| Acquired NDI, postobstructive polyuria (98)                                           |

* 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 V2 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,
holders 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 hypotonicity 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 hydraulic 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 V2 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-V2 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 AQ2P2 expression could be a factor in the increased water reabsorption and reduced dilutional ability. However, as will be discussed, the changes in AQ2P2 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 AQ2P2 protein and AQ2P2 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, AQ2P2 mRNA levels correlated with the amount of ascites, suggesting that AQ2P2 may play a role in the development of water retention. As with congestive heart failure, treatment with the vasopressin V2 receptor antagonist OPC31260 reversed the increase in AQ2P2 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 AQ2P2 expression. This reduction in collecting duct water channels was consistent with functional data demonstrating a reduced effect of vasopressin-V2 receptor antagonist treatment (118). In yet a third model, using CCl4 inhalation, hepatic cirrhosis was associated with ascites and hyponatremia (evidence of excess water retention). However, no change in AQ2P2 expression was observed in this model of hepatic cirrhosis, but AQ1P1 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 AQ2P2 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 V2 receptor antagonist OPC31260 effectively downregulates AQ2P2 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 V2 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 HgCl2 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.

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