Aquaporins in the Kidney: From Molecules to Medicine

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Nielsen, Søren, Jørgen Frøkiær, David Marples, Tae-Hwan Kwon, Peter Agre, and Mark A. Knepper. Aquaporins in the Kidney: From Molecules to Medicine. Physiol Rev 82: 205–244, 2002; 10.1152/physrev.00024.2001.---The discovery of aquaporin-1 (AQP1) answered the long-standing biophysical question of how water specifically crosses biological membranes. In the kidney, at least seven aquaporins are expressed at distinct sites. AQP1 is extremely abundant in the proximal tubule and descending thin limb and is essential for urinary concentration. AQP2 is exclusively expressed in the principal cells of the connecting tubule and collecting duct and is the predominant vasopressin-regulated water channel. AQP3 and AQP4 are both present in the basolateral plasma membrane of collecting duct principal cells and represent exit pathways for water reabsorbed apically via AQP2. Studies in patients and transgenic mice have demonstrated that both AQP2 and AQP3 are essential for urinary concentration. Three additional aquaporins are present in the kidney. AQP6 is present in intracellular vesicles in collecting duct intercalated cells, and AQP8 is present intracellularly at low abundance in proximal tubules and collecting duct principal cells, but the physiological function of these two channels remains undefined. AQP7 is abundant in the brush border of proximal tubule cells and is likely to be involved in proximal tubule water reabsorption. Body water...
balance is tightly regulated by vasopressin, and multiple studies now have underscored the essential roles of AQP2 in this. Vasopressin regulates acutely the water permeability of the kidney collecting duct by trafficking of AQP2 from intracellular vesicles to the apical plasma membrane. The long-term adaptational changes in body water balance are controlled in part by regulated changes in AQP2 and AQP3 expression levels. Lack of functional AQP2 is seen in primary forms of diabetes insipidus, and reduced expression and targeting are seen in several diseases associated with urinary concentrating defects such as acquired nephrogenic diabetes insipidus, postobstructive polyuria, as well as acute and chronic renal failure. In contrast, in conditions with water retention such as severe congestive heart failure, pregnancy, and syndrome of inappropriate antidiuretic hormone secretion, both AQP2 expression levels and apical plasma membrane targeting are increased, suggesting a role for AQP2 in the development of water retention. Continued analysis of the aquaporins is providing detailed molecular insight into the fundamental physiology and pathophysiology of water balance and water balance disorders.

I. INTRODUCTION

The discovery of aquaporin membrane water channels by Agre and co-workers (6, 7, 191, 192) answered a long-standing biophysical question of how water crosses biological membranes specifically, and provided insight, at the molecular level, into the fundamental physiology of water balance and the pathophysiology of water balance disorders. Out of at least 10 aquaporin isoforms, at least 7 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 be the main site for proximal nephron water reabsorption. It is also present in the descending vasa recta. AQP2 is abundant in the collecting duct principal cells and is the chief target for the regulation of collecting duct water reabsorption by vasopressin. Acute regulation involves vasopressin-induced trafficking of AQP2 between an intracellular reservoir in vesicles and the apical plasma membrane. In addition, AQP2 is involved in chronic/adaptational control of body water balance, which is achieved through regulation of AQP2 expression. Importantly, multiple studies have now underscored a critical role of AQP2 in several inherited and acquired water balance disorders. This includes inherited forms of nephrogenic diabetes insipidus, acquired states of nephrogenic diabetes insipidus, and other diseases associated with urinary concentrating defects where AQP2 expression and 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 represent exit pathways for water reabsorbed via AQP2. Several additional aquaporins have been identified in kidney, including AQP6, which is expressed at lower abundance in the collecting duct intercalated cells. Four additional aquaporins (AQP7, -8, -9, and -10) are also expressed in kidney, but less is known about expression sites and their pathophysiological function. In this review we focus mainly on the role of collecting duct aquaporins in water balance regulation and in the pathophysiology of water balance disorders.

II. DISCOVERY OF THE FIRST MOLECULAR WATER CHANNEL

The molecular identity of membrane water channels long remained elusive until the pioneering discovery of AQP1 by Agre and colleagues around 1989–1991, and the “route” of this discovery has been covered in several reviews (6).

Previous attempts to purify water channel proteins from tissues, or to isolate water channel cDNAs by expression cloning in oocytes, were unsuccessful (see reviews in Refs. 4, 8, 15). The identification of the first water channel took place via a convoluted route. The Agre lab at that time worked on identifying the function of Rhesus polypeptides and in the process of isolating a 32-kDa bilayer-spanning polypeptide component of the red cell Rh blood group antigen (7, 205); a 28-kDa polypeptide was partially copurified (46) which displayed a number of biochemical characteristics (6). It was comprised of hydrophobic amino acids and exhibited an unusual detergent solubility allowing purification and biochemical characterization. The 28-kDa polypeptide was found to exist as an oligomeric protein with the physical characteristics of a tetramer. The NH$_2$-terminal amino acid sequence was identified (213) that subsequently allowed cDNA cloning (191). This protein was first known as “CHIP28” (channel-like integral protein of 28 kDa), but was later redubbed aquaporin-1 or AQP1 (8). The Human Genome Nomenclature Committee has accepted this nomenclature for all related proteins.

The *Xenopus laevis* oocyte expression system, largely developed by Wright et al. (254), was used by Preston et al. (191) and oocytes injected with cRNA for “CHIP28” (or AQP1), exhibited remarkably high osmotic water permeability causing the cells to swell rapidly and burst in hypotonic buffer. In contrast, control oocytes injected with water alone exhibited water permeability one order of magnitude lower. Thus this strongly suggested that AQP1 was a molecular water channel. Moreover, the oocyte studies demonstrated that AQP1 behaves like the water channels in native cell membranes (192). The biophysical characteristics of AQP1 in oocytes revealed a series of the water channel specific characteris-
AQP1 contains an internal repeat with the NH2- and the COOH-terminal halves being sequence related and each containing the signature motif Asn-Pro-Ala (NPA) (181, 252). This is consistent with earlier observations on the homologous major intrinsic protein from lens, (MIP, now referred to as AQP0). When evaluated by hydropathy analysis, six bilayer-spanning domains are apparent (Fig. 1); however, the apparent interhelical loops B and E also exhibit significant hydrophobicity. Critical to the topology is the location of loop C which connects the two halves of the molecule. Epitope mapping has been extensively used for analyzing the AQP1 structure by adding a peptide epitope at various sites that can be localized to intracellular or extracellular sites with antibodies and by selective proteolysis of intact membranes or inside-out membrane vesicles. Using such an approach, Preston et al. (194) demonstrated that loop C resides at the extracellular surface of the oocytes, confirming the obverse symmetry of the NH2- and COOH-terminal halves of the molecule. Site-directed mutagenesis of AQP1 expressed in oocytes led to the observations that Cys-189 in the E loop is the site of mercurial inhibition (193), and this has been found by other workers (271). Thus loop E has been implicated as a structural component of the aqueous pathway. Moreover, it has been demonstrated that loops B and E were functionally essential for water permeability, leading to the “hour-glass” model (107) in which these domains overlap midway between the leaflets of the bilayer, creating a narrow aqueous pathway (Fig. 1).

B. Cryo-electron Microscopy and Atomic Force Microscopy of AQP1

Reconstitution of highly purified red cell AQP1 into membranes forms crystals with very uniform lattices that retain full osmotic water permeability (248). High-resolution electron microscopic evaluation of such membranes subjected to negative staining allowed the production of a three-dimensional reconstruction (247). Image projections revealed the presence of multiple bilayer-spanning domains, and atomic force microscopy further defined the orientation and extramembranous dimensions of AQP1 (249). Electron crystallographic analysis of cryopreserved specimens has been undertaken which allowed determination of the three-dimensional structure of AQP1 at 6-Å resolution (21, 134, 246). These images confirm the tetrameric organization, within which each subunit is comprised of six tilted, bilayer-spanning α-helices that form a right-handed bundle surrounding a central density, an arrangement which is strikingly similar to the original proposed hour-glass arrangement of the B and E loops (107). This approach eventually provided the AQP1 structure at 3.8-Å resolution. By modeling the AQP1 sequence within the electron density, the three-dimensional atomic structural model of AQP1 has emerged (162a). This model first provided a molecular answer to the long-standing problem, How can water channels avoid passage of protons (H3O+)? As predicted, loops B and E are associated by Van der Waals interactions between the two NPA motifs. Free hydrogen bonding occurs in the column of water within the pore, except at the very center where a single water molecule transiently reorients to bond with the two asparagines residues of the NPA motif. This results in minimum resistance to the flow of water, thus permitting kidneys to perform their important physiological roles of reabsorbing water while excreting acid (see below).

C. Cryo-electron Microscopy and Atomic Force Microscopy of Other Aquaporins

The structural organization of other aquaporins such as bacterial aquaporin-Z and plant aquaporins have also been studied, providing further information about the
general structure of aquaporins. They display marked similarities to AQP1. This work has been reviewed in detail previously (200). Virtually simultaneous to the studies of AQP1, the atomic structure of GlpF, the glycerol-translocating homolog of *Escherichia coli*, was solved at 2.2-Å resolution by X-ray crystallographic analysis (78a). The

![Diagram](image)

**FIG. 1.** *A*: schematic representation of the structural organization of aquaporin-1 (AQP1) monomers in the membrane (top and bottom). Aquaporins have six membrane-spanning regions, both intracellular NH$_2$ and COOH termini, and internal tandem repeats that, presumably, are due to an ancient gene duplication (top). The topology is consistent with an obverse symmetry for the two similar NH$_2$- and COOH-terminal halves (bottom). The tandem repeat structure with two asparagine-proline-alanine (NPA) sequences has 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 aqueous pathway within a symmetric structure that resembles an “hourglass.” *B*: AQP1 is a multisubunit oligomer that is organized as a tetrameric assembly of four identical polypeptide subunits with a large glycan attached to only one.
structure of GpP is virtually identical to AQP1; however, the aqueous pore is slightly wider, and key hydrophobic residues provide a “slippery slide” for the carbon backbone of glycerol. Although the physiological need for glycerol transport is not understood in some tissues, it may confer important properties to other tissues (e.g., glycerol release from adipocytes in response to starvation).

D. Oligomeric Organization of AQP1, AQP2, and AQP4

As discussed above, the crystallographic studies have confirmed the tetrameric organization of AQP1 in the membrane (Fig. 1). Studies by Brown and colleagues (235, 239, 258) using Chinese hamster ovary (CHO) cells transfected with AQP1 to -5 have indicated that AQP2, -3, and -5 may also form tetramers in the membrane.

Not all aquaporins appear to assemble in the plasma membrane as tetramers. Recently, several studies revealed that AQP4 forms larger oligomeric structures in the plasma membrane. It is well established that freeze-fracture analyses of glial cells and other cells, later found to express high amounts of AQP4, have a high density of intramembrane particle square arrays also called orthogonal arrays (clusters of intramembrane particles in a special systematic/geometric organization). The subsequent demonstration that square arrays are absent in cells from transgenic knockout mice lacking AQP4 protein (240) supports 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 et al. (198). It is not yet clear if these square arrays are themselves made up of tetramers.

Similarly, structures (referred to as linear parallel grooves) exist in the “intramembrane particle arrays” described in toad bladder (which is a functional homolog of the mammalian collecting duct) and thought to represent the sites of antidiuretic hormone (ADH)-induced water flow in this tissue (22, 110). Although suspected, it remains to be proven that these represent members of the aquaporin family.

IV. BIOPHYSICS OF AQUAPORIN FUNCTION

A. Discovery and Biophysical Characterization of the First Molecular Water Channel AQP1

Expression of AQP1 in X. laevis oocytes by Preston et al. (192) demonstrated that AQP1-expressing oocytes exhibited remarkably high osmotic water permeability ($P_f \approx 200 \times 10^{-4}$ cm/s), causing the cells to swell rapidly and explode in hypotonic buffer. The osmotically induced swelling of oocytes expressing AQP1 occurs with a low activation energy and is reversibly inhibited by HgCl$_2$ or other mercurials. Only inward water flow (swelling) was examined, but it was predicted that the direction of water flow through AQP1 is determined by the orientation of the osmotic gradient. Consistent with this, it was later demonstrated that AQP1-expressing oocytes swell in hypomolar buffers but shrink in hyperosmolar buffers (160). Highly purified AQP1 protein from human red blood cells was reconstituted with pure phospholipid into proteoliposomes and were compared with liposomes without AQP1. The analyses were performed by rapid transfer from hyperosmolar buffer (267). The unit water permeability (conductance per monomeric AQP1) was extremely high ($P_3 \approx 3 \times 10^9$ water molecules subunits/s). As demonstrated in oocytes, AQP1 reconstituted in liposomes also displayed sensitivity to HgCl$_2$, which dramatically and reversibly reduced the osmotic water permeability. The AQP1-containing liposomes also exhibited a low activation energy for water permeation. This was confirmed in other studies (234). Zeidel et al. (268) also demonstrated that AQP1 proteoliposomes are not permeable to various small solutes or protons, thus revealing that AQP1 is water selective. Together, these studies indicated that AQP1 is both necessary and sufficient to explain the well-recognized membrane water permeability of the red blood cell and strongly suggests that AQP1 water channels are of fundamental importance for transmembrane or transcellular water transport in tissues where it is expressed.

B. Selectivity

Over the past 4 years a series of studies have explored the issues of selectivity and polytransport function of aquaporins. This has led to a division of aquaporins (4) into a group that transports water relatively selectively (the “orthodox” set or “aquaporins”) and a group of water channels that also conduct glycerol and other small solutes in addition to water (the “cocktail” set or aquaglyceroporins). This appears to represent an ancient phylogenetic divergence between glycerol transporters and pure water channels (185). Recently, it has become clear that transport properties are even more diverse, since AQP6 has been demonstrated to conduct anions as well (263), and it has also been demonstrated that aquaporins can be regulated by gating, as discussed below.

AQP1 is generally believed to be a constitutively active, water-selective pore. Nonetheless, some observations contradict this. A small degree of permeation by glycerol has been seen in AQP1 oocytes, which may represent opening or presence of a leak pathway (1), but the biological significance of this remains unclear (160). Yool et al. (206) reported that forskolin treatment of oocytes expressing AQP1 induces a cation current, indicating the
presence of cation permeation. However, several other research groups were unable to reproduce this effect (5).

Yasui et al. (263) recently demonstrated that AQP6 expressed in oocytes displays unique biophysical properties. It has a low, but significant, basolateral water permeability. Treatment of AQP6 oocytes with mercurials dramatically increases the osmotic water permeability but interestingly also induces membrane conductance for other small anions. Thus AQP6 can function as an anion channel. Moreover, it was demonstrated that reducing the pH to \( <5.5 \) resulted in a marked increase in anion conductance revealing that changes in pH may provide a physiological mediator of these effects. The physiological role of anion conductance remains to be established, but it is suspected that this may be involved in vesicle acidification, since AQP6 is expressed only on intracellular vesicles.

The “aquaporin of the toad bladder” may allow the passage of protons. Gluck and Al-Awqati (86) showed an increase in proton permeability that occurred in parallel with the ADH-induced increase in water permeability of this tissue. This proton permeability was blocked by methohexital, an inhibitor of water permeability, but not by inhibitors of urea or sodium permeation. The authors concluded that the protons were crossing through the water channels, but direct proof of this remains elusive.

Recently, Yool and colleagues (10) provided evidence that an additional water channel (viz AQP1) can also provide a pathway for anions. Using two-electrode voltage-clamp analyses, the authors showed activation of an ionic conductance in AQP1-expressing oocytes after direct injection of cGMP. Current activation was not observed in control (water-injected) oocytes or in AQP5-expressing oocytes with osmotic water permeabilities equivalent to those seen with AQP1. Patch-clamp recordings revealed large-conductance channels in excised patches from AQP1-expressing oocytes after the application of cGMP to the internal side. These results indicate that AQP1 channels have the capacity to participate in ionic signaling after the activation of cGMP second messenger pathways. These observations were qualitatively confirmed using purified AQP1 reconstituted into planar bilayers (208a); however, the stoichiometry (only one ion channel per \( 10^7 \) AQP1 molecules) and permeation through a pathway distinct from the aqueous pore raise serious doubts about possible physiological significance.

Recently, the possible permeation of small gases through aquaporins has been investigated using CO\(_2\). The rates of pH change are \( \sim40\% \) higher in oocytes expressing AQP1 (166) than in control oocytes. As described in a follow-up paper (41), this observation is consistent with several interpretations. The authors found that expressing the AQP1 in \( Xenopus \) oocytes increases the CO\(_2\) permeability of oocytes in an expression-dependent fashion, whereas expressing the K\(^+\) channel ROMK1 has no effect. The mercurial \( p\)-chloromercuribenzenesulfonate (PCMBs), which inhibits the water conductance through AQP1, also blocks the AQP1-dependent increase in CO\(_2\) permeability. The mercury-insensitive C189S mutant of AQP1 increases the CO\(_2\) permeability of the oocyte to the same extent as does the wild-type channel, but the C189S-dependent increase in CO\(_2\) permeability is unaffected by treatment with PCMBs. These results therefore suggest that CO\(_2\) passes through the same pore in AQP1 as water does. It should be mentioned that experiments on the CO\(_2\) permeability of the erythrocytes and lung cells from AQP1 null mice and wild-type mice revealed no differences, arguing against a major CO\(_2\) permeability through AQP1 (260). The potential physiological relevance of AQP1 permeation by gases warrants more study (190). Thus, although the evidence that AQP1 functions as a water channel is incontrovertible, the possibility of yet undiscovered transport functions cannot be excluded.

Whereas some previously cloned aquaporins are only permeable to water, some homologs are permeated by water, glycerol, and other small solutes. AQP3 was noted to be genetically closer to the \( E. \ coli \) glycerol transport protein GlpF. The structural explanation for how AQP3 may permit transport of water and glycerol is debated (60, 99, 160); however, the atomic structure of GlpF now provides a clear explanation (78a). Multiple other aquaglyceroporins are now being identified by cDNA cloning and computer search of expressed sequence tagged (EST) cDNA libraries. A cDNA encoding AQP7 was isolated from rat testis (100, 218) and AQP9 from liver (97, 122, 123, 225, 226). These homologs are also thought to function as glycerol transporters as well as water channels.

C. Regulation by Gating

A major research topic has been the elucidation of how aquaporins are regulated. In the kidney collecting duct it became clear in the mid 1990s that AQP2 is regulated by vasopressin-induced shuttling from an intracellular reservoir in vesicles to the apical plasma membrane, thereby increasing the osmotic water permeability of the membrane and the collecting duct (see sects. \( vB \) and \( vL \)). Moreover, it has been demonstrated that the expression levels of AQP2 (and of AQP3) are also tightly regulated, as described in sections \( vB \) and \( vL \), \( B–D \). However, it was unclear whether aquaporins are also subject to regulation by gating.

Early, but indirect, evidence for the gating of water channels came from studies looking at the effect of pH on toad bladder water permeability. Lowering the pH both inhibited the increase in water permeability induced by ADH and reversed the increase after it had been established. These changes were also seen after stimulation with cAMP instead of ADH, indicating that the effect of pH was at a step distal to the activation of adenylate

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cyclase. This correlated with the prevention of the appearance of particle aggregates thought to represent the sites of water flow in the apical plasma membrane, or their disappearance in an established response. However, at low temperatures, when removal of the aggregates from the plasma membrane was slowed, lowering the pH led to a fall in water permeability without a parallel fall in aggregate area, suggesting that the channels could be closed. Furthermore, raising the pH led to a rapid restoration of water flow, consistent with the channels reopening (23, 182–184). Thus pH appeared to affect both the trafficking of the water channels and the permeability of the channels themselves. These effects could be caused by lowering either mucosal or serosal pH, but only if a buffer system, such as bicarbonate/CO₂, which could modify cytosolic pH, was used. This implies that it is the cytosolic pH that is responsible for the altered channel permeability, whereas extracellular pH has no effect.

A breakthrough discovery came in a recent report by Agre and associates on AQP6 (264), a water channel residing in intracellular vesicles in kidney collecting duct intercalated cells (263). When expressed in X. laevis oocytes, AQP6 exhibits low basal water permeability (P_f), but (surprisingly) when treated with mercurials, known inhibitors of many aquaporin water channels, the osmotic water permeability of AQP6 oocytes rapidly rises ~10-fold (and is accompanied by ion conductance, as described above). Because AQP6 is present in intercalated cells, which are engaged in renal acid/base regulation, it was tested if pH may be a physiological parameter that may alter the conductance. At pH <5.5, water permeability and anion conductance are rapidly and reversibly activated in AQP6-expressing oocytes. Site-directed mutation of lysine to glutamate at position 72 in the cytoplasmic mouth of the pore changes the cation/anion selectivity but leaves low pH activation intact. These studies demonstrated unprecedented biophysical properties of an aquaporin, viz regulation by gating, and in addition provided evidence for anion channel function.

Evidence has also appeared that additional aquaporins, such as AQP3, are also regulated by gating in response to pH changes (270).

V. AQUAPORINS IN KIDNEY

Absorption of water out of the renal tubule depends on the osmotic driving force for water reabsorption and the equilibration of water across the tubular epithelium (119). 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 over the past 10 years has made it clear that osmotic water transport across the tubule epithelium is chiefly dependent on aquaporin water channels.

At least seven aquaporins are expressed in the kidney (Table 1). AQP1 (46, 191, 192) is present in the proximal tubule and in the descending thin limb (Fig. 2) but is absent in highly water-impermeable segments of ascending thin limb, thick ascending limb, and distal tubule (175). Moreover, it is absent from the collecting duct, making it clear that AQP1 does not participate in the regulation by vasopressin of renal water excretion (175). Subsequently, additional aquaporins were identified by homology cloning approaches. These include AQP2 (82),

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<th>TABLE 1. Distribution of aquaporin-1 to -10 in kidney and other organs</th>
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<td>Renal aquaporins</td>
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APM, apical plasma membrane; BLM, basolateral plasma membrane; VES, intracellular vesicles. Most of the renal aquaporins have been cloned from several species (human, rat, and mouse).
which is abundant in kidney collecting duct principal cells (Fig. 2), and this water channel has been found to be the chief target by which vasopressin regulates kidney collecting duct water permeability and hence renal regulation of body water balance (121, 169, 171). Two additional aquaporins are expressed in the collecting duct principal cells, AQP3 and AQP4 (59, 60, 73, 99, 137, 223, 224, 261). Both of these channels are present in the basolateral plasma membranes and are likely to represent exit pathways for water entering the cells through AQP2 (Fig. 2). AQP5 is not present in the kidney as determined by survey screening methods (immunohistochemistry). AQP6 is present in collecting duct intercalated cells type A (264). At the subcellular level, AQP6 is unique compared with the early aquaporins (AQP1 to -5) in that it is exclusively present in an intracellular location with no expression in the plasma membrane. The physiological role of AQP6 is undefined. However, as discussed above, its location and anion permeability have suggested a role in the acidification of vesicles. Several further new aquaporins have recently been identified, presumably by homology cloning approaches or as a consequence of the human genome project. AQP7 appears to be expressed in the brush border of the proximal tubule, but little is known about its role in the kidney. AQP8 through AQP12 have also been identified (see sect. vE for references), and some of these homologs appear by Northern blot or RT-PCR survey to be expressed in kidney although at low abundance. Their functional role remains to be determined.

A. AQP1

The archetypal member of the aquaporin family, AQP1 (46, 191, 213), is highly abundant in the proximal tubule (Figs. 3 and 4) and descending thin limb (Figs. 3 and 4) (175, 176), and it constitutes almost 1% of total membrane protein in kidney cortex (175). AQP1 is absent in other nephron segments and absent from the collecting duct (175) and is thus exclusively expressed in segments of the kidney nephron that are constitutively, and highly, water permeable and is not involved in the vasopressin regulation of kidney water transport. Immuno-electron microscopic analysis has documented that AQP1 is abundant in both apical and basolateral plasma membranes in proximal tubules and descending thin limbs (Fig. 4), consistent with a role for AQP1 in the movement of water across both surfaces of the cells (175).

Immunocytochemistry (174, 175, 204) revealed that AQP1 immunolabeling is especially abundant in the seg-

FIG. 2. Diagrammatic representation of the localization of different aquaporins in the nephron and collecting duct system. AQP1 (blue) is present in the proximal tubule and descending thin limb. AQP2 (green) is abundant in the apical and subapical part of collecting duct principal cells, whereas AQP3 (red) and AQP4 (purple) are both present in the basolateral plasma membrane of collecting duct principal cells. AQP7 (orange) is confined to the apical brush border of straight proximal tubules. ADH, antidiuretic hormone.
ment 3 proximal tubule, although high levels are also present in segments 1 and 2. This is the case both in rat kidney (see above) and in human kidney (159). Importantly, there is an axial heterogeneity and segment-to-segment difference in the expression levels of AQP1 in the descending thin limb segments (174, 175). As described below, this heterogeneity parallels closely the water permeability characteristics in the different segments, as established using isolated perfused descending thin limbs (28, 29, 32). AQP1 is extraordinarily abundant in type II descending thin limb epithelium (174, 175), which is also known to display an extremely high osmotic water permeability (32). Type II descending thin limb epithelium (present in the inner stripe of the outer medulla from long looped nephrons) continues into type III descending thin limb epithelium, which has a much simpler ultrastructure and has a lower osmotic water permeability (albeit still very high in absolute terms) than the type II epithelium. The labeling density of AQP1 in this segment (DTL III) is lower than in type II, consistent with the lower water permeability. Finally, type I epithelium, found in descending thin limbs from short loops, has a very simplified epithelium and a much lower osmotic water permeability than type II and type III (28, 94). Consistent with this, the

**FIG. 3.** Immunoperoxidase labeling of AQP1 in thin cryosections from kidney cortex (A) and inner stripe of the outer medulla (B). A: extensive labeling is seen in both of the apical plasma membrane domains and in the basolateral plasma membrane domains (arrows) in proximal tubule cells. Arrows point to basolateral plasma membrane domains, and arrowheads point to apical plasma membranes. Inset: apical and basolateral AQP1 labeling of multiple cross-sectioned proximal tubules. B: in descending thin limbs, both apical and basolateral plasma membrane domains are labeled. Arrows point to basolateral plasma membrane domains, and arrowheads point to apical plasma membranes. Asterisks indicate vascular structures, and C indicates collecting duct. Magnification: ×1,000.
AQP1 labeling is less intense than in type II and type III. The short loop descending limb itself appears heterogeneous with greater labeling in the early part than in the late part (242). Thus the abundance of AQP1 parallels the differences in the osmotic water permeability, consistent with the hypothesis that AQP1 is providing the main route for water movement across the tubule wall.

The absolute abundance of AQP1 in individual microdissected renal tubule segments of rat has been measured with a fluorescence-based enzyme-linked immunosorbent assay (ELISA) standardized using AQP1 purified from red blood cells (144). In general, these measurements confirmed the very high abundance of AQP1 in proximal tubules and descending limbs previously inferred from immunocytochemical observations. In proximal tubule segments, AQP1 levels (in molecules/mm tubule length \( \times 10^9 \)) averaged the following: S-1, 6.5; S-2, 6.0; and S-3, 12.8. In descending limb segments, the values in the same units were as follows: type I, 7.8; type II, 52.1; and type III, 25.9.

The biophysical properties, the overall extraordinary abundance of AQP1 in the proximal tubule and descending thin limb, combined with its expression levels in different segments of the descending thin limb, strongly supported the view that AQP1 is essential for proximal nephron water handling and urinary concentration (119, FIG. 4. Immunoelectron microscopic localization of AQP1 in segment 3 proximal tubule cell (A) and descending thin limb cell (B) (cryosubstituted and low-temperature Lowicryl HM20 embedded tissue). A: AQP1 is extremely abundant in the apical plasma membrane of the brush border (BB). M, mitochondrion. B: in descending thin limb (DTL) cell, very strong labeling is seen in both the apical and basolateral plasma membrane (BM). L, lumen. Magnification: \( \times 54,000 \).
The critical role of AQP1 in urinary concentration was recently confirmed in humans lacking AQP1 and in transgenic knockout mice lacking AQP1. Humans have been identified who totally lack the AQP1 protein (195). The human AQP1 gene was localized to chromosome 7p14, and the Co blood group antigens were previously linked to 7p, suggesting a molecular relationship. It was established that the Co blood group antigen results from an Ala/Val polymorphism of AQP1 (214). Only six individuals have been shown to lack Co, and most of these Co null individuals are women who developed anti-Co during pregnancy. Three of these Co null individuals were found to have mutations in the AQP1 gene (195). Recent studies have indicated that these Co null individuals have a urinary concentrating defect in response to vasopressin or water deprivation (116a).

The AQP1-deficient mice were polyuric (140) and were unable to concentrate urine to more than ~700 mosmol/kgH2O even in response to water deprivation, during which they become rapidly dehydrated; plasma osmolalities increased dramatically up to 400–500 mosmol/kgH2O. Thus AQP1 is required for the formation of a concentrated urine. It is presumed that lack of AQP1 undermines the countercurrent multiplication process, which depends on the rapid equilibration of water across the descending thin limb of Henle’s loop. Subsequent studies have demonstrated that the osmotic water permeability of perfused proximal tubules isolated from AQP1 knockout mice were only one-fifth of the permeabilities in proximal tubules dissected from kidneys of normal mice (210). Recently, Chou et al. (30) also demonstrated that the osmotic water permeability of descending thin limb (dissected from kidneys of AQP1-deficient animals) is reduced by 90%. These studies in AQP1-deficient mice and the subsequent studies in AQP1-deficient humans 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. Additional support for a critical role of AQP1 for osmotic equilibration across the tubular epithelium came from free-flow micropuncture experiments in AQP1-deficient mice by Schnermann and colleagues (231). They determined that the osmolality difference between the plasma and the kidney proximal tubule luminal fluid is excessively increased in the AQP1-deficient mice compared with control mice, strongly supporting the view that AQP1 is important for efficient water transport across the tubular epithelium (231).

In addition to its presence in the proximal tubule and descending thin limb, AQP1 is also expressed in the descending vasa recta of rat kidney but not in the ascending vasa recta (174). Functional studies were performed using isolated perfused descending vasa recta briefly fixed with glutaraldehyde. This fixation was performed to eliminate the cellular toxicity of mercurials, and it has been shown in collecting duct (124), toad urinary bladders (241), and peritoneum (19) that fixation at least partially maintains the osmotic water permeability. The fixed descending vasa recta displayed a marked sensitivity for mercurials on the transendothelial water permeability, confirming that AQP1 at this site may play a significant role for water transport. This was later confirmed by Pallone et al. (180) using isolated perfused descending vasa recta from wild-type mice and from mice lacking AQP1. Water equilibration across the vasa recta may also play a critical role in avoiding the disruption of the osmotic gradient established by the countercurrent exchanger. It should also be mentioned that the demonstrated role of aquaporins, including AQP1 for peritoneal transport of water in experimental peritoneal dialysis (19), was also later confirmed in AQP1-deficient mice (259).

B. AQP2

AQP2 (82) is abundant in the apical plasma membrane and apical vesicles in the collecting duct principal cells (169) (Figs. 5 and 6) and at lower abundance in connecting tubules (117, 135). In addition to its presence in the apical plasma membrane and intracellular vesicles (Fig. 6), some AQP2 immunostaining has also been found to be associated with the basolateral plasma membrane, especially in the inner medullary collecting duct principal cell (151, 169) (Fig. 5C).

AQP2 is the primary target for vasopressin regulation of collecting duct water permeability. This conclusion was solidly established in studies showing 1) the cellular and subcellular distribution (82, 169), 2) a direct correlation between AQP2 expression and collecting duct water permeability in rats (50), 3) a direct correlation between the osmotic water permeability and AQP2 levels in the apical plasma membrane of collecting duct principal cells in isolated perfused collecting ducts (168) and in whole animal experiments (only of the onset phase, Refs. 151, 203, 256), and 4) in studies demonstrating that humans with mutations in the AQP2 gene (45) or rats with 95% reduction in AQP2 expression (149) have profound nephrogenic diabetes insipidus. AQP2 regulation is described in detail below.

C. AQP3 and AQP4

AQP3 and AQP4, which are expressed in cells in a wide range of organs (see, for example, Refs. 73, 74, 88, 158, 165, 170, 173, 228), are also present in the collecting duct principal cells (59, 60, 73, 99, 137, 223, 224, 261) where they are abundant in the basolateral plasma mem-
branes (Fig. 5, D and E) and represent potential exit pathways from the cell for water entering via AQP2. There is some heterogeneity in the segmental and subcellular localization of these two aquaporins in kidney collecting duct. AQP3 is very abundant in connecting tubule as well as cortical, outer medullary, and inner medullary collecting duct (59, 223). In contrast, AQP4 is mainly abundant in the inner medulla, although some expression is also noted in the more proximal segments (223). At the subcellular level, AQP3 is abundant in both the basal and in the lateral plasma membranes of collecting duct principal cells. In contrast, AQP4 is mainly present in the basal plasma membrane of collecting duct principal cells in rat. In mice, AQP4 was also present in the collecting duct principal cell, but interestingly also in basolateral membranes of proximal tubule S3 segments (223). This was corroborated by freeze-fracture electron microscopy, revealing orthogonal arrays of intramembrane particles (OAPs; known to represent AQP4) on the basolateral membranes of the S3 segment in normal mice but lack of AQP4
imunostaining and OAPs in collecting duct and proximal tubule in AQP4 knockout mice. Neither AQP3 nor AQP4 is found in significant amounts in cytoplasmic vesicles, and there is no evidence for a rapid increase in labeling of the plasma membrane in response to vasopressin.

A number of studies have also focused on the issue of whether the expression of AQP3 and AQP4 are regulated and whether this correlates with changes in renal water balance regulation. In brief, it has been demonstrated that the expression of AQP3 is regulated by changes in vasopressin levels (224). Brattleboro rats, which lack endogenous vasopressin, have lower expression levels of AQP3 than do Long Evans rats (the parent strain of the Brattleboros, which have normal vasopressin expression). Long-term vasopressin treatment of Brattleboro rats (using implantable osmotic minipumps to deliver vasopressin for 5 days) results in a significant increase in AQP3 expression, in parallel with an increase in AQP2 expression. Thirsting of normal rats is also associated with increased AQP3 expression (224). In contrast, AQP4 appears not to be regulated or at least not as markedly as AQP2 and AQP3. Long-term vasopressin infusion did not affect AQP4 expression.

To obtain further information about the physiological role of AQP3 and AQP4, gene knockout mice have been produced (31, 138, 139, 240). Although such studies are complicated by potential interfering compensatory mechanisms during fetal and postnatal development, the use of these gene knockout mice by a variety of research groups has been informative. Transgenic knockout mice lacking AQP4 showed a mild urinary concentrating defect (139), and studies using isolated perfused collecting ducts from inner medulla (IMCDs) from AQP4-lacking mice revealed a fourfold reduction in the vasopressin-stimulated osmotic water permeability (31). This indicates that AQP4 is responsible for a substantial majority of the basolateral membrane water movement in IMCDs under maximal vasopressin stimulation. The lower abundance of AQP4, together with higher abundance of AQP3 in cortical and outer medullary collecting duct, raises the possibility that AQP3 may play a more significant role at these more proximal segments of the collecting duct. Recently, AQP3 knockout mice were produced, and they revealed a marked urinary concentrating defect with very severe polyuria (138). After deamino-8-D-arginine vasopressin (dDAVP) administration or water deprivation, the AQP3-deficient mice were able to concentrate their urine partially to ~30% of that in wild-type mice. Osmotic water permeability of dissected, nonperfused cortical collecting duct measured by an optical method was reduced in IMCDs from AQP3 knockout mice. One complicating factor is that in the AQP3 knockout mice AQP2 expression in the cortical collecting duct is also reduced extensively, raising the possibility that part of the polyuria in these AQP3 knockout mice may be caused by the reduced expression of AQP2.

D. AQP6

Three further aquaporin cDNAs have been identified in kidney. One of these is AQP6. Initially a cDNA from rat kidney (WCH3) and the human homolog (hKID) was identified, and when expressing these in X. laevis oocytes they increased the osmotic water permeability slightly, but unsuccessful attempts to raise antibodies led the authors to conclude that the protein is not immunogenic (136, 141). The International Human Genome Nomenclature Committee adopted the name aquaporin (symbol AQP) as the referencing system (8). AQP6 was designated for rat WCH3 and human hKID (3), although the cellular locations were not established at the time. While searching for new aquaporins in kidney by PCR, a rat cDNA clone was isolated encoding a water channel protein (AQP6), which is closely related to rat WCH3 and human hKID. Production of antibodies against AQP6 allowed the definition of the cellular and subcellular localization of AQP6 in rat kidney. AQP6 was expressed in collecting duct intercalated cells in cortical, outer medullary, and inner medullary collecting duct (264). The presence in collecting duct intercalated cells in the outer medulla and inner medulla allowed the conclusion that AQP6 was present in type A intercalated cells and in the type A-like intercalated cell found in the inner medullary collecting duct. A major surprise was that AQP6 was almost exclusively found associated with intracellular vesicles in the cells described above, with an almost complete absence of AQP6 immunogold labeling in the plasma membranes. Thus AQP6 appears, at least in rat kidney, to be an exclusively intracellular water (and ion) channel.

E. AQP7 to AQP9

AQP7 is highly abundant in spermatocytes (96, 100, 218), but it may also be present in other tissues. Preliminary studies using antibodies to rat or mouse AQ7 have indicated that AQP7 is expressed in the proximal tubule brush border especially in the segment 3 proximal tubule (Table 1). The cellular and subcellular localization of AQP8 (98, 142) has recently been established and AQP8 is present mainly in intracellular domains of proximal tubule and collecting duct cells (63a) in addition to being present in many other organs (63a, 83a). AQP9 (97, 122, 123, 225, 226) is not expressed in kidney but is abundant in many other tissues. Some of them are expected to be present in the kidney based on RT-PCR analysis, but the exact presence and abundance needs to be clarified using immunolabeling methods.
A. Acute Regulation of Collecting Duct Water Permeability: Role of AQP2 Trafficking

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 evidence that the vasopressin-induced increase in permeability depended on the appearance of specific water channels in the apical plasma membrane of the ADH-responsive cells.

Much of the early work on vasopressin action was done in amphibian skin or bladder, which are functional analogs of the kidney collecting duct. Using freeze-fracture electron microscopy, Chevalier et al. (22) identified large clusters of particles, arranged in characteristic arrays (linear parallel grooves), which appeared in the apical plasma membrane of ADH-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 (93, 109). In summary, these and subsequent studies in amphibian tissues revealed 1) that 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 Wade et al. (244) to propose the “membrane shuttle hypothesis,” 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 and subsequently AQP2, later shown to be the predominant vasopressin-regulated water channel (for recent review, see Ref. 171), made it possible to prepare antibodies and investigate the effects of vasopressin in mammalian collecting ducts directly. As shown in Figure 5, AQP2 is present in the apical and subapical parts of collecting duct principal cells, and immunoelectron microscopy (Fig. 6) showed that AQP2 is very abundant both in the apical plasma membrane and in small subapical vesicles (169).

Water flow out of the collecting duct is determined by the apical plasma membrane of the collecting duct cells (Fig. 7), which provides the rate-limiting barrier to water movement (71). Regulation of the osmotic water permeability of this membrane could, in principle, occur via one or both of two distinct mechanisms: either 1) the permeability of existing channels could be altered, probably by chemical modification (e.g., phosphorylation of the chan-
The number of water channels present in the membrane could be altered by vasopressin or by insertion or removal of channels from an intracellular store, as proposed by the membrane shuttle hypothesis. The presence of AQP2 in small vesicles favored the latter hypothesis, and several in vitro and in vivo studies have now underscored 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, while there were reciprocal changes in the intracellular labeling for AQP2 (Fig. 8) (168). 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 dDAVP treatment (151, 203, 256).
off-set response has been examined in vivo using acute treatment of rats with vasopressin V₂ receptor antagonist (35, 91) or acute water loading (to reduce endogenous vasopressin levels, Ref. 206). These treatments (both reducing vasopressin action) resulted in a prominent internalization of AQP2 from the apical plasma membrane to small intracellular vesicles further underscoring 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 trafficking, two studies have attempted to address this issue. Kuwahara et al. (126) demonstrated that protein kinase A (PKA)-induced phosphorylation of AQP2 in Xenopus oocytes was associated with only a very small (∼30%) increase in water permeability (126). Because vasopressin causes much greater changes in collecting duct water permeability (3- to 10-fold increase), this demonstrated that changes in water conductance of AQP2 by PKA-mediated phosphorylation could not explain more than a small fraction of the hormonal effect on the collecting duct. Consistent with this, Lande et al. (130) found that treatment with PKA caused no change in the water permeability of AQP2-bearing vesicles harvested from kidney inner medulla (130). 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 body water balance, is acutely regulated primarily by vasopressin-regulated trafficking of AQP2.

Several groups have now successfully reconstituted the AQP2 delivery system using cultured cells transfected with either wild-type AQP2 or AQP2 tagged with a marker protein or a fluorescent protein (43, 114–116, 229). 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.

1. Signal transduction pathways involved in vasopressin regulation of AQP2 trafficking

The signal transduction pathways have been described thoroughly in previous reviews (e.g., Ref. 121). cAMP levels in collecting duct principal cells are increased by binding of vasopressin to V₂ receptors (62, 125). The synthesis of cAMP by adenylate cyclase is stimulated by a V₂ receptor-coupled heterotrimeric GTP-binding protein, Gₛα, Gₛ interconverts between an inactive GDP-bound form and an active GTP-bound form, and the vasopressin-V₂ receptor complex catalyzes the exchange of GTP for bound GDP on the α-subunit of Gₛ. This causes release of the α-subunit, Gₛα-GTP, which subsequently binds to adenylate cyclase, thereby increasing cAMP production (Fig. 9A). 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 subunits.

Early studies demonstrated that PKA induces phosphorylation of various membrane proteins in bovine kidney (52) and that vasopressin treatment of saponin-permeabilized outer medullary collecting duct segments induced phosphorylation of at least two 45- and 66-kDa proteins (83). It has also been shown that inhibition of PKA activity with H-89 inhibits the vasopressin-induced increase in water permeability in isolated perfused rabbit collecting ducts (215). AQP2 contains a consensus site for PKA phosphorylation (RRQS) in the cytoplasmic COOH terminus at serine-256 (82). Recent studies using 32P 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 (178). This agrees well with the time course of vasopressin-stimulated water permeability of kidney collecting ducts (245). As described above, PKA-induced phosphorylation of AQP2 apparently does not change the water conductance of AQP2 significantly. Importantly, it was recently demonstrated that vasopressin or forskolin treatment failed to induce translocation of AQP2 when serine-256 was substituted by alanine (S256A) in contrast to a significant regulated trafficking of wild-type AQP2 in LLC-PK₁ cells (115). A parallel study by Sasaki and colleagues (81) also demonstrated the lack of cAMP-mediated exocytosis of mutated (S256A) AQP2 transfected into LLC-PK₁ cells. Thus these studies indicate a specific role of PKA-induced phosphorylation of AQP2 for regulated trafficking. To explore this further, an antibody was designed that exclusively recognizes AQP2, which is phosphorylated in 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 mainly located in intracellular vesicles as shown by immunocytochemistry and immunoblotting using membrane fractionation (37). Surprisingly, there was no significant increase in the total amount of phosphorylated AQP2 after vasopressin treatment in normal rats (37). However, recent evidence that at least three of the subunits in each tetramer need to be phosphorylated to be transported to the plasma membrane (Fig. 9B) (111) may explain this; if there is a low level of phosphorylation and dephosphorylation occurring continuously,
such that many tetramers are close to the threshold for insertion, only a very modest increase in phosphorylation may be needed to induce substantial transfer to the plasma membrane. 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 (37). Conversely, treatment with V2-receptor antagonist induced a marked decrease in expression of phosphorylated AQP2 (36) likely to be due to

**FIG. 9.** Signaling cascades and molecular apparatus involved in vasopressin regulation of AQP2 trafficking. See text for details. A: signaling cascades involving vasopressin V2 receptors (V2R), adenylyl cyclase (AC), cAMP, and protein kinase A (PKA). B: protein kinase A phosphorylates AQP2 monomers in Ser-256 of AQP2. Phosphorylation of 3 or 4 monomers of the homotetramers is necessary for trafficking to the apical plasma membrane. C: other kinases are likely also to play a role in regulation of AQP2 trafficking. D: cytoskeletal elements are involved in AQP2 trafficking and include microtubule-based motor proteins (dynein and dynactin) as well as actin filament binding proteins such as myosin-1. E: the docking mechanism may involve vesicle targeting receptors that have been found in the collecting duct cells associated with membrane domains housing AQP2. This includes VAMP2 and syntaxin-4. F: regulation of intracellular calcium is involved in AQP2 trafficking and appears to involve a calcium sensing receptor.
reduced PKA activity and/or increased dephosphorylation of AQP2, e.g., by increased phosphatase activity.

PGE$_2$ inhibits vasopressin-induced water permeability by reducing cAMP levels (for detailed review, please see Ref. 121). In preliminary studies, Zelenina et al. (269) investigated the effect of PGE$_2$ on PKA phosphorylation of AQP2 in kidney papilla, and the results suggest that the action of prostaglandins is associated with retrieval of AQP2 from the plasma membrane, but that this appears to be independent of AQP2 phosphorylation by PKA.

Phosphorylation of AQP2 by other kinases, e.g., protein kinase C or casein kinase II, may potentially participate in regulation of AQP2 trafficking (Fig. 9C). Phosphorylation of other cytoplasmic or vesicular regulatory proteins may also be involved. These issues remain to be investigated directly.

2. 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 fashion, 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 where $<5\%$ of total AQP2 is present in the apical plasma membrane; Refs. 87, 203, 256). Thus there must be some kind of a “clamp” preventing fusion in the unstimulated state and/or a “trigger” when activation occurs.

3. 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 and in the mammalian collecting duct (189, 190). Because microtubule-disruptive agents inhibit the development of the hydromosotic response to 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 (230), 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 fibroblastic 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 (2). If this is the case in collecting duct cells, and there is some evidence that it is (153), 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 Ref. 152) and that both dynein and dynactin, a protein complex believed to mediate the interaction of dynein with vesicles, associate with AQP2-bearing vesicles (152). Furthermore, both vanadate, a rather nonspecific inhibitor of ATPases, and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), a relatively specific inhibitor of dynein, inhibit the antidiuretic response in toad bladder (47, 147). Thus it seems likely that dynein may drive the microtubule-dependent delivery of AQP2-bearing vesicles toward the apical plasma membrane (Fig. 9D).

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 hydromosotic response, since disruption of microfilaments with cytochalasins inhibits the response in the toad bladder (108, 186, 241). Cytochalasins can also inhibit an established response, and even the offset of the response (162). 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 the subsequent endocytic retrieval of the water channels (187). Interestingly, vasopressin itself causes actin depolymerization (51), suggesting that reorganization of the terminal web is an important part of the cellular response to vasopressin, a conclusion reached on morphological grounds by DiBona (49).

4. 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 SNAP/SNARE system described there (201) 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 (72, 101, 172). tSNAREs are also present. Syntaxin 4, but not syntaxins 2 or 3, is present in the apical plasma membrane of collecting duct principal cells (145, 146). Another putative tSNARE, SNAP23, has also been found in collecting duct principal cells both in the apical plasma membrane and in AQP2-bearing vesicles (95). Some soluble components of the fusion complex, including NEM-sensitive factor (NSF) and α-soluble NSF-associated protein (SNAP), 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 (Fig. 9E), although considerable work remains to be done in isolating and characterizing the components, their regulation, and prime physiological function.

In addition to increasing cAMP levels in collecting duct principal cells, vasopressin acting through the V2 receptor has also been demonstrated to transiently increase intracellular Ca^{2+} (20, 56, 143, 216). The increase occurs in the absence of activation of the phosphoinositide signaling pathway (33) and has recently been demonstrated to be due to activation of ryanodine-sensitive calcium release channels in the collecting duct cells (27). Buffering intracellular calcium with BAPTA or inhibition of calmodulin completely blocked the water permeability response to vasopressin in isolated perfused inner medullary collecting ducts, suggesting a critical role for calcium at some step in the process of AQP2 vesicle trafficking (34).

B. Long-Term Regulation of Urinary Concentration: Role of AQP2

In addition to the acute regulation of collecting duct water permeability brought about by the trafficking of AQP2 described above, it is now clear that there are longer term adaptational changes that modulate this acute response. These occur during prolonged changes in body hydration status and form an appropriate physiological response to such challenges. However, similar long-term changes also appear to be important in a wide variety of pathological conditions, discussed below, and an understanding of the mechanisms involved in these adaptational responses may provide the basis both for a better understanding of, and for potential therapeutic approaches to, pathological disorders of water balance.

It has been known for many years that chronically water-loaded patients, such as those with compulsive polydipsia, have a markedly impaired maximal urinary concentrating capacity (48). Conversely, prolonged dehydration or fluid restriction resulted in an increased maximal concentrating capacity (64, 106). These results have been confirmed more recently in rat models (50, 120, 131, 151, 169, 243). Such studies have demonstrated that the total amount of AQP2 present in the kidney also decreases during overhydration and increases after dehydration. There is a rapid change in AQP2 mRNA expression, followed more slowly by protein levels (58, 79, 137, 257), suggesting that the long-term alterations in concentrating capacity are due to changes in total AQP2 protein levels, which are caused in turn by changes in gene transcription.

Nephrogenic diabetes insipidus (NDI) is the term given to conditions where the kidney is unable to respond to vasopressin by producing a concentrated urine. In addition to the hereditary forms of the disease discussed below, there are a wide variety of acquired forms of NDI. In animal models of several of these, it has been demonstrated that AQP2 levels are decreased, in some cases dramatically so (54, 75, 77, 149, 150). There are also some models in which water retention is associated with increased AQP2 expression (12, 177, 179, 255). Such changes in AQP2 may be of etiological importance in the disease, but such experiments can also give us important information about the mechanisms governing AQP2 expression.

C. Regulation of AQP2 and AQP3 Expression

Although the evidence for long-term regulation of AQP2 expression is now compelling, evidence for regulation of the other renal aquaporins is more patchy. There is good evidence that expression of AQP3, the major basolateral water channel in the cortical and outer medullary collecting duct, is regulated by vasopressin (59). Its level changes in parallel with that of AQP2 in some, but not all, of the pathological models discussed below, providing further evidence that vasopressin is only one of the signals regulating AQP2 expression. In contrast, the expression of AQP4, the major basolateral water channel in the inner medullary collecting duct, does not appear to be regulated (223, 224), although one study found parallel changes in AQP2, -3, and -4 mRNA with changes in hydration (163). The expression of the major water channel found in the proximal tubule, AQP1, also appears to be unaffected by changes in hydration status (223), although levels of AQP1 are markedly reduced in some pathological conditions (68, 76, 127, 128), as discussed below.
D. Signaling Pathways Involved in Regulation of AQP2 Expression

1. Regulation of AQP2 expression via vasopressin-dependent signaling pathways

The most obvious signal for the long-term regulation of AQP2 expression would be vasopressin itself. During chronic dehydration, vasopressin levels will be high, while vasopressin release will be suppressed by water loading, providing a mechanism by which changes in hydration state could modulate AQP2 levels. There is clear evidence that vasopressin does indeed increase AQP2 expression. Brattleboro rats have a defect in the neurophysin gene from whose gene product vasopressin is cleaved (84). Thus these animals lack endogenous vasopressin and are profoundly polyuric. Their AQP2 expression is about one-half to one-third of that found in their parent strain (Long Evans) (50, 118, 196). Chronic infusion of vasopressin via osmotic minipumps for 5 days both reversed their polyuria and increased their AQP2 expression to that of the normal animals (Fig. 10) (50). Chronic vasopressin infusion also increases AQP2 expression in normal rats (58), whereas administration of V2-receptor antagonists decreases it (91, 148), albeit only by ~50%.

The effect of vasopressin is probably mediated by the same V2 receptors that mediate the acute shuttling response to vasopressin (Figs. 9 and 11). Evidence for this comes from experiments with DI +/+ severe mice, a strain with an activating mutation of cAMP phosphodiesterase. This means that there is no rise in cytosolic cAMP and they are profoundly polyuric. Their AQP2 expression is about one-half to one-third of that found in their parent strain (Long Evans) (50, 118, 196). Chronic infusion of vasopressin via osmotic minipumps for 5 days both reversed their polyuria and increased their AQP2 expression to that of the normal animals (Fig. 10) (50). Chronic vasopressin infusion also increases AQP2 expression in normal rats (58), whereas administration of V2-receptor antagonists decreases it (91, 148), albeit only by ~50%.

Although Brattleboro rats have a lower level of AQP2 than rats with normal levels of vasopressin, at 50% it is still surprisingly high if vasopressin itself is the main or only stimulus for AQP2 expression. Since the DI +/+ severe mice showed a greater downregulation of AQP2 (up to 85% in the male mice), it seemed likely that some vasopressin-independent effect might also be acting via the actions of cAMP production, and thus be being blocked in the DI +/+ severe mice. Such an effect might either be due to other ligands binding at the V2 receptor, or due to the action of other pathways also mediated by cAMP. To investigate this, the effect of V2-receptor antagonists was investigated in Brattleboro rats (196). The antagonist SR121463A caused both an increase in polyuria and a fall of ~50% in AQP2 expression, as indeed had the slightly less potent antagonist OPC31260 in an earlier study (148, 196). This clearly suggests that even in Brattleboro rats some other ligand [perhaps oxytocin, as suggested by Chou and co-workers (25, 26), Valtin and Edwards (232), and Lencer et al. (132)] has a significant effect at V2 receptors. Interestingly, both receptor antagonists almost completely eliminated the presence of phosphorylated AQP2, which remains at moderately high levels in Brattleboro rats, suggesting that under normal circumstances there is some ongoing activation of PKA as a result of ligand binding to V2 receptors (37, 196). It is
perhaps surprising that Brattleboro rats show virtually no trafficking of AQP2 to the apical plasma membrane despite the activity of PKA, but it has been calculated that three of the four components of a tetramer need to be phosphorylated to be transported to the plasma membrane (111). Presumably in Brattleboro rats this threshold is rarely reached, but PKA activity remains high enough to provide a significant stimulus for AQP2 gene transcription.

3. Regulation of AQP2 expression via vasopressin-independent signaling pathways

Although it is clear that vasopressin, or other ligands acting at V2 receptors, provide one stimulus for AQP2 expression, it is not the only one. Evidence for this comes from a variety of experiments which show that AQP2 expression can be altered independent of the activity of vasopressin. One of the most clear-cut involved the so-

![Graphs and images showing regulation of AQP2 expression](image)

**FIG. 10.** Vasopressin regulation of AQP2 expression in Brattleboro rats treated with exogenous vasopressin in osmotic minipumps for 5 days. This induces a marked increase in AQP2 expression levels corresponding to a 3-fold increase (A). In parallel, there was a 3- to 4-fold increase in the maximal vasopressin-stimulated osmotic water permeability in isolated perfused inner medullary collecting ducts dissected from Brattleboro rats receiving long-term vasopressin treatment (B, AVP) or vehicle (B, VEH). Immunocytochemistry also demonstrates a marked increase in AQP2 labeling including apical plasma membrane domain labeling after vasopressin stimulation (D and E, AVP) compared with vehicle-treated controls (C, VEH). *P < 0.05.
called vasopressin-escape phenomenon (55, 58, 238). In these experiments, rats were given a chronic vasopressin infusion, but then given a water load mixed with their diet. These animals initially retain water and become hyponatremic, but after a few days they start to excrete water. This is accompanied by a striking fall in renal AQP2 levels, despite the continued high level of circulating vasopressin. The trafficking response is still intact, showing that the vasopressin continues to be effective, but the cells are able to reduce their AQP2 expression despite this. Furthermore, the levels of AQP3 do not fall in parallel with the decrease in AQP2, providing further evidence that the cells remain responsive to vasopressin.

Earlier evidence for a non-vasopressin-mediated effect on AQP2 levels had come from experiments with a lithium-induced NDI model (129, 149). In these experiments, lithium produced a profound (>90%) downregulation of AQP2, in parallel with a massive polyuria. In addition to the decrease in expression, lithium treatment also impairs the trafficking response, and it is the combination of these two effects that causes the severity of the polyuria. It has been suggested that the effects of lithium on the kidney are a consequence of inhibition of adenylate cyclase (16, 39). Thus, in principal, both the impairment of trafficking and the reduction in AQP2 expression could be mediated by decreased cAMP production. Treatment with high doses of dDAVP, a V₂-specific vasopressin analog which can be given in high doses because of its lack of pressor action, could cause efficient trafficking of AQP2 and allow reasonable urinary concentration. There was also some (~2-fold) increase in AQP2 expression. However, dehydration for 2 days was able to cause a fivefold increase in AQP2 expression, despite proving unable to increase the efficiency of delivery of the AQP2 to the plasma membrane. Because dehydration induced a greater increase in AQP2 expression without inducing trafficking, it is clear that dehydration can induce AQP2 expression via a mechanism independent of the cAMP-

![Diagram](http://physrev.physiology.org.org/)

**FIG. 11.** Regulation of AQP2 trafficking and expression in collecting duct principal cells. AVP acts on V₂ receptors (V₂R) in the basolateral plasma membrane (A and B). A: through the GTP-binding protein Gₛ adenyl cyclase (AC) is activated, which accelerates the production of cAMP from ATP. Then cAMP binds to the regulatory subunit of protein kinase A (PKA), which activates catalytic subunit of PKA. PKA phosphorylates AQP2 in intracellular vesicles and possible other cytosolic or membrane proteins. Specifically, cAMP participates in the 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 factor is thought to increase gene transcription of AQP2 resulting in synthesis of AQP2 protein which in turn enters the regulated trafficking system. In parallel, AQP3 synthesis and trafficking to the basolateral plasma membrane takes place. PDE, phosphodiesterase. B: AQP2 is excreted into urine or recycled from the apical plasma membrane.
mediated pathway that drives the shuttling process. Other NDI models (discussed below) have also demonstrated that changes in AQ2P expression are not always correlated with impaired shuttling, as would be expected if both were being driven by vasopressin, acting via cAMP. In particular, hypokalemia causes about a 75% decrease in AQ2P expression while apparently leaving AQ2P shutting intact (150), whereas hypercalcemia inhibits shuttling, but produces only about a 50% decrease in AQ2P (54). Both produce a similar degree of polyuria, emphasizing that the interaction between long- and short-term regulation of AQ2P is critical in the maintenance of body water balance.

Because the reduction of AQ2P expression caused by lithium treatment is much greater than can be obtained by the use of V2-receptor blockers, it seems likely that it is affecting expression via some other mechanism. Although it is possible that this also depends on the inhibition of cAMP production, this seems unlikely in the light of the effects of dehydration and vasopressin infusion described above. Thus the downregulation of AQ2P expression during lithium treatment also appears to be partly via a vasopressin-independent pathway, which may or may not be the same as that responsible for the non-vasopressin-mediated effects of body hydration status. Further evidence for this comes from the observation that lithium treatment also causes a profound downregulation of AQ2P in Brattleboro rats (196). In the light of the evidence described above that Brattleboro rats still have some stimulation via V2 receptors, this result must be treated with caution, but again the effect of lithium on AQ2P levels was much greater than the effect of receptor blockade.

A range of other factors might provide stimuli for increased or decreased AQ2P expression, but these remain to be determined. One possible signal would be tubular flow itself, either by some mechanical process or by altering the concentration of solute delivered to the tubule. However, the osmotic diuresis (glycosuria) seen with streptozotocin-induced diabetes mellitus is associated with a modest increase in AQ2P rather than a decrease, as seen in NDI models (167). In this case, urine osmolality remains high (~1,000 mosmol/kgH2O), because of the glucose, but tubular flow is markedly increased. Since water is lost because of the osmotic diuresis, the animals presumably become slightly dehydrated and have increased vasopressin levels. This probably explains the increase in AQ2P expression observed. Consistent with this, there was also an increase in AQ3 expression. Furthermore, furosemide, a loop diuretic, had no significant effect on AQ2P expression, despite causing a profound polyuria (148, 150). This latter treatment will also result in washout of the medullary osmotic gradient, since loop diuretics inhibit salt uptake in the thick ascending limb of Henle’s loop. Thus the absence of any alteration in AQ2P levels implies that interstitial tonicity is not a major determinant of AQ2P expression in the inner medulla. In the cortex, changes in interstitial tonicity will be very modest, yet cortical collecting ducts also undergo a profound downregulation of AQ2P expression during lithium treatment or hypokalemia (150), further indicating that the signal driving at least this downregulation is not dependent on changes in interstitial tonicity. Although a hypertonicity responsive element has been described in the 5’-flanking region of the AQ2P gene (157), these results indicate that it is not functionally important under most circumstances.

Results from models of unilateral and bilateral ureteric obstruction provide valuable insights into the mechanisms of AQ2P regulation. These experiments are discussed in more detail below, but the results indicate that some (but not all) of the factors involved in the downregulation are probably local to the kidney, at least in this condition. This might suggest that local metabolites or ischemia are important signals. One candidate had been suggested by earlier work in NDI models: a number of these models are associated with increased renal production of prostaglandins, so it seemed possible that prostaglandins were responsible for decreased AQ2P expression. This would also be consistent with the known ability of prostaglandins to inhibit the shuttling response via G-protein-coupled receptors (155, 207). However, experiments with nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandin synthesis, caused a decrease in AQ2P (D. Marples and C. A. Ecelbarger, unpublished data). This would imply that prostaglandins actually provide a stimulus for AQ2P expression. Consistent with this, NSAIDs prevent the dehydration-induced increase in AQ2P expression, suggesting that prostaglandins may play an important role in non-vasopressin-mediated, dehydration-induced AQ2P expression. Further work is in progress to characterize this further.

An interesting recent observation came from studies on mice in which the AQ2P gene has been knocked out (138). These animals have a striking polyuria (10-fold greater than controls) but were able to concentrate their urine reasonably well (although still only to one-third of the level of controls) in response to dDAVP or dehydration. The water permeability of the basolateral membrane was reduced to about one-third of that in the controls. In these animals, AQ2P expression is reduced to about one-fourth of control levels in the cortex, but not in the inner medulla (where basolateral water permeability is provided predominantly by AQ2P). This might suggest that swelling of the cells and/or cytosolic dilution, caused by influx of water through the apical plasma membrane without parallel efflux through basolateral membrane, causes a decrease in AQ2P expression, although the signal for this remains to be determined. One possibility would be a dilution of cytosolic cAMP, a mechanism suggested for...
“flux inhibition,” the reduction in the acute increase in permeability of the toad bladder in response to ADH when there is an osmotic gradient (and therefore transcellular water flow) relative to the permeability increase in the absence of a gradient (63). It is also possible that physical distortion of the cells leads to signaling from adhesion molecules or cytoskeletal components.

The results to date leave open the question of what causes the non-vasopressin-mediated changes in AQP2 expression seen in a number of pathophysiological states. The observation that the DI +/- severe mice, which have constitutively low levels of cAMP, have lower AQP2 levels than seen in rats treated with high doses of V2-receptor antagonists suggests that other signals may also act via the cAMP cascade, which may provide a final common pathway. It is also possible that changes in the rate of AQP2 degradation play an important part in the regulation of AQP2 levels in the cells. Some support for this comes from the observation that in most of the models described above the decrease in mRNA levels is greater than the decrease in protein, whereas in lithium-treated Brattleboro rats, the decrease in mRNA is rather smaller than the decrease in protein, consistent with part of the effect of lithium being due to increased degradation.

VII. PATHOPHYSIOLOGY OF RENAL AQUAPORINS

There are a variety of disorders in which water handling is abnormal (Table 2). Some of these are primary renal disorders, whereas others reflect changes in other organs or systems. The renal handling of water may be considered abnormal in all of them, since there is a breakdown of the body’s normal homeostatic mechanisms. The role of changes in the expression and/or function of aquaporins has been investigated in a range of these conditions, including genetic defects, acquired defects of renal responsiveness (acquired NDI), and conditions in which there is an inappropriate retention of water.

A. Inherited NDI and Central Diabetes Insipidus

There are two significant inherited forms of diabetes insipidus (DI): central and nephrogenic. In central (or neurogenic) DI, there is a defect of vasopressin production or release. Central DI is rarely hereditary in humans, usually occurring as a consequence of head trauma or disease in the hypothalamus or pituitary. However, the Brattleboro rat provides an excellent model of this condition.

<table>
<thead>
<tr>
<th>TABLE 2. Physiological or pathophysiological conditions associated with altered abundance and/or targeting of AQP2</th>
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<td>Hepatic cirrhosis (CBDL, compensated)</td>
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<td>Ischemia-induced acute renal failure (oliguric phase in rat model)</td>
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AQP2, aquaporin-2; CBDL, common bile duct ligation; CCL4, carbon tetrachloride; DI, diabetes insipidus; DM, diabetes mellitus; NDI, nephrogenic diabetes insipidus; PAN, puromycin aminonucleoside; SIADH, syndrome of inappropriate secretion of antidiuretic hormone. * Reduced V2-receptor density has profound effect on the AQP2 targeting and expression. † Mild increase in urine production rates.
dition. These animals have a total or near-total lack of vasopressin production. As discussed above, experiments by DiGiovanni et al. (50) showed that AQP2 levels in Brattleboro rats were substantially lower than those in the parent strain (Long Evans) and that this deficit was reversed by chronic vasopressin infusion, suggesting that patients lacking vasopressin are likely to have decreased AQP2 expression. The subsequent work showing that expression of AQP3 is also regulated by vasopressin implies that the level of these channels will also be decreased in patients with CDI. However, the most important denominator is the absence of AQP2 trafficking to the apical membrane. These deficits are likely to be the main causes of the polyuria from which these patients suffer, but they will also be reversed by the treatment given for their condition (i.e., desmopressin).

The second form of DI is called NDI and is caused by the inability of the kidney to respond to vasopressin stimulation. The most common hereditary cause of this is a mutation of the V2 receptor, making the collecting duct cells insensitive to the hormone. This gene is found on the X chromosome in humans and shows the classical pattern expected, with males affected much more often, while females are usually asymptomatic carriers, because they have inherited a normal gene from their father. Although there is no direct evidence, it is likely that this form of NDI will be associated with decreased expression of AQP2, since the cells are unable to respond to circulating vasopressin. This will compound the lack of trafficking of the AQP2. Consistent with this, urinary AQP2 levels are very low in patients with X-linked NDI (44, 113). However, because the amount of AQP2 in the urine appears to be determined largely by the response of the collecting duct cells to vasopressin (250), rather than their content of AQP2, this data must be interpreted with caution with respect to predicting AQP2 expression levels. Rather, this reflects reduced trafficking of AQP2 and hence less excretion into urine. It may be possible to increase AQP2 expression by stimulating non-vasopressin-mediated pathways, but the delivery of AQP2 is likely to remain low, so this may be of limited value. Furthermore, such pathways may well be maximally activated in these patients anyway, since they will tend to be rather dehydrated much of the time.

Less commonly (~10% of hereditary NDI), there is a genetic defect in the AQP2 gene. In most cases, this results in an autosomal recessive condition (45, 236), although a few cases have been reported that show an autosomal dominant inheritance (161). It is thought that the recessive forms on NDI are due to mutations in which either the mutant protein is unable to form tetramers with the normal form, or in which the normal protein functions normally. In contrast, in the dominant cases, it has been shown that heterotetramers between the mutant and normal forms can be created but that they are unable to travel to the plasma membrane (112). Thus the mutant protein can prevent the function of the normal form.

Of the 19 mutations so far reported, about one-half are found in the B or E loops of the protein, where it is likely that they will destroy the pore function of the molecule. The remainder are scattered throughout the molecule, although three occur together on the C loop. This is consistent with a previous report suggesting that this region of the molecule is closely associated with the channel (13).

Most mutations in AQP2 result in impaired trafficking to the plasma membrane, even though some of the mutants will act as functional water channels (42). This has led to the idea that it may be possible to treat patients with such mutations by using chemical chaperones to increase the number of channels which the cell can package successfully, and transport to the surface. Such methods have been used successfully in cultured cells transfected with mutant aquaporins (219), but much work remains to be done before such methods are available clinically.

An interesting form of NDI with no known human parallel occurs in the DI +/+ severe mouse line. As discussed above, these animals have an activating mutation of cAMP phosphodiesterase, which breaks down cAMP in the collecting duct principal cells. As a consequence, these animals are unable to increase the intracellular cAMP levels in response to vasopressin, and hence are unable to mount an antidiuretic response. They have greatly reduced levels of AQP2 protein and mRNA, as well as AQP3 (78), consistent with the hypothesis that the regulation of the expression of these proteins is at least partly driven by vasopressin acting via the production of cAMP. It is interesting that the downregulation in these animals is greater than can be induced by V2-receptor antagonist treatment in rats, suggesting that adenylyl cyclase may act as a final common pathway for a number of stimuli of AQP2 and -3 expression.

A range of other genetic defects results in urinary concentrating defects that are secondary to the failure of another transport process. For example, Bartter’s and Gitelman’s syndromes are both caused by defective NaCl uptake in the thick ascending limb and distal convoluted tubules, respectively. Both also cause hypokalemia, which is known to cause NDI, in association with decreased AQP2 expression, as discussed below. Impaired salt uptake in the loop of Henle will also cause a concentrating defect because of a reduced medullary osmotic gradient. Although humans lacking functional AQP1 do not have any gross functional disturbance (195), the results from AQP1 knockout mice suggest that during dehydration a concentrating defect would be revealed, due to impaired function of the loop of Henle caused by reduced water permeability in the descending limb (140). Water handling by the vasa recta is also impaired (61,
AQP3 levels were progressively reduced to concentrating defect, i.e., NDI. We examined the effect of oral
39) primarily due to a vasopressin-resistant urinary-con-
these develop serious side effects including polyuria (16,
20 –30% of
of manic-depressive disease. It is estimated that 1 in 1,000
lev-
el in the various models. In addition to DI, a few other
different mechanisms are responsible for AQP2 dysregu-
lation as well as the inhibition of targeting to the plasma mem-
brane in response to lithium treatment. This is consistent
with the presence of a cAMP-responsive element in the
15% of control

B. Acquired NDI

Acquired NDI is the consequence of several condi-
tions (Table 2) that are characterized by an increased
water output and reduced urine osmolality, despite elevated levels of AVP. In many of these conditions, the
kidney is unable to handle water due to an impaired
responsiveness to vasopressin. As discussed below, a
number of rat models with NDI have been evaluated, and
common for all is a reduced expression of AQP2 in the
principal cells of the collecting ducts. However, as is
discussed, the degree of AQP2 downregulation as well as
the intracellular localization of the protein differs signifi-
cantly among the various conditions, suggesting that dif-
ferent mechanisms are responsible for AQP2 dysregu-
lation in the various models. In addition to DI, a few other
serious conditions are associated with reduced AQP2 lev-
els and urinary concentrating defects (see Table 2).

1. Lithium-induced NDI

Lithium administration is a very common treatment
of manic-depressive disease. It is estimated that 1 in 1,000
of the population receive lithium, and roughly 20–30% of
these develop serious side effects including polyuria (16,
39) primarily due to a vasopressin-resistant urinary-con-
centrating defect, i.e., NDI. We examined the effect of oral
lithium treatment of rats for 25 days (129, 149). AQP2 and
AQP3 levels were progressively reduced to ~5% of levels
in control rats after 25 days of lithium treatment (129,
149). The downregulation of AQP2 expression was paral-
leled by a progressive development of severe polyuria.
With serum lithium levels in the therapeutic range, rats
showed that there was a reduction in AQP2 expression in both
apical plasma membrane (a lot of AQP2 was found in
the membrane than seen in control animals), but there
was only a modest increase in AQP2 expression relative
to animals treated with lithium alone. On the contrary,
with lithium from the diet (149).

Treating rats on a potassium-deficient diet for 11 days
resulted in downregulation of AQP2 expression in both
inner medulla and cortex (27 ± 3 and 34 ± 15% of control
regulation of both AQP2 and AQP3 appears to play a
significant role in the development of lithium-induced
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Thus reduction of AQP2 in both the apical and the basolateral plasma membrane may participate in the overall reduced water resorption (149). The reduced AQP3 expression was also confirmed by immunocytochemistry (129). Thus downregulation of both AQP2 and AQP3 appears to play a significant role in the development of lithium-induced diabetes insipidus (DI). Lithium administration is a very common treatment of manic-depressive disease. It is estimated that 1 in 1,000 of the population receive lithium, and roughly 20–30% of these develop serious side effects including polyuria (16, 39) primarily due to a vasopressin-resistant urinary-concentrating defect, i.e., NDI. We examined the effect of oral lithium treatment of rats for 25 days (129, 149). AQP2 and AQP3 levels were progressively reduced to ~5% of levels in control rats after 25 days of lithium treatment (129, 149). The downregulation of AQP2 expression was paralleled by a progressive development of severe polyuria. 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Thus reduction of AQP2 in both the apical and the basolateral plasma membrane may participate in the overall reduced water resorption (149). The reduced AQP3 expression was also confirmed by immunocytochemistry (129). Thus AQP2 in both the apical and the basolateral plasma membrane may participate in the overall reduced water resorption (149). The reduced AQP3 expression was also confirmed by immunocytochemistry (129). Thus
levels, respectively). Thus hypokalemia is associated with significantly less downregulation of AQP2 compared with that seen in lithium-treated rats. In parallel with the less extensive AQP2 downregulation, urine production increased moderately from 11 ± 1 to 30 ± 4 ml/day, i.e., much less increase in urine output compared with lithium-treated animals. The increase in urine output was associated with an impaired urine-concentrating ability. In response to 12-h water deprivation, urine osmolality was significantly lower in hypokalemic rats compared with controls. Treating rats with a normal potassium containing diet for 7 days after an 11-day period on a potassium-deficient diet showed normal urinary concentrating capacity and normalization of AQP2 levels. Taken together, the results support the view that there is a causative link between the inability to concentrate urine and the reduced AQP2 levels.

Another electrolyte disturbance, hypercalcemia, is also frequently associated with a urinary concentrating defect and polyuria. An experimental model of vitamin D-induced hypercalcemia in rats has been used by two groups to investigate if dysregulation of AQP2 may also participate in this condition (54, 208). The molecular mechanisms resulting in vasopressin resistance in hypercalcemic conditions remain incompletely understood. Rats treated orally for 7 days with dihydrotachysterol produced a significant hypercalcemia with a 15 ± 2% increase in plasma calcium concentration compared with controls. Hypercalcemic rats demonstrated a threefold increase in urine production, whereas urine osmolality decreased from 2,007 to 925 mosmol/kgH₂O. Consistent with this, immunoblotting and densitometry of membrane fractions revealed a 50% reduction in AQP2 expression in kidney inner medulla from hypercalcemic rats. Recently, the molecular defects associated with vitamin D-induced hypercalcemia in rats were further elucidated. Using the same model, Wang et al. (249a) demonstrated that in addition to downregulation of collecting duct AQP2 expression, there was also a significant downregulation of the bumetanide-sensitive Na⁺-K⁺-2Cl⁻ cotransporter BSC-1 in membranes from inner stripe of the outer medulla in response to hypercalcemia. This defect in the thick ascending limb may participate in the development of the urinary concentrating defect.

Both hypokalemia and hypercalcemia are associated with downregulation of AQP2 expression, and immunolocalization studies of AQP2 demonstrated similar features although there were also differences between the two models. In kidneys from hypokalemic and hypercalcemic animals, immunocytochemistry confirmed that there was a marked reduction in the expression and showed a change in the subcellular distribution of AQP2. There was a significant reduction in the labeling of the apical plasma membrane domains of collecting duct principal cells similar to what was found in kidneys of lithium-treated animals, although much less extensive in hypercalcemic (and hypokalemic) animals. This supports the view that downregulation of AQP2 and reduced apical plasma membrane levels of AQP2 are common features among many forms of acquired NDI and are likely to play a critical role in the development of these NDI conditions. However, it should be emphasized that other defects are also likely to participate in the conditions, e.g., downregulation of BSC-1 in hypercalcemia.

3. NDI caused by urinary tract obstruction

Urinary tract obstruction is a serious clinical condition associated with complex changes in renal function involving marked alterations in both glomerular and tubular function, and it may be associated with long-term impairment in the ability to concentrate urine (262). To examine whether altered AQP2 expression was associated with urinary concentrating defect, AQP2 expression levels were examined in a rat model in which both ureters were reversibly obstructed (77). After bilateral obstruction for 24 h, AQP2 expression levels were markedly reduced, even before obstruction was released (Fig. 13). During this period of obstruction urine production is zero, and the result supports the view that diuresis per se is not the cause of decreased AQP2 levels. This is consistent with the lack of downregulation of AQP2 in response to short-term (24 h) or long-term (5 days) polyuria induced by furosemide treatment (148, 150).

After release of the obstruction, there is a marked polyuria that persists for several days, and there is an increased solute-free water clearance, indicating an impaired ability to reabsorb water at the collecting duct level (Fig. 12). Although urine output in this particular study was down to normal 7 days after release of obstruction, the animals still had an impaired urinary concentrating defect in response to 24 h of thirsting. These results are consistent with the measurements of AQP2, which were reduced to ~20% of control levels 2 days after release of obstruction, before increasing to ~50% 7 days after release of obstruction. Thus the persisting urinary concentrating defect is likely to be related to the continued reduction in AQP2 levels (Fig. 13). Therefore, it seems very likely that the reduction in total AQP2 available combined with a reduced osmotic gradient driving the water reabsorption is responsible for the concentrating defect seen in these animals. In addition to the reduced AQP2 expression, AQP3 and AQP1 expression levels have also been shown to be downregulated in response to bilateral ureteral obstruction (BUO). Expression of AQP2 and AQP3 tends to normalize within 30 days after release of BUO, whereas the urinary concentrating capacity is still reduced, although the concentrating defect is marginal at this stage.

AQP1 remains downregulated coinciding with a sig-
nificant impairment in urinary concentrating capacity (J. Frøkjaer, unpublished data), supporting the view that AQP1 may play a major role in maintaining a normal urinary concentrating capacity (30, 210).

In contrast to BUO, conditions with unilateral ureteral obstruction are not associated with changes in the absolute excretion of sodium and water, since the non-obstructed kidney compensates for the reduced ability of the obstructed kidney to excrete solutes. To examine whether the previously identified reduction in AQP2 expression in bilateral ureteral obstruction was caused by local factors (increased tissue pressure and changes in renal hemodynamics or parenchymal biochemistry) or systemic changes in the animal, experiments were performed to investigate the effects of unilateral ureteral obstruction for 24 h (75). In this case, there was a pro-

**FIG. 12.** AQP2 levels (**A**) and urine output (**B**) during 24 h of bilateral ureteral obstruction (BUO) and 24 h, 48 h, and 7 days after release (REL) of BUO compared with time-matched sham-operated controls. Twenty-four hours of BUO resulted in a dramatic decrease in AQP2 expression. Low levels of AQP2 persisted 24 and 48 h after release of obstruction and coincided with a marked increase in urine production. Seven days after release of bilateral ureteral obstruction, AQP2 expression had partially normalized compared with sham-operated control rats. At this time, urine production had normalized. *P < 0.05.

**FIG. 13.** Effect of α-melanocyte stimulating hormone (α-MSH) treatment of AQP2 levels and changes in urine output and urine osmolality in rats with acute renal failure (ARF) (2 days after 40-min bilateral ischemia). **A:** immunoblot is reacted with anti-AQP2. **B:** densitometric analysis of all samples from ARF (ARF 40/2), either in the absence of α-MSH treatment (ARF) or with α-MSH treatment (ARF+MSH), and sham-operated rats. In absence of α-MSH treatment, rats with ARF have markedly decreased AQP2 expression levels (13 ± 3% of sham levels, *P < 0.05). AQP2 expression is 7-fold higher in response to α-MSH of ARF rats compared with untreated rats. **C** and **D:** time courses of the changes in urine output (**C**) and urine osmolality (**D**). Urine output is significantly increased, and urine osmolality is significantly decreased after 40-min bilateral renal ischemia in ARF rats (both α-MSH treated and nontreated). ARF rats treated with α-MSH showed a reduced urine output and a higher urine osmolality compared with untreated ARF rats. *P < 0.05, ARF compared with sham-operated rats. †P < 0.05, nontreated ARF rats compared with α-MSH-treated ARF rats. [From Kwon et al. (127).]
found downregulation of AQP2 levels in the obstructed kidney (23% of control) and a moderate AQP2 reduction (75% of control) in the nonobstructed kidney. Consistent with this, urine production was increased by 150% from the nonobstructed kidney. Additional experiments revealed that changes in AQP2 expression are reciprocal to the changes in free water clearance, demonstrating a functional association between these two parameters. The decrease in AQP2 expression in the nonobstructed kidney may participate in the increased urine output, by decreasing free water reabsorption, thus compensating for the loss of excretion from the obstructed kidney.

These results support the view that local factors play an important role in the downregulation of AQP2 expression during obstruction, but the signals leading to this decrease remain to be determined. However, the reduction in AQP2 expression in the contralateral, nonobstructed, kidney may suggest a systemic effect, which may potentially involve decreased circulating vasopressin or washout of metabolites from the obstructed kidney, or may be a consequence of renorenal nerve activity, known to play a role in the compensation for unilateral obstruction.

C. Urinary Concentrating Defects in Renal Failure

1. Experimental ARF induced by ischemia and reperfusion injury in rat

Renal failure, both acute and chronic, is associated with polyuria and a urinary concentrating defect, and in both cases there is a wide range of glomerular and tubular abnormalities that contribute to the overall renal dysfunction. Ischemia-induced experimental ARF in rats is a model that is widely used. In this model there are structural alterations in renal tubules, in association with an impairment in urinary concentration. The proximal tubule (S3 segment) and thick ascending limb are known to be main sites of ischemic injury (17, 237).

Several studies have shown defects both in collecting duct water reabsorption and proximal tubule water reabsorption, as well as defects in solute handling in postischemic kidneys (90, 102, 221, 237). In an isolated tubule microperfusion study of the rabbit, it was observed that water reabsorption in the proximal tubule and cortical collecting duct was significantly reduced after ischemia (90). Moreover, it has been demonstrated that there are no differences in either basal or vasopressin-induced cAMP levels in outer or inner medulla in rats with ARF compared with sham-operated rats (9), further supporting the view that there are defects in collecting duct water reabsorption in postischemic kidneys. It is also well known that kidneys subjected to injury by ischemia are unable to establish or maintain a high medullary solute content (14). Moreover, a decreased ability of the thick ascending limb to lower perfusate chloride ion concentration was observed as well (90). These studies suggest that there are defects in both countercurrent multiplication and collecting duct water permeability in response to ischemic damage.

Consistent with these findings, it was recently demonstrated that the levels of AQP2 and AQP3 in the collecting duct as well as AQP1 in proximal tubule are significantly reduced in response to renal ischemia (67, 127). The decreased levels of aquaporins were associated with impaired urinary concentration in rats with both oliguric or nonoliguric ARF. To further examine the role of aquaporin downregulation in the development of a urinary concentrating defect, rats with ischemia-induced ARF were treated with α-melanocyte stimulating hormone (α-MSH) to prevent the establishment of a urinary concentrating defect (24). Both the reduced expression of AQP1 to -3 and the reduced urinary concentration capacity was significantly prevented by cotreatment with α-MSH (Fig. 13) (127). The results suggest that decreased levels of aquaporins in both the proximal tubule and collecting duct in postischemic kidneys may play a significant role in the impairment of urinary concentration encountered in oliguric, maintenance, and polyuric phases of experimental ischemia-induced ARF.

2. Experimental chronic renal failure induced by 5/6 nephrectomy in rat

Patients with advanced chronic renal failure (CRF) have urine that remains hypotonic to plasma despite the administration of supramaximal doses of vasopressin (220). This vasopressin-resistant hypostenurina specifically implies abnormalities in collecting duct water reabsorption in CRF patients. Consistently, Fine et al. (70) observed that isolated and perfused cortical collecting ducts dissected from remnant kidneys of severely uremic rabbits exhibited a significantly decreased water flux and adenylyl cyclase activity in the response to vasopressin. Importantly, they demonstrated that 8-bromo-cAMP failed to induce a normal hydrosomatic response in cortical collecting duct from remnant kidneys. Several micropuncture and microcatheterization studies have also indicated that the impaired urinary concentrating ability may, at least partly, be caused by impairment of vasopressin-stimulated water reabsorption in the collecting duct in CRF (18, 251). As an extension of these observations, Teitelbaum and McGuinness (222) demonstrated that RT-PCR of total RNA from the inner medulla of CRF rat kidneys revealed virtual absence of V2-receptor mRNA. Thus these studies provide firm evidence for significant defects in the collecting duct water permeability. Consistent with these observations, recent studies have shown both decreased collecting duct water channel AQP2 and AQP3 expression and a vasopressin-resistant downregulation of AQP2 in a 5/6 nephrectomy-induced CRF rat.
model (128). Immunocytochemistry and immunoelectron microscopy confirmed a marked reduction in AQP2 and AQP3 expression in the principal cells. This suggests that reduced AQP2 and AQP3 expression levels may be important factors involved in the impaired collecting duct water permeability and in reduced vasopressin responsiveness in CRF.

**D. States of Water Retention**

1. **Chronic heart failure**

Severe chronic heart failure is characterized by defects in renal handling of water and sodium resulting in extracellular fluid expansion and hyponatremia. An increased baroreceptor-mediated vasopressin release is believed to play a critical role for the renal water retention and contribute to the development of hyponatremia. Two studies have looked at changes in renal aquaporin expression in rats with congestive heart failure (CHF) induced by ligation of the left coronary artery (177, 255) to test if upregulation of AQP2 expression and targeting may play a role in the development of CHF. Both studies demonstrated that the renal water retention in severe CHF in rats is associated with marked dysregulation of AQP2 in the renal collecting duct involving both an increase in the abundance of AQP2 water channel protein in the collecting duct principal cells and a marked redistribution of AQP2 water channels in the collecting duct principal cells with most channels located to the apical plasma membrane (177, 202, 255). Rats with severe heart failure had significantly elevated left ventricular end-diastolic pressures (LVEDP) compared with sham-operated animals (26.9 ± 3.2 vs. 4.1 ± 0.3 mmHg) and had reduced plasma sodium concentrations (177). Immunoblotting revealed a threefold increase in AQP2 expression compared with sham-operated animals. These changes were markedly related to elevated LVEDP or hyponatremia, since animals without changes in LVEDP and plasma sodium did not have increased AQP2 levels compared with sham-operated controls (177). Furthermore, this study showed a marked increase in plasma membrane targeting, providing an explanation for the increased permeability of the collecting duct causing an increase in water reabsorption. This may provide an explanation for excess free water retention in severe CHF and for the development of hyponatremia. In parallel, the other study showed upregulation of both AQP2 protein and AQP2 mRNA levels in kidney inner medulla and cortex in rats with chronic heart failure (255). These rats had significantly decreased cardiac output and, importantly, increased plasma vasopressin levels. Furthermore, in this study administration of the V2 antagonist OPC 31260 was associated with a significant increase in diuresis, a decrease in urine osmolality, a rise in plasma osmolality, and a significant reduction in AQP2 protein and AQP2 mRNA levels compared with untreated rats with CHF. Thus there is abundant evidence for a major role for vasopressin in the upregulation of AQP2 in experimental CHF in the rat.

2. **Hepatic cirrhosis**

Hepatic cirrhosis is another serious chronic condition associated with water retention. It has been suggested that an important pathophysiological factor in the impaired ability to excrete water could be increased levels of plasma vasopressin. However, unlike CHF, the changes in expression of AQP2 protein levels vary considerably between fundamentally different models of hepatic cirrhosis. Several studies have looked at changes in renal aquaporin expression in rats with cirrhosis induced by common bile duct ligation (CBDL) (69, 104, 105). In these studies hepatic cirrhosis caused significant sodium retention (104) consistent with significant hypertrophy of the thick ascending limb (103). The rats displayed impaired vasopressin-regulated water reabsorption despite normal plasma vasopressin levels. This was determined by testing the effect of a vasopressin V2-receptor antagonist OPC 31200. An impaired effect was observed in cirrhotic rats. Consistent with this, immunoblotting and semiquantitative densitometry showed a significant decrease in AQP2 abundance in rats with hepatic cirrhosis (69, 104). In addition, the expression levels of AQP3 and AQP4 located to the basolateral plasma membrane of the collecting duct principal cells were downregulated in CBDL rats, which may predict a reduced water permeability of the collecting duct independent of the effects due to AQP2 dysregulation in this model (69). Thus these results support the view that AQP2, AQP3, and AQP4 play important roles for abnormal collecting duct water transport as evidenced from studies using AQP3 and AQP4 knockouts (31, 138). In contrast, the expression levels of AQP1 were not decreased (69). Thus dysregulation of multiple water channels may play a role in water balance abnormalities associated with CBDL-induced cirrhosis in rat. The results with CBDL-induced cirrhosis are similar to what has been demonstrated in rats with nephrotic syndrome, supporting the view that increased expression of AQP2 is not a uniform finding in rat models of volume-expanded states. In the study by Fernandez-Lama et al. (69), CBDL was also associated with hyponatremia in response to water loading, showing that in the case of extracellular fluid volume expansion, excessive water retention can occur in the absence of increased AQP2 levels. Importantly, in a recent study where rats with CBDL were treated with the aldosterone receptor antagonist canrenone, an increased urine production was demonstrated that was associated with a marked reduction in AQP2 expression; this strongly supports the view that aldosterone may play a significant role for vasopressin-mediated water reabsorption.

In contrast, Fujita et al. (79) demonstrated that he-
Nephrotic syndrome induced by intraperitoneal administration of carbon tetrachloride and olive oil twice a week for 12 wk was associated with a significant increase in both AQP2 protein levels and AQP2 mRNA expression (79). Interestingly, AQP2 mRNA levels correlated with the amount of ascites, suggesting that AQP2 may play a role in the abnormal water retention followed by the development of ascites in hepatic cirrhosis (12). In a different model of carbon tetrachloride-induced cirrhosis, using carbon tetrachloride inhalation, AQP2 abundance was not increased (68). There was, however, evidence for increased trafficking of AQP2 to the plasma membrane consistent with the presence of elevated levels of vasopressin in the plasma. Interestingly, there was a marked increase in AQP3 expression that is likely to be due to increased vasopressin levels. The pattern of high AQP3 without elevation of AQP2 is consistent with previous findings in vasopressin escape (55), suggesting that the lack of increase in AQP2 abundance is a result of a normal compensatory response related to the escape phenomenon. Another finding in carbon tetrachloride-inhalation-induced cirrhosis was a marked increase in cortical AQP1 expression, a potential contributing factor to increased proximal fluid absorption in this segment. Although the explanation for the differences between cirrhosis induced by CBDL and carbon tetrachloride remains to be determined, it is well known that the dysregulation of body water balance depends on the severity of cirrhosis (85, 253).

CBDL results in a compensated cirrhosis characterized by peripheral vasodilation and increased cardiac output, whereas cirrhosis induced by 12 wk of carbon tetrachloride administration may be associated with the late decompensated state of liver cirrhosis characterized by sodium retention, edema, and ascites (85, 133). Thus the downregulation of AQP2 observed in milder forms of cirrhosis (i.e., in a compensated stage without water retention) may represent a compensatory mechanism to prevent development of water retention. In contrast, the increased levels of vasopressin seen in severe “noncompensated” cirrhosis with ascites may induce an inappropriate upregulation of AQP2 that would in turn participate in the development of water retention.

3. Experimental nephrotic syndrome in rats

Nephrotic syndrome (NS) is characterized by extracellular volume expansion with excessive renal water and sodium reabsorption. The mechanisms of water and sodium retention are poorly understood; however, it was expected to be associated with dysregulation of aquaporins and sodium transporters (11, 66). In contrast to CHF and liver cirrhosis, where extracellular volume expansion and hyponatremia have been reported to be associated with upregulation of AQP2 (12, 79, 177), rats with NS 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 the renal inner medulla in the rats with puromycin aminonucleoside (PAN)-mediated NS and adriamycin-induced NS (11, 65). This reduced expression of collecting water channels could represent a physiologically appropriate response to extracellular volume expansion. Circulating vasopressin levels are high in rats with PAN-induced NS. Nevertheless, AQP2 levels are dramatically reduced in the experimental NS, thus suggesting that this represents an “escape” phenomenon. Certainly, it indicates that there is a signal other than vasopressin than can cause changes of AQP2 expression.

4. Vasopressin escape

Recently, Ecelbarger et al. (58) examined the mechanisms behind vasopressin escape, a condition where the normal hydromotic response to vasopressin is suspended to prevent water intoxication. Rats infused with dDAVP in osmotic minipumps were either forced water loaded or allowed free access to water. Despite that all rats had the same levels of circulating dDAVP (i.e., clamping of plasma ADH levels), the water-loaded rats had a dramatic downregulation of AQP2 and developed polyuria compared with the antidiuretic control rats. This downregulation of AQP2 may therefore represent a physiologically appropriate way to reduce the capacity to reabsorb water (58). The signaling transduction pathways involved in the altered long-term regulation of AQP2 during vasopressin escape are at present unknown, but the study strongly suggests that a vasopressin-independent signaling pathway may be involved. Thus the existence and potential importance of a vasopressin-independent signaling pathway (149) has gained considerable support (58).

5. Regulation of AQP2 by female sex hormones

Pregnancy is characterized by a 30–50% increase in extracellular fluid, plasma, and blood volume in different mammalian species, including humans and rats (211). Other characteristic changes during pregnancy are arterial vasodilation together with sodium and water retention and a decrease in plasma osmolality both in rats and humans. In parallel with the arterial vasodilation, the renin-angiotensin-aldosterone system is activated, and it has been suggested that the decrease in plasma osmolality is caused by resetting of the threshold for vasopressin secretion during pregnancy (53). Therefore, the hypothesis that AQP2 expression could be increased during pregnancy was tested in rats on days 7, 14, and 20 of pregnancy (179). Plasma osmolality, sodium, and body weight were consistently lower in pregnant rats, and associated with this, AQP2 mRNA and AQP2 levels significantly in-
creased during pregnancy. Administration of the V2-receptor agonist suppressed the increase in both AQP2 mRNA and AQP2 despite normal levels of plasma vasopressin (179). Thus this study suggests that the upregulation of AQP2 contributes to water retention during pregnancy in part through a V2 receptor-mediated effect, but also AVP independent factors (such as oxytocin) may be important for this upregulation (212).

Another common condition in females where the circulating levels of sex hormones is altered is onset of menopause, which may also be associated with changes in regulation of body water balance. Ovariectomy has been shown to reduce plasma vasopressin concentrations in rats and suppress diurnal changes in hormone concentrations (188). Sardeli et al. (209) therefore hypothesized that AQP2 dysregulation could be associated with changes in renal water handling after ovariectomy. One and two weeks after ovariectomy a significant increase in AQP2 and phosphorylated AQP2 levels in kidneys of rats was demonstrated, which was associated with a reduction in urine output and a significant increase in body weight (209). These results therefore suggest that AQP2 dysregulation may play an important role in renal water handling after ovariectomy.

6. Urinary excretion of aquaporins

Kidney diseases and conditions associated with altered kidney function are often characterized by urinary excretion of distinct renal and extrarenal proteins. A number of studies have demonstrated that the response of urinary AQP2 excretion to dDAVP infusion is an index of vasopressin action on the kidney (197). Urinary excretion was increased during dehydration or vasopressin treatment and decreased after hydration, and CDI patients treated with vasopressin had increased urinary AQP2 excretion (113). Furthermore, it was shown that the amount of daily excretion of AQP2 in the urine was the same in men and women, and urinary AQP2 content was not affected by age of the subjects. Importantly, urinary AQP2 showed a positive correlation with urine osmolality (197). The fraction of AQP2 excreted in the urine compared with whole kidney content was determined in rats, and ~3–4% of AQP2 in the kidney was excreted daily (197, 250). Thirsting or vasopressin treatment markedly increased urinary AQP2 excretion, whereas water loading suppressed AQP2 excretion, supporting the view that urinary AQP2 excretion closely parallels changes in vasopressin action (250). There is strong evidence that urinary AQP2 excretion is mediated by an apical pathway that is tightly regulated by vasopressin, since AQP3 located to the basolateral plasma membrane is undetectable (250). Urinary AQP2 excretion may be a valuable marker in clinical conditions with altered water balance. In a recent study where patients with CHF were treated with the V2-receptor antagonist VPA-985, urinary AQP2 excretion was dramatically decreased in a dose-dependent manner (154). This decrease correlated with increased urinary excretion of solute-free water supporting a functional association between AQP2 levels and water reabsorption at the collecting duct level. Future studies will determine whether urinary AQP2 excretion will be a useful parameter.

VIII. CONCLUSION

The discovery of aquaporins by Agre and colleagues has allowed enormous progress in the understanding of how water is transported across biological membranes and epithelial cells at the molecular level and how renal water handling occurs at the physiological level as well as in multiple diseases associated with severe derangement of body water balance. Currently more than 10 mammalian aquaporins have been identified and at least 5 renal aquaporins have been well characterized. It may be speculated that several more aquaporins are to be identified. Rough estimates based on the number of aquaporin proteins identified in organisms where the entire genome has been completely identified (e.g., Drosophila, Escherichia coli, or yeast) may allow a prediction that several more aquaporin homologs may exist in the human/mammalian genome. Future studies will concentrate on 1) defining the structure-function relationship at molecular resolution to define the structural determinants involved in selective water and ion conductivity, 2) defining the signaling cascades and the molecular apparatus involved in aquaporin regulation in various cells, 3) defining the physiological and pathophysiological roles at the integrated level of each aquaporin, and 4) identifying novel aquaporins and establishing their regulation and physiological and pathophysiological roles at the cellular and integrated level.

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