MINIREVIEW

Aquaporins (Water Channels): Role in Vasopressin-Activated Water Transport

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Abstract. **The discovery of water channels (aquaporins) was a breakthrough in research on water transport. Aquaporins are a family of intrinsic membrane proteins that function as water-selective channels (except aquaporin-3 and aquaporin-7, which are permeable to urea and glycerol as well) in the plasma membranes of many cells. Aquaporin-0 (MIP26) functions to maintain fluid balance in the lens. Aquaporin-1 is involved in water reabsorption in the kidney's proximal tubules and the thin descending Henle's loop, aqueous humor formation in eye, cerebrospinal fluid formation in brain, and airway hydration in lung. Aquaporin-2 is the only water channel that is activated by vasopressin to enhance water reabsorption in the kidney collecting duct. Aquaporin-3 also contributes to water reabsorption in the kidney collecting duct but is unresponsive to vasopressin. It also appears that aquaporin-3 may contribute to cornea transparency. Aquaporin-4 is involved in cerebrospinal fluid transport in brain, water transport in the kidney collecting duct, aqueous humor transport in the eye, and airway hydration in the lung. Aquaporin-5 apparently is coupled to fluid secretion in exocrine tissues. Although the exact function of aquaporin-6 is not known due to its uncertain localization, its restricted presence in the kidney may suggest a potential role in water transport. Aquaporin-7 appears to play a role in the cryopreservation of the sperm whereas aquaporin-8 is responsible for the secretion of pancreatic juice. The major focus of this review is a discussion of aquaporins in renal epithelia, and particularly the mechanisms associated with vasopressin-mediated water transport involving aquaporin-2 and the signal transduction pathways linked to vasopressin action.** [P.S.E.B.M. 1998, Vol 219]

Although water can cross cells through simple diffusion across membrane phospholipids, measurements of water permeability of lipid membranes suggested the existence of a facilitated transport mechanism. Contributing to this finding was a difference between the permeability coefficient of water in most plasma membranes (50 μm/s) as compared to membranes of chinchilla's descending Henle's loop (1500–2000 μm/s) (1, 2). Such a several hundred-fold difference in permeability could not have been explained by simple diffusion. In addition, this rapid transport of water was attenuated by mercury chloride and restored by adding β-mercapoethanol (3–5). This led to further investigations of such phenomena that led to the first selective water channel being purified, cloned, and termed aquaporin-1 (6). Earlier studies purified a water channel protein called MIP26 (membrane integral protein, lens-specific, now known as AQP-0), but it was found to be a nonselective water channel (permeable to sucrose, KCl,

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polyethylene glycol, ascorbate) (7–9). To date, there are at least nine aquaporins that have been identified (AQP0–AQP8). AQP-2 is the vasopressin-sensitive channel and is localized only in the kidney collecting duct (10). Other water channels that participate in water reabsorption in the kidney are AQP-1, AQP-3, and AQP-4 (11). A new AQP-6 has also been cloned and appears to be restricted to the kidney, but the nephron localization has not been established; therefore, its physiological role has yet to be elucidated (12–13). Interestingly, Northern blotting of AQP-7 mRNA expression has detected a novel transcript of a different size in the kidney, but whether this represents an AQP-7-like channel needs further investigation (14). The second richest tissue containing aquaporins is the eye (AQP-0, AQP-1, AQP-3, AQP-4, and AQP-5), where aquaporins may be responsible for aqueous humor formation (15). However, the focus of the current review is on vasopressin actions in the kidney and the proposed mechanisms involved in water reabsorption.

Renal Absorption

The renal concentrating mechanism occurs at three different nephron segments: 1) the proximal tubules (designated area A in Figure 1); 2) thin descending Henle's loop (area B); and 3) collecting duct (area C) (16). Although water reabsorption at the proximal tubules and thin descending Henle's loop are not subjected to hormonal regulation (vasopressin-insensitive), water transport at the collecting duct is absolutely vasopressin-sensitive (17). Of the 180 liters of glomerular filtrate a day, 80%–90% is reabsorbed in the proximal tubule and descending thin limb of Henle's loop. The remaining 10%–20% of glomerular filtrate is reabsorbed in the collecting duct as shown in Figure 1 (18). Water reabsorption at areas A and B is carried out by a single aquaporin channel known as AQP-1 (AQP-1 constituted 4% of the brush border proteins in the proximal tubules (19–21)). By contrast, vasopressin-regulated water permeability in the collecting duct is carried out by at least three different aquaporins (AQP-2, AQP-3, and AQP-4). It is believed that vasopressin induces the trafficking of vesicles containing AQP-2 between an intracellular compartment and the apical plasma membrane (22)). AQP-3 and AQP-4 are not shuttled by vasopressin but are constitutively active and localized at the basolateral membranes. The dehydration of rats increased the mRNA levels of AQP-2 and AQP-3, whereas the mRNA levels of AQP-1 and AQP-4 remained unchanged (23–24). This suggests that both AQP-2 and AQP-3 are responsible for the kidney's concentrating urine. However, the lack of aquaporins in the ascending thin and thick limbs of Henle's loop, distal convo-

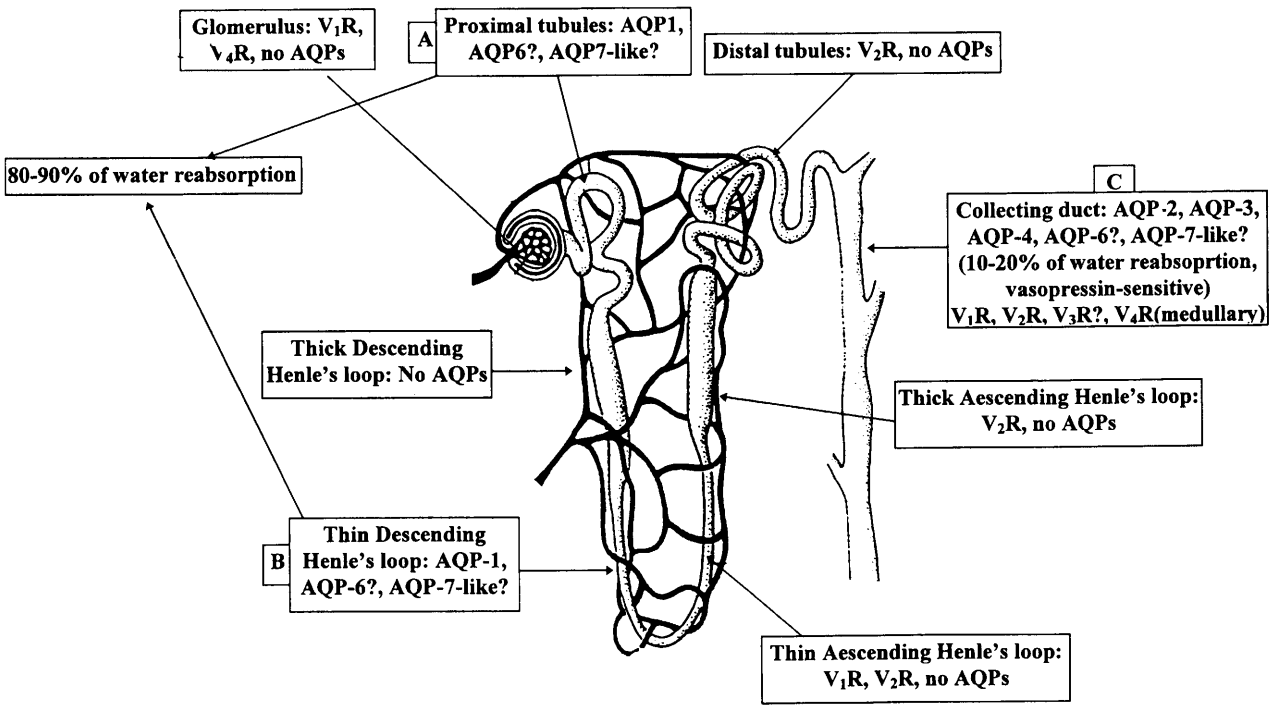

Figure 1. Water reabsorption in mammalian nephron, aquaporin and vasopressin receptors distribution. Water reabsorption occurs at three different areas in the nephron designated A, B, and C. Area A represents the proximal tubules, and water reabsorption is carried out by AQP-1. Area B includes the thin descending Henle's loop, and water reabsorption is carried out also by AQP-1. Both Areas A and B help reabsorb 80%–90% of the glomerular filtrate and are not regulated by vasopressin. The third area that is responsible for the remaining 10%–20% water reabsorption (vasopressin-activated) is the collecting duct and involves at least three water channels (AQP-2, AQP-3, and AQP-4). The vasopressin V_1 and V_2 receptors are present in the collecting duct (area C) and the thin ascending Henle's loop. Whereas the V_2 receptor is present in the distal tubules, the V_1 receptor is present in the glomerulus. The V_4 receptor has been localized to the medullary portion of the collecting duct and the glomerulus. However, the localization of the V_3 receptor is still not established.

luted tubules, and connecting tubules explains their impermeability to water (18). The functions of AQP-6 and possibly the AQP-7-like channels in the kidney have yet to be described. It is known that vasopressin-mediated effects on water channels are initiated through actions on selective receptors.

Vasopressin Receptors Mediating Vasopressin Actions

Vasopressin (or antidiuretic hormone—ADH) is a neuropeptide of nine amino acids. It is synthesized in the neurons and stored at the posterior pituitary gland. Upon hypovalamia, osmoreceptors release vasopressin to help regulate water reabsorption (25–26). Vasopressin carries out its functions by acting on selective membrane receptors. At least four different vasopressin receptors have been identified. The V_{1a} vasopressin receptor is found in liver, vascular smooth muscle cells, and most peripheral tissues (27–28); however, in humans, the V_{1a} receptor is restricted to only the mesenteric artery (27). The V_2 receptor is present in the kidney and platelets (29–30), and the V_3 receptors (termed previously V_{1b}) are present in the pituitary, kidney, heart, thymus, heart, lung, spleen, uterus, and breast in rat tissues (31–34) whereas, in humans it is absolutely restricted to the pituitary (32). Recently, a fourth receptor has been cloned from rabbit kidney medulla and has been termed VACM receptor (termed vasopressin-activated calcium mobilizing), and in this review we will refer to this new protein as the V_4 receptor (35). The V_4 receptor is present in heart, brain, and skeletal muscles (36). While V_1, V_3, and V_4 receptors are coupled primarily to a phospholipase C (PLC) and phospholipase A_2 (PLA_2) enzymes, the V_2 receptor is coupled to an adenylyl cyclase enzyme. Despite the presence of three different receptors in kidney, the V_2 receptor appears to be solely responsible for water transport (26). A classification of vasopressin receptors is shown in Figure 2.

Distribution of Vasopressin Receptors in the Kidney

Plasma Membranes. The V_2 receptor has been localized at the luminal and basolateral membranes in the rat terminal inner medullary collecting duct (37). It appears that the luminal V_2 receptor exerts a negative feedback inhibition of the basolateral V_2 receptor with a decrease in cAMP production. The presence of the V_{1a} receptor at the luminal site in the rat cortical collecting duct has been identified also, but it is not known whether it is also involved in inhibiting the V_2 receptor actions (38). However, the plasma cellular localization of V_3 and V_4 receptors is still not clear.

Nephron Segments. Using OPC-21268 and OPC-31260 selective nonpeptide antagonists of V_1 and V_2 receptors, respectively, Mimura *et al.* have localized the different vasopressin receptors in the Wistar rat kidneys (39). This group has shown that the V_2 receptor is the predominant receptor in the kidney with V_2 receptors visualized in the collecting ducts and medullary tubules, whereas, the V_1 receptor was localized to the glomerulus, cortical vessels, interstitial cells, and the medullary vessels. However, based on adenylyl cyclase measurements and PCR studies, it appears that the V_2-receptors are localized to different segments of the nephron: the collecting duct, ascending limb of Henle's loop (thick and thin), the distal tubule occasionally, but not other segments (40). In the rat, the V_{1a} receptor appears to be present in the thin ascending limb, cortical and outer medullary portions of the collecting duct, but absent from the glomerulus, the proximal tubule, the thick ascending limb of Henle's loop, and the terminal portion of the papillary collecting duct (40). The contradiction of observations on the presence or absence of the V_1 receptor in the glomerulus may have resulted from the two different techniques used to study receptor distribution. In addition, the potential binding of OPC-21268 (V_1 antagonist) to V_3 or V_4 receptors has not been ruled out. Shwartz *et al.* have developed a selective V_3 antagonist that would be useful in studying V_3 receptor distribution in the kidney (41), and offer a potential solution to the previous observations. Immunostaining has shown that the V_4 receptor is present in the medullary collecting duct portion of rabbit kidney and absent from the cortical collecting duct (42). The V_4-receptor was also detected in the glomerulus but not in the Henle's loop. Table I is a summary of the vasopressin receptor distribution proposed in the kidney.

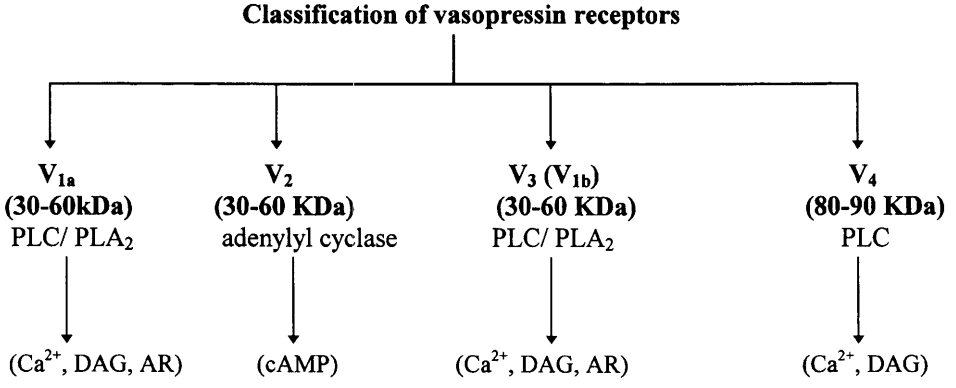

Figure 2. Classification of vasopressin receptors. To date, there are at least four vasopressin receptors that have been identified. Whereas V_1, V_3, and V_4 receptors are coupled to PLC/ PLA_2 enzymes, the V_2 receptor is coupled to an adenylyl cyclase protein. Although both V_1 and V_3-receptors are present in rat kidney, in human, the V_1 receptor and the V_3 receptor are present only in the mesenteric and the pituitary, respectively (27, 32)(AR: arachidonic acid, DAG: diacylglycerol).

Table I. Distribution of Vasopressin Receptors at the Nephron

Method of Detection	V_1R	V_2R	V_3R	V_4R
OPC-21268/OPC-31260 antagonists	Glomerulus, cortical vessels, interstitial cells, medullary vessels	Collecting ducts/medullary tubules	?	?
Adenylyl cyclase/RT-PCR	Thin ascending limb, cortical and outer medullary portions of the collecting duct	Collecting duct, ascending limb of Henle's loop (thick and thin), distal tubule	?	?
Western blotting/ immunocytochemistry	—	—	?	Medullary collecting duct, glomerulus

Vasopressin V_2 Receptor Coupled to cAMP Generation and the Activation of Cyclic AMP-Dependent Protein Kinase (PKA) is Responsible for Water Transport

It is widely accepted that an increase in cAMP is critical to vasopressin-induced increase in water channel trafficking and water transport (43–44). Vasopressin-activated water transport is mimicked by dibutyryl cAMP or other permeable analogs of cAMP. Since cAMP activates cyclic AMP-dependent protein kinase (PKA), and inhibition of PKA results in the attenuation of vasopressin-activated water transport, different isoforms of PKA may have selective roles in the kidney. First, PKAα appears to be expressed in toad urinary bladder cells (popular model for mammalian collecting duct cells) as detected by Western blotting (Fig. 3A). Second, using liposomes (lipofactamine, Gibco Inc., Grand Island, NY) to introduce antibodies directed against the catalytic subunit of PKAα intracellularly, vasopressin-activated water transport was inhibited as shown in Figure 3B. This observation is consistent with previous studies showing that cholera toxin, a potent activator of $G_{\alpha s}$ protein (GTP-binding protein activating adenylyl cyclase), potentiated vasopressin-activated water transport (45).

Cyclic AMP-dependent protein kinase has been purified and cloned from a number of different tissues (46) and consists of an inactive R_2C_2 tetramer (R: regulatory and C: catalytic) that is activated and dissociated by cAMP: $R_2C_2 + 4cAMP \rightarrow R_2cAMP_4 + 2C$. The C subunit exists as a monomeric protein with an M_r of 40,800 (46). At least three forms of the catalytic subunit have been identified in mammalian tissues: Cα, Cβ, Cγ (47, 48). While Cα and Cβ are

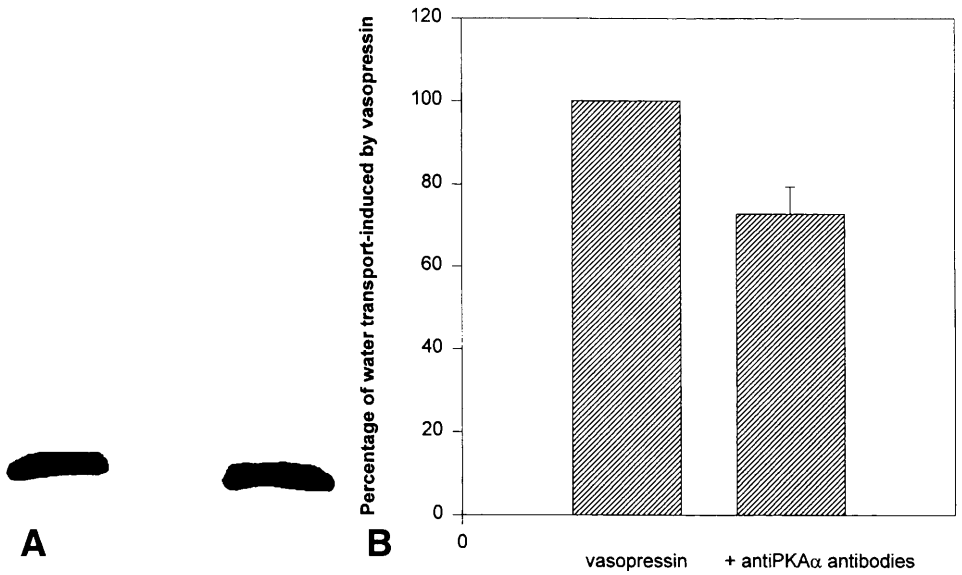

Figure 3A. PKAα subunit is expressed in toad urinary bladders. Immunoblotting using antipolyclonal antibodies against PKAα (Santa Cruz, CA), detected a 40-kDa protein.

Figure 3B. Antibodies against PKAα subunit inhibited vasopressin-induced water transport of toad urinary bladders. Antibodies (polyclonal antibodies against PKAα, Santa Cruz, CA), (10 μg/100 μl (dialyzed overnight against N-Ringer solution to remove sodium azide)) were mixed with lipofactamine (50 μg/25 μl) by vortexing for 20–40 min at room temperature. Isolated bladders, set up as sacs, received lipofactamine alone (50 μg) or lipofactamine/antibodies mixture for 2 hr at room temperature. The bladders were then removed, and 1/10 Ringer solution was added to the mucosal (apical) side while the serosal side received N-Ringer. Vasopressin (10 mU/ml) was added to the serosal side, and water transport was measured after 30 min. As shown, water transport was inhibited by 25%.

expressed in most tissues, Cγ appears to be tissue-specific and is restricted to the testis (48). The catalytic subunit undergoes at least two covalent modifications and can be phosphorylated at a number of serine sites (position 10, 197, 338) (49). Interestingly, the catalytic subunit can be myristoylated, which may explain the localization of the catalytic subunits at the plasma membrane (50). There are also two categories of regulatory subunit, type I, and II (51), each of which can be further classified into α and β subtypes as shown in Figure 4. Type I and II can be distinguished by autophosphorylation. Whereas type I holoenzyme undergoes autophosphorylation, type II has no autophosphorylation sites (52). A classification of cAMP-dependent protein kinase A isoforms is shown in Figure 4.

Role of V_1 Receptor in Vasopressin-Induced Water Transport in Nonhuman Water-Transporting Epithelia

While the V_2 receptor is the predominant receptor in the kidney, and the V_{1a} (V_1) and $V_{3(1b)}$ are absent from the human kidney, a role for the V_1 receptor in regulating water transport may be only relevant in rat, rabbit kidney, and toad urinary bladders (37, 53, 54). The V_1 receptor is typically coupled to PLC and PLA_2 [activation results in the hydrolysis of phospholipids and the generation of diacylglycerol (DAG is a PKC activator), inositol 1, 4, 5-trisphosphate (IP_3) mobilizes intracellular calcium, and arachidonic acid (can be converted into eicosanoids)]. The presence of a calcium/phospholipid-activated protein kinase C (PKC) was first reported by Yorio *et al.* in toad urinary bladders (55). In addition, phorbol esters (PMA), potent activators of protein kinase C (PKC), inhibited vasopressin-activated water transport in toad urinary bladders when added serosally, an effect mediated by the inhibition of adenylyl cyclase and the decrease in the generation of cAMP (56). A similar effect was also observed in isolated rabbit cortical collecting tubules (54). Such an effect could be due to PKC activation of phospholipase A_2 (57). PLA_2-released arachidonic acid metabolites have been shown to inhibit adenylyl cyclase activities (58–61). It was also demonstrated that mezerein, a nonphorbol activator of PKC, when added apically, potentiated cAMP-mediated water transport (53). Furthermore, the addition of other activators of PKC added apically, induced hydrosmosis in toad urinary bladders in the absence of vasopressin (62). Therefore, whereas PKC at the serosal site inhibited vasopressin-induced cAMP production, activation of PKC apically increased the transepithelial water transport. The cross-talk between these two events is interesting, however, the fine regulation of both phenomena is yet to be elucidated. The addition of A23187 (10 μ*M*), a calcium ionophore, which increases intracellular calcium, resulted in a 30% inhibition of vasopressin-activated water transport (63). This suggests the presence of a calcium-sensitive adenylyl cyclase. Northern blotting analysis of mRNA expression of all known eight isoforms of adenylyl cyclase have detected types IV, V, and VI in the kidney (64). Adenylyl cyclase type V and VI are inhibited by low micromolar concentrations of calcium (65) suggesting that these isoforms may be important for vasopressin-mediated water transport. However, since type V is activated by PKC (66), and PKC activators inhibited adenylyl cyclase in the kidney as well as toad urinary bladders (55–56), it is anticipated that type VI may be coupled to the V_2 receptor in epithelial-transporting cells (see Iyengar *et al.* and Taussig *et al.* for excellent reviews on adenylyl cyclase). However, further investigation is needed to verify such a suggestion.

Why Study Water Transport in Toad Urinary Bladder?

The toad's urinary bladder is a popular model of the mammalian collecting duct and is a valuable tissue for studying transepithelial water transport phenomena. In contrast to mammals, the major organs of osmoregulation in adult amphibians are its skin and urinary bladder (67–71). Similar to mammals, the apical membrane is the major barrier to tissue water flow. The basal P_f is low (<10 μm/s), but greatly increases after vasopressin stimulation and could reach 250 μm/s (72–74). In the last 30 years, numerous published reports have used toad urinary bladders to study vasopressin-activated water transport. A comparison of water transport characteristics between toads and mammals is shown in Table II. In both tissues, vasopressin-induced water transport is mimicked by cAMP and inhibited by $HgCl_2$ and colchicine (75, 76). Isolated toad endocytotic vesicles that transport water contain at least 12 different proteins of unknown roles (77). The identification of a vasopressin-

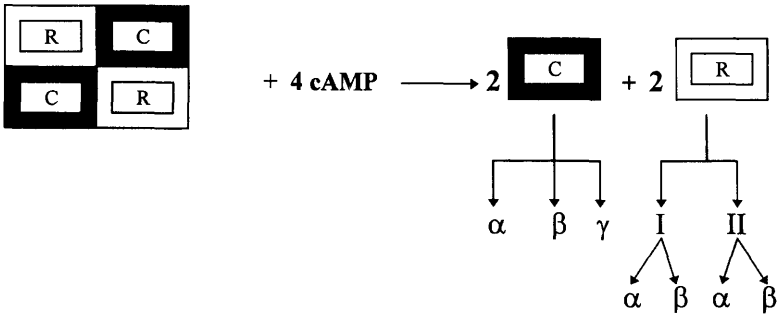

Figure 4. Activation and classification of cAMP-dependent protein kinase A isoforms. cAMP-dependent protein kinase A (PKA) consists of an inactive R_2C_2 tetramer (R: regulatory and C: catalytic) that is activated and dissociated by cAMP: R_2C_2 + 4cAMP → R_2cAMP_4 + 2C. There are at least three isoforms of the catalytic subunit (Cα, Cβ, and Cγ) and two isoforms of the regulatory subunit (RI and RII). While Cα and Cβ proteins are expressed in many tissues, Cγ isoform is restricted to the testis. In addition, each of the regulatory subunits is classified into α and β subtypes.

Table II. Comparison between Mammals and Toads

Characteristic	Mammals	Toads
Organs of osmoregulation	Kidney	Skin & bladder
AVP-AQP sensitive	AQP-2, AQP-6?	AQP-TB
Constitutive AQP	AQP-1, AQP-3, AQP-4, AQP-6?, AQP-7-like?	AQP-t1
Basal P_f	10–30 μm/s (80–81)	<10 μm/s
AVP P_f	170–230 μm/s	~250 μm/s
Known inhibitors of water transport	$HgCl_2$, colchicine	$HgCl_2$, wortmannin, ML9, HELSS, manoalide, α_2-agonists (82), somatostatin (83), colchicine, EHNA, mastoparan, compound 48/80
Phosphorylation	AQP-2	AQP-TB, 34-, 28-, 7-kDa proteins
Shuttling of water vesicles	Vesicles with AQP-2	Vesicles with AQP-TB

sensitive water channel in toad urinary bladders was first reported by Harris *et al.* (suggested to be the AQP-2 mammalian homolog) (78). More recently, Verkman *et al.* cloned the AQP-1-mammalian homolog from toad bladders and termed it as AQP-t1 (79). Interestingly, both channels have 78% homology.

Role of Post-Translational Modification of Mammalian AQP-2 in Water Transport

Glycosylation of AQP-2 is Not Necessary for Vasopressin-Activated Water Transport. Aquaporins undergo a number of post-translational modification including glycosylation (84) and phosphorylation (85). In fact, in a number of published studies, immunoblotting has detected at least two forms of each aquaporin isoform (85). The low molecular weight corresponded to the nonglycosylated form. Glycosylation has been shown to regulate protein sorting (86–87). In fact, tunicamycin, a potent inhibitor of glycosylation, impaired the expression of functional thrombin and platelet-activating factor receptors. The effect of tunicamycin (1.5 μg/ml) on vasopressin-induced water transport was investigated (88). MDCK cells stably transfected with mammalian AQP-2 were stimulated with vasopressin, and results have shown that inhibition of glycosylation has minimal effect on cAMP-activated water transport. Tunicamycin increased the levels of low M_rAQP-2 as detected by Western blotting (88), a response that was reproduced using endoglycosidase-F-treated plasma membranes. Furthermore, the elimination of the N-glycosylation site in AQP-1 had no effect on the trafficking or function in the *X. laevis* expression system (89).

Phosphorylation of AQP-2 is Required for its Translocation to Apical Membrane and Not Directly Activating. The presence of a PKA phosphorylation site at Ser^{256} on AQP-2 and the observed translocation of such a channel to the apical membrane in response to cAMP has suggested the involvement of phosphorylation in the translocation process (85). This was confirmed by Katsura *et al.* (85). H89, a potent inhibitor of PKA, inhibited the translocation of AQP-2 in LLC-PK_1 stably transfected cells. However, the transfection of LLC-PK_1 cells with a mutant AQP-2 in which the Ser^{256} was replaced with Ala, resulted in the loss of the translocation response to cAMP. By contrast, Lande *et al.* have shown that the phosphorylation of AQP-2 by PKA in isolated rat papillary water channel–containing vesicles did not alter the membrane water permeability (90). Therefore, it is suggested that the phosphorylation of AQP-2 may mediate the translocation of AQP-2 to the apical membrane rather than potentiating the channel function. In addition, Harris *et al.* also observed that vasopressin increased the phosphorylation of three proteins in toad urinary bladder cells; 14, 28-kDa integral proteins and another 34-kDa protein (91, 92). Nevertheless, the significance of such phosphorylation and the identity of the proteins are yet to be elucidated.

Involvement of Microtubules in Translocation of Water Channels

In nonrenal tissues, there are considerable data supporting a role for microtubule-associated proteins as vehicles to mobilize vesicles. The best characterized proteins are kinesin and dynein (93). The first evidence for the potential role of microtubules in water transport was based on the observed inhibition of vasopressin-activated water transport by colchicine and colcemid, microtubule disrupting agents (94–98). Lumicolcemid, an inactive analog of colcemid, had no effect on water transport. Furthermore, Taylor *et al.* have detected dynein in mammalian collecting duct by Western blotting (99). Moreover, the use of EHNA (erythro-9-[3-(2-hydroxynonyl)]adenine, a potent inhibitor of dynein, inhibited water transport in toad urinary bladders (100). EHNA inhibited the ATPase and motor activities of dynein as assayed *in vitro.* These observations suggested that the delivery of water channel–containing vesicles to the apical membrane is mediated in part by microtubule-associated proteins involving dynein.

Involvement of Microfilaments in Translocation of Water Channels

A role for the microfilament involvement in vasopressin-induced water transport has been made based on the depolymerization of F-actin to G-actin by cAMP and vaso-

pressin (101–104). Further support for such an involvement was also based on the inhibitory effects produced by cytochalasin B, a fungal metabolite that caps the growing ends of actin filaments, and thus causes the depolymerization of actin filaments that are actively turning over (43, 105–107). However, although such inhibition was observed by at least four different groups, recently, Hays *et al.* have shown that cytochalasin B has no effect on water transport (105). The reason for this contradiction is not known. However, recently we probed the role of myosin light chain kinase (MLCK) in vasopressin-activated water transport in toad urinary bladder. Wortmannin and ML9, potent inhibitors of MLCK, dose-dependently inhibited vasopressin-induced water transport (unpublished data). Therefore, a role for MLCK in vasopressin-activated water transport is suggested.

Involvement of G-Proteins in Water Channel-Vesicle Translocation

The first evidence for the involvement of G-proteins in vasopressin-induced water transport was reported by Yorio *et al.* who demonstrated that fluoride (an activator of G-proteins) inhibited vasopressin-induced transport in toad urinary bladders (108). More recently, G-proteins have been detected in AQP-2 vesicles. $G_{\alpha s}$, $G_{\alpha ii}$, and $G_{\alpha iii}$, but not $G_{\alpha 0}$, were colocalized with AQP-2 in the mammalian collecting duct (109). Pertussis toxin treatment attenuated vasopressin-induced translocation of AQP-2 to the apical membranes (110) suggesting a role for $G_{\alpha ii}/G_{\alpha iii}$ in water channel translocation. In addition, mastoparan, a tetradecapeptide toxin from wasp venom, and the synthetic polymer compound 48/80 (poly-p-methoxyphenylethylmethyl-amine), both potent direct activators of G-proteins (111), inhibited vasopressin-activated water transport in toad urinary bladders while at the same time enhancing vasopressin-induced endocytosis. Furthermore, the presence of synaptobrevin (VAMP-2: vesicle-associated membrane GTP-binding protein that mediates synaptic vesicle exocytosis in the brain), has been detected in rat AQP-2-containing endosomes (112). A similar observation has been demonstrated by Nielsen *et al.* using double-labeling immunoelectron microscopy and immuno-isolation of vesicles (113). These observations strongly suggest a role for both large and small G-proteins in water channel trafficking, translocation, and endocytosis.

Vasopressin-Induced Water Transport in Toad Urinary Bladders is Insensitive to Brefeldin A, an Endoplasmic Reticulum (ER) Inhibitor of Vesicular Transport

Brefeldin A, a known inhibitor of vesicular transport from the endoplasmic reticulum (ER) (114), was tested on vasopressin-induced water transport and short-circuit current (I_{sc}). The I_{sc} in response to vasopressin was not affected by brefeldin A, but subsequent challenges detected inhibition of I_{sc}. By contrast, brefeldin A had no effect on vasopressin-activated water transport, suggesting that endoplasmic reticulum vesicular transport is not directly involved in the short-term effects of vasopressin on water transport (114).

Vasopressin-Induced Water Transport (Short-Term) in Toad Urinary Bladders is Likely Insensitive to Cycloheximide, a Protein Synthesis Inhibitor

Cycloheximide, a protein synthesis inhibitor (1 μg/ml, 30 min), has no effect on water transport at least under short-term incubations (114, 115). However, high concentrations (200 μg/ml, 30 min) of the drug have been shown to impair water transport (115). Such high concentrations of cycloheximide have nonspecific effects and are toxic to cells; therefore, protein synthesis effects may not be implicated. Hence, it is suggested that vasopressin-mediated short-term effects on water transport do not involve protein synthesis.

Role of PLA_2 in Water Transport

Vasopressin stimulation of water transport in amphibian urinary bladder has been shown to be accompanied by arachidonic acid release (59). Interestingly, this mechanism appears to exert a negative feedback inhibition on water transport as prostaglandins impair adenylyl cyclase activity. However, Schlondorff *et al.* suggested the involvement of a different mechanism for inhibition of water transport involving a site subsequent to the formation of cAMP (116). However, it is still unclear how such a mechanism regulates water transport. Recently, we observed that HELSS and manoalide (117, 118), two potent inhibitors of PLA_2, attenuated cAMP-induced water transport from toad urinary bladders (Figs. 5A, and 5B, respectively). Therefore, a PLA_2 may be involved in water transport through a non-prostaglandin/thromboxane pathway. Although it is suggested that vasopressin-induced increases in arachidonic acid are mediated *via* a V_1-receptor (119), forskolin stimulation of rat renal mesangial cells and rat cortical collecting duct cells resulted in the release of arachidonic acid exogenously (120). Furthermore, vasopressin induces arachidonic acid formation in a number of renal tissues including: rat cortical, outer medullary collecting tubule, and thick ascending limb (121). Therefore, increases in arachidonic acid may also result from the activation of vasopressin V_2 receptor.

Are Caveolae Involved in Water Transport?

The recent discovery of a new endocytotic pathway or potocytosis mediated by specialized structures known as caveolae (122), has led us to test the potential role of caveolae in water transport in toad urinary bladders. Caveolae are abundant cell structures present along the basal-lateral membranes and the apical membrane surface in toad urinary bladder (Figs. 6A and 6B, respectively). The major component of caveolae is a 20-kDa cholesterol-binding protein

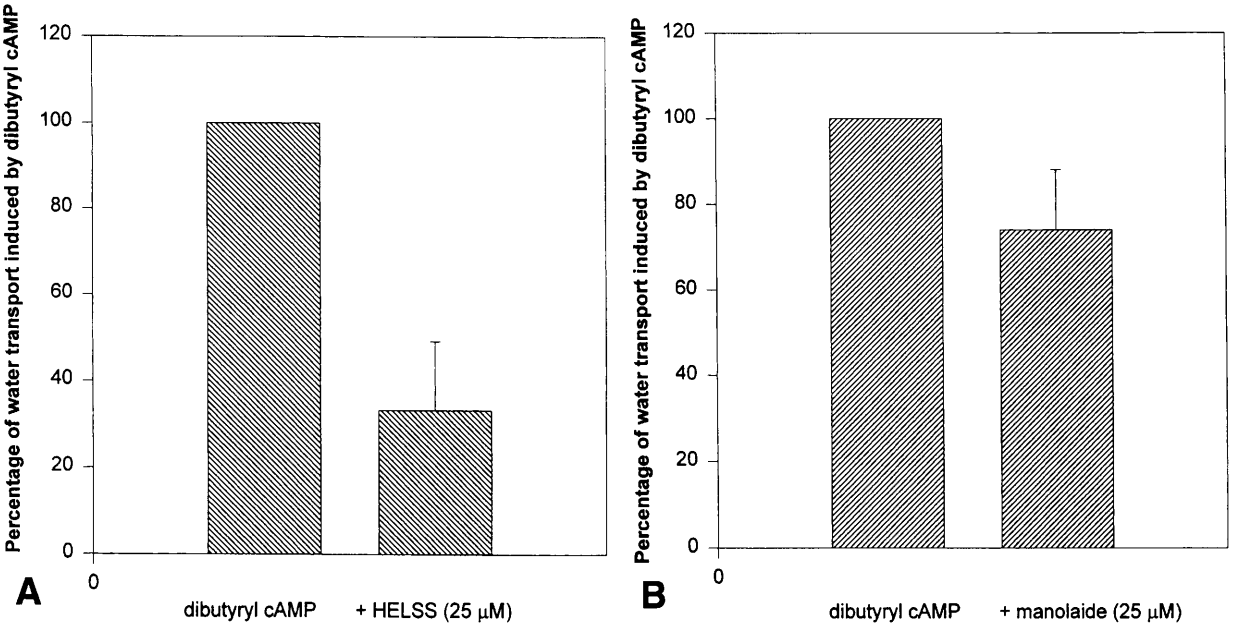

Figure 5A. HELSS (10–25 μ*M*) inhibited dibutyryl cAMP-induced water transport of toad urinary bladders. Isolated toad urinary bladders were set as sacs and HELSS (10–25 μ*M*) was added to the serosal solution 30 min prior to dibutyryl cAMP addition. HELSS had no effect on basal water transport (data not shown). After, the addition of dibutyryl cAMP (0.5 m*M*) to the serosal solution water transport was measured after 30 min and was found to be inhibited by 72% (n = 4).

Figure 5B. Manolaide (25 μ*M*) inhibited dibutyryl cAMP-induced water transport of toad urinary bladders. Isolated toad urinary bladders were set as sacs and manoalide (25 μ*M*) was added to the serosal solution 30 min prior to dibutyryl cAMP addition. Manoalide had no effect on basal water transport (data not shown). After, the addition of dibutyryl cAMP (0.5 m*M*) to the serosal solution, water transport was measured after 30 min and was found to be inhibited by 25% (n = 4).

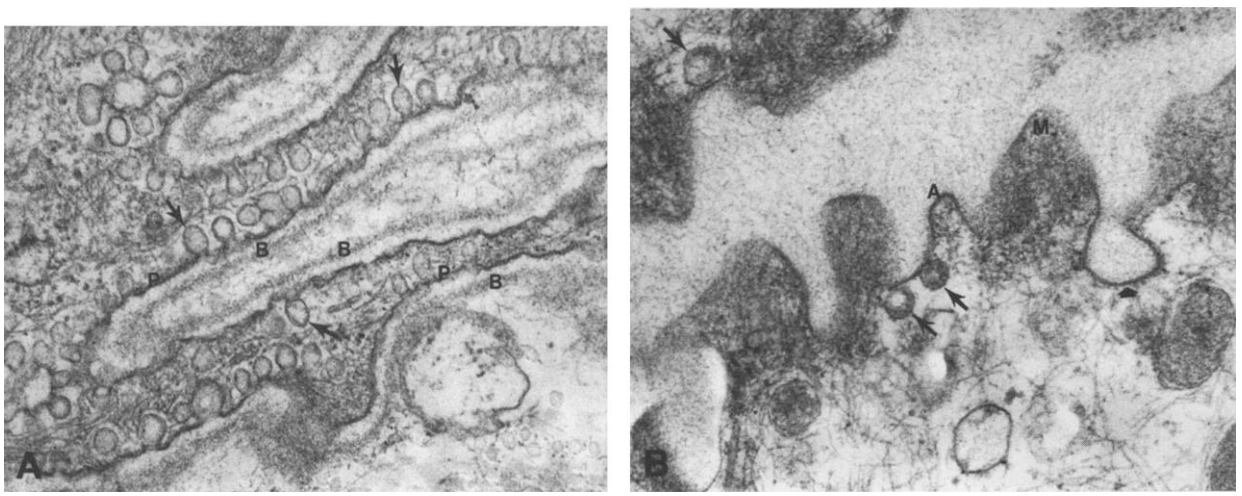

Figure 6A. Caveolae is present at the basal plasma membranes in toad urinary bladder granular cells. Ultrathin section of epon-embedded toad urinary bladder through several granular cells showing sac-like caveolae (arrows) along the basal plasma membranes (P) inner to basement membrane (B). 71,000X.

Figure 6B. Caveolae are present at the apical plasma membranes in toad urinary bladder granular cells. Ultrathin section of epon-embedded toad urinary bladder through two granular cells showing sac-like caveolae (arrows) in positions to fuse with the apical plasma membrane (A) along the microville (M). The short arrow indicates the presence of clathrin-coated pits. 71,000X.

termed caveolin (122). Stetson *et al.* have localized cholesterol in toad urinary bladders using filpin antibiotic and have found no consistent redistribution of cholesterol after vasopressin-induced water transport (123). Therefore, it is possible to suggest that no consistent redistribution of a caveolin-like protein after cAMP production was observed. However, the role of caveolae in vasopressin-mediated water transport needs further investigation.

A Model of Water Transport in Kidney Collecting Duct and Toad Urinary Bladders

In response to an increase of serum osmolarity or hypovolaemia, vasopressin (AVP) is secreted from the neurohypophysis. This hormone binds to the vasopressin type 2 receptor in basolateral membranes (Fig. 7). *Via* stimulatory G-proteins (G_s), adenylyl cyclase is activated, resulting in

the generation of cyclic AMP (cAMP). cAMP-dependent protein kinase (PKA) activation results in the depolymerization of F-actin to G-actin (101–106), a mechanism that may be mediated by phosphorylating myosin light chain kinase resulting in its inhibition (124). Also, PKA phosphorylates AQP-2 or AQP-TB, and this somehow induces the translocation of water channels–containing vesicles (85). The vehicle used to drive these vesicles is a protein known as dynein and is an ATP-dependent process (99–100). Water vesicles are associated with a number of GTP-binding proteins that appear important for vesicle fusion and trafficking at the apical membrane (125). Fusion at the plasma membrane is likely involving a PLA_2 enzyme. The exact sequence of events between cAMP production and the fusion of water channels at the luminal membrane is largely unknown. However, Harris *et al.* (125) have shown that vasopressin induced the insertion of at least 3 proteins of 55, 14–17, and 7 kDa at the apical membrane as detected by ^{125}I surface labeling. However, whereas one report has detected changes in the iodination patterns (126), another study failed to detect any changes in the apical membrane insertion using similar methods (127). The luminal membrane, which is nearly water tight in the absence of AVP, is rendered water permeable. Since the basolateral membrane is constitutively water permeable (mediated by mammalian AQP-3 and AQP-4 or AQP-t1 in toad bladders), and the renal medulla or serosal side in toad bladders is kept hypertonic, reabsorption of water will result. Continuous reabsorption is maintained *via* a mechanism involving shuttling of endosomes containing water channels (128). Based on freeze-fracture studies in the toad urinary bladder, Wade *et al.* observed that vasopressin-induced increase of water flow across these epithelia was associated with the appearance of intramembranous particle clusters (129). These particles were found to originate from subapical cytoplasmic vesicles, termed aggrephores (130). Subsequently, similar observations were made in apical membranes of the mammalian collecting duct (131). More recently, this shuttling mechanism was observed in HCD cells, MDCK cells, and LLC-PK_1 cells, stably transfected with AQP-2 (132–135). Finally, the feedback inhibition of vasopressin actions appears to involve V_1 or V_2 receptors localized at the apical membrane (39). This suggestion, which may be true in toads and rats, remains to be shown in humans due to the potential

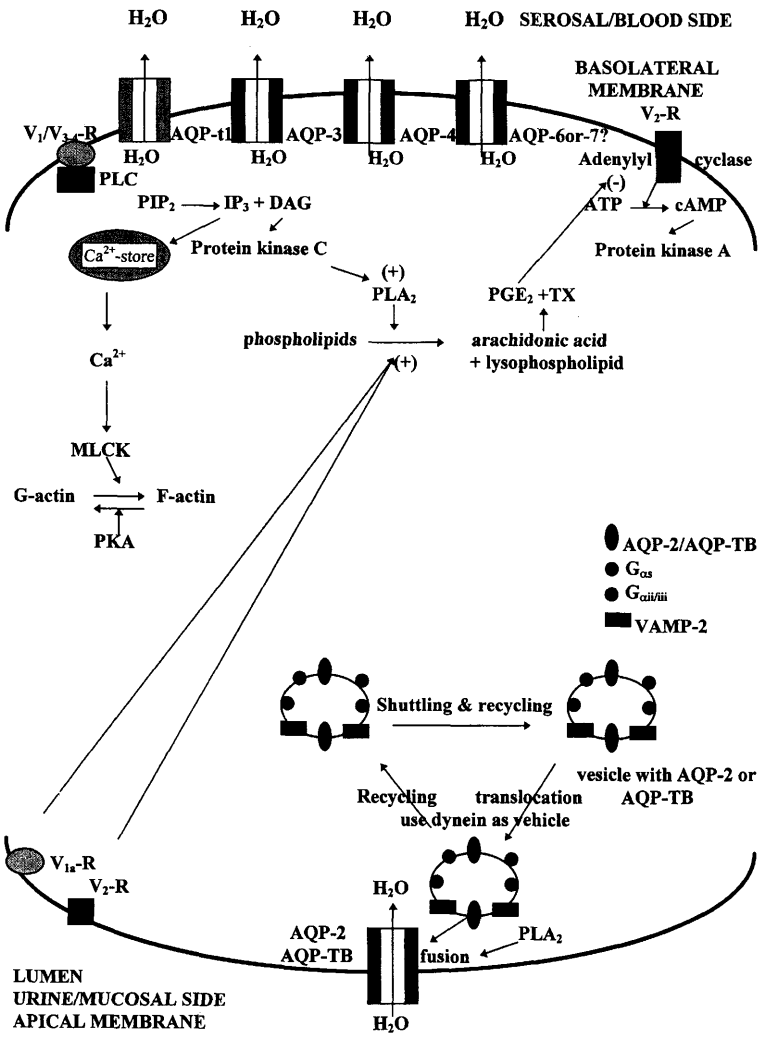

Figure 7. A working model for the signal transduction pathway of vasopressin-stimulated water transport in mammalian collecting duct and toad urinary bladder epithelia. Vasopressin activation of V_2 receptor (coupled to adenylyl cyclase) results in the generation of cAMP and stimulation of PKA. The exact steps between cAMP generation and the shuttling of water channels to the apical site are not known but appear to involve myosin light chain kinase, dynein, GTP-binding proteins, PLA_2, and PKC proteins. Mammalian AQP-2 or toad bladder AQP-TB channels undergo shuttling to the apical (urine side), whereas at the basolateral side, AQP-3 and AQP-4 (mammal) or AQP-t1 (toad bladders) are constitutively active allowing the exit of water to the serosal or blood side. The roles of mammalian AQP-6 or AQP-7-like are still questionable. Note that the presence of V_1 and V_2 receptors at the apical membranes may exert negative feedback inhibition of the basolateral V_2 receptor.

lack of V_1 and V_3 receptors in the kidney (27, 32, respectively). The role of kidney AQP-6 and possibly AQP-7-like in water transport is not known yet.

Recycling or Shuttling of Water Channels

At least three different groups were successful to develop a cell line that is stably transfected with AQP-2. Valenti *et al.* have developed HCD cells (Human cortical collecting duct cell-line) (132), Deen *et al.* have developed MDCK cells (133), and Katsura *et al.* have developed LLC-PK_1 cells, stably expressing AQP-2 (134, 135). In all three transfected kidney cells, AQP-2 was translocated to plasma membranes in response to cAMP stimulation. Whereas MDCK and HCD cells showed translocation of AQP-2 to the apical membranes, surprisingly, LLC-PK_1 cells showed a translocation to the basolateral membranes. The reason for behavior is not known. However, these culture models are extremely valuable to further study the cellular mechanisms mediating translocation. For instance, Brown *et al.* have transfected LLC-PK_1 cells with AQP-2 tagged with a green fluorescent protein (GFP) and have shown that when GFP was fused to the N-terminus of AQP-2, translocation of AQP-2 was observed in response to cAMP (135). However, when GFP was fused to the carboxy terminus, no translocation of AQP-2 was detected. Therefore, it was suggested that the signal for the translocation of AQP-2 lies in the carboxy terminus, and the disruption of the sequence will impair its translocation. Additional evidence supporting this observation was recently shown by Toriano *et al.* who have transfected LLC-PK_1 cells with a chimeric AQP-1 containing the carboxy terminus of AQP-2 (136). The AQP1-AQP2 chimeric protein showed translocation in response to cAMP or forskolin (136). Therefore, the carboxy terminus of AQP-2 contains the cAMP-sensitive domain. Using this chimeric models, interactions with membrane proteins involved in the translocation process and membrane fusion could be elucidated.

Regulation of AQP-2 Expression by Vasopressin

The detection of a cAMP-responsive element in the AQP-2 gene has suggested a potential role for cAMP in regulating the expression of aquaporin-2 (137). Yasui *et al.* have transiently transfected LLC-PK_1 cells with a fragment of the human AQP-2 promoter (nucleotide −547 to +5)-CAT reporter fusion gene (138). Vasopressin activated the promoter transcription, a mechanism that was mediated in part by the phosphorylation of the cAMP-responsive element protein (CREB protein), providing direct evidence for the regulation of gene transcription of AQP-2 by cAMP. Dehydration increased AQP-2 levels in rats (23), toad AQP-TB in toad urinary bladders (71), and FA-CHIP28 (mammalian AQP-1 homolog) in frogs (139). Dehydration also increased AQP-3 mRNA levels in rats (23). A similar effect was observed in rats using a V_2 agonist (138). Verbavatz *et al.* have shown a marked increase in water transport activities in the skin and bladder of salt-acclimated frogs or toads, and this was accompanied by an increase in the bladder surface (140). Interestingly, Brattleboro rats (deficient in vasopressin synthesis), treated with vasopressin for 5 days showed a 3-fold increase in AQP-2 as detected by Western blotting and immunocytochemistry (141). By contrast, treatment of rats and DI +/+ Severe mice (have a genetic defect resulting in high phosphodiesterase activity) with a V_2 antagonist OPC31260 (OPC) for 60 hr resulted in a great reduction of the AQP-2 levels in both mice and rats as detected by Western blotting and immunocytochemistry (142). In addition, Lithium treatment for 10 days or 25 days reduced the expression of AQP-2 to 31% or 4%, respectively (143). This is consistent with an older study showing that Lithium decreased vasopressin receptor numbers in rat renal medulla membranes (144). Thus, modulation of the AQP-2 gene expression is another mechanism related to water homeostasis in the kidney. A summary of factors affecting the gene expression of different aquaporins is shown in Table III.

Mammalian Aquaporins

Mammalian aquaporins are a subfamily of the MIP family (membrane integral proteins) (145). The MIP family can be classified into at least four subgroups: 1) animal MIP (contains mammalian aquaporins) and BIP protein (big brain in Drosophila, responsible for neurogenesis); 2) plant (contains NOD protein (allows communication between host plants and symbiotic nitrogen-fixing bacteria), TIP protein (tonoplast integral proteins, responsible for water transport during seed and root development), and W-TIP protein (water stress–induced resistance to desiccation in plants); 3) yeast (contains FPS1 (may transport fermentable sugars into yeast); and 4) bacteria (contains GlpF (glycerol transporter) and AQP-Z (bacterial aquaporin) (146). There are many common features of these aquaporin-like proteins and others that distinguish each member of the group. However, the similarities and differences are beyond the scope of this review. There are at least nine mammalian aquaporins that have been described (AQP0–AQP8), and the role of each aquaporin is discussed below. The human aquaporin genes are present on four different chromosomes as a single-copy

Table III. Regulation of Aquaporin-Gene Expression

Factors affecting AQP expression	Known effects	Aquaporins not affected
Water deprivation	Increased AQP-2, AQP-3, AQP-TB, AQP-6 expression	AQP-1, AQP-4
Salt acclimation	Increased AQP-2, FA-CHIP28 expression	—
Vasopressin, V_2 agonist, cAMP	Increased AQP-2, AQP-3, AQP-6	—
V_2 antagonist (OPC31260)	Decreased AQP-2 expression	—
Lithium	Decreased AQP-2 expression	—

gene: AQP-1 at 7p14 (147); AQP-3 at 9p12 (148); AQP-4 at 18q22 (149); AQP-0 (150), AQP-2 (150), AQP-5 (151), and AQP-6 at 12q13 (13). However, the chromosomal location of AQP-7 and AQP-8 is yet to be determined.

Aquaporin-0 or MIP26. The first aquaporin to be isolated and cloned was from bovine lens and termed MIP26 (membrane integral protein) in 1984 by Gorin *et al.* (7). AQP-0 is a gap junction protein (connexin) in the lens fiber of human, cow, and rat and plays an important role in the transport of water and other small molecules (cAMP, IP_3, calcium) between cells. Such a function helps synchronize cells actions (8). AQP-0 is also permeable to sucrose, ascorbate, and polyethylene glycol (8). The phosphorylation of AQP-0 has been shown to convert the voltage-independent Na^+ channel of AQP-0 into a voltage-dependent channel (152). AQP-0 constitutes over 60% of the membrane protein of lens fiber cells, and it is suggested to play a role in maintaining fluid balance in the lens (153). Deficiencies in MIP proteins have also been associated with cataract and presbyopia (154–155).

Aquaporin-1 or CHIP28 (Channel Forming Integral Protein). The first selective water channel from red blood cells identified by Agre *et al.* (6) was termed CHIP28. This 28-kDa protein is now known as aquaporin-1 (AQP-1). AQP-1 is poorly stained with coomassie blue, typical of plasma membrane proteins, and insoluble in Triton X-100 (6). AQP-1 exists as a homotetramer and is found in the proximal tubules and thin descending Henle's loop where it functions to reabsorb 80%–90% of water in kidney (18). However, AQP-1 is absent from the thick descending and ascending loop, which explains the lack of water transport in these nephron segments and the collecting duct. AQP-1 is localized at both the apical and basolateral membranes and is a constitutively active water channel (not regulated by vasopressin or other hormones) (19–21). The channel is inhibited by $HgCl_2$, and it appears that cystein 73 and 189 are the targets of covalent modification by $HgCl_2$ (156, 157). While several studies suggested the selectivity of AQP-1 for water (158–163), at least two groups have reported otherwise. Abrami *et al.* (164) have reported glycerol permeability whereas Yoon *et al.* have reported ion-conductance characteristics (165). Although AQP-1 functions to reabsorb water in kidney, it may serve different important functions in other tissues. AQP-1 may be involved in the transport of aqueous humor in the eye (15) and cerebrospinal fluid in the brain (166–168). In addition, AQP-1 has been implicated in regulating interstitial fluids, airway hydration, and clearance of alveolar liquid in the lung (169).

Aquaporin-2 or AQP-CD (aquaporin collecting duct). Fushimi *et al.* cloned a novel aquaporin from rat kidney medulla; this clone has a 42% homology to AQP-1 and was termed aquaporin-2 (10). AQP-2 is absolutely restricted to kidney collecting duct and absent from proximal tubules or Henle's loop (170, 171). AQP-2 is localized predominantly to the apical membrane and to a lesser extent to subapical vesicles. Although the exact translocation mechanism induced by vasopressin is not well elucidated, AQP-2 vesicles contain GTP-binding proteins that may participate in vesicle translocation (109–113). HCD cells and MDCK cells transfected with AQP-2 showed a consistent translocation to the apical membrane after vasopressin stimulation (132–133). A similar translocation of AQP-2 was observed in LLC-PK, cells but to the basolateral membrane (134–135). Dehydration of rats increased AQP-2 levels by 5-fold suggesting the regulation of the AQP-2 gene by vasopressin (23). This was further confirmed by Yausi *et al.* who have shown the presence of a cAMP-responsive element in the 5′ flanking region of the AQP-2 gene (129). The AQP-2 channel was detected in normal urine but greatly reduced in urine of patients with nephrogenic diabetes insipidus (172). In addition, lithium used for treatment of manic depression decreased AQP-2 mRNA (143), which appeared the result of downregulation of the kidney V_2 receptors (144). To date, AQP-2 is the only water channel that is regulated by hormone action and hydration state.

Aquaporin-3. Aquaporin-3 was cloned by three groups and appears to be permeable to water, glycerol, and urea (173–175). Upon dehydration of rats, mRNA of AQP-3 increased 2-fold. Whereas water transport was increased 5-fold, urea transport was not affected, suggesting that water transport is likely the key function of AQP-3 (23). AQP-3 has been localized to the basolateral membranes of the collecting duct, and unlike AQP-2 is absent from intracellular vesicles (174–175). Whereas Ishibashi *et al.* have detected AQP-3 in spleen, stomach, small intestine, colon, kidney cortex, and kidney medulla (174), Echevarria *et al.* have shown that AQP-3 is restricted only to kidney (175). The difference between these reports is not yet known. AQP-3 is also $HgCl_2$ sensitive. In addition, AQP-3 was also detected in the cornea and may help in the removal of small metabolites, such as glycerol phosphates, to maintain corneal transparency (15). AQP-3 is also present in the trachea, but the exact role for the channel is not yet known (176).

Aquaporin-4. Unlike AQP-1, AQP-2, and AQP-3, AQP-4 is $HgCl_2$-insensitive (177). AQP-4 was first cloned from rat brain by Jung *et al.* (178) and may play an important role in the formation of cerebrospinal fluid in the brain. Northern blot analysis has detected AQP-4 in the eye, kidney, intestine, and lung. AQP-4 was localized at the basolateral membranes in the kidney medullary collecting duct but was absent from intracellular vesicles, descending Henle's loop, and proximal tubules (23, 179). Similar to AQP-2, AQP-4 has a potential PKA phosphorylation at the B-loop (178). However, no physiological significance of such phosphorylation has yet been observed. AQP-4 was also detected in ciliary epithelium in the eye suggesting a role in the formation of aqueous humor (15). The detection of AQP-4 in the retina has been reported, but its function is not yet clear.

Aquaporin-5. AQP-5 was first cloned from salivary glands by Raina *et al.* (180). AQP-5 also has a PKA phos-

phorylation site and was detected in submandibular, sublingual, parotid, and lacrimal glands, as well as trachea, eye (cornea), and lung, by Northern blot analysis. Although AQP-5 is absent from kidney, it is also inhibited by $HgCl_2$. The presence of AQP-5 in secretory organs may suggest a role in fluid secretion in exocrine glands (177, 181).

Aquaporin-6. A novel cDNA that is homologous to other aquaporins was isolated from human kidney and first named hKID (12) or WCH3 (182). This channel is now known as aquaporin-6 and is similar to AQP-2 (52% homologous). It is absolutely restricted to kidney and absent from other tissues (12). This 282 amino-acid protein is sensitive to $HgCl_2$ and selective for water transport. The exact function of AQP-6 is not known, but its presence in the medulla and cortex may suggest a role for this channel in water reabsorption. Such a suggestion is further supported by the upregulation of AQP-6 in dehydrated rats, a response shared by both AQP-2 and AQP-3 channels. The localization of AQP-6 at the nephronal segments will help identify its physiological significance.

Aquaporin-7. A new channel cloned from rat testis (269 amino-acid protein) is now called AQP-7 (14). Not only is AQP-7 permeable to water, but also it is permeable to urea and glycerol, similar to AQP-3 (shares greatest homology with AQP-3 (48%)). In fact, both AQP-7 and AQP-3 induced a 5-fold increase in the uptake of glycerol when injected into oocytes (14, 174). However, the degree of urea uptake induced by AQP-7 injection was much greater than that induced by AQP-3 injection (14). Northern blot analysis has detected intense labeling of AQP-7 in the heart and kidney but with a different transcript size. Whether or not these are also specialized AQP-7 in the kidney and heart is yet to be determined. AQP-7 is similar to AQP-4 in its insensitivity to $HgCl_2$. AQP-7 has been localized to the inner surface of seminiferous tubules but is absent from interstitial tissues. AQP-7 is suggested to play a crucial role in the cryopreservation of sperm. However, further investigation is needed to characterize its functions.

Aquaporin-8. The latest aquaporin to be cloned was from rat pancreas and liver by Koyama *et al.* (183) and was named AQP-8. AQP-8 is a 263 amino-acid protein that is sensitive to $HgCl_2$ (similar to most aquaporins). The channel was detected in hepatocytes, acinal cells of pancreas, salivary glands, and colonic cells. AQP-8 is absolutely water-selective and not permeable to urea or glycerol. Localized to the glandular lobules but not to the islets of pancreas. AQP-8 may play a role in the large volume of water secreted by the pancreas (pancreatic juices). AQP-8 was also detected in parencymal cells and not in the portal area. The localization of AQP-8 in the salivary gland and absorptive epithelial cells in the colon suggests a role in secreting the saliva and water reabsorption (183). It appears that AQP-8

Table IV. A Comparison of Mammalian Aquaporins

Name	Other names	Transport	Tissues	HgCl inhibition	Phosphorylation	Function
AQP-0	MIP26 GAP proteins	H_2O, sucrose, PEG, KCl, ascorbate	Lens	+	PKA	Fluid balance
AQP-1	CHIP28	H_2O, glycerol?	Most tissues	+	PKC	Water reabsorption in kidney, aqueous humor formation in eye, CSP[a] formation in brain, airway hydration in lung
AQP-2	AQP-CD WCH-CD	H_2O, H^+	Only in kidney collecting tubule	+	PKA	Water reabsorption in kidney collecting duct
AQP-3	—	H_2O, urea, glycerol	Kidney, eye	+	casein Kinase II	Water transport in kidney collecting duct, cornea transparency
AQP-4	MICW	H_2O	Kidney, brain, lung, skin, heart, intestine	–	PKA	CSF formation in brain, water transport in kidney collecting duct, aqueous humor formation in eye, airway hydration in lung
AQP-5	—	H_2O	Salivary gland, lacrimal, submandibular sublingual, parotid, trachea, eye, lung	+	PKA	Fluid secretion in exocrine glands
AQP-6	hKID, AQP2L, WCH3	H_2O	Only kidney	+	PKC	Water transport in kidney?
AQP-7	—	H_2O, urea, glycerol	Testis, kidney?, heart?	–	PKC	Cryopreservation of sperm?
AQP-8	—	H_2O	Liver, pancreas, colon, salivary gland	+	?	Secretion of pancreatic juice & fluids

[a] Cerebrospinal fluid

will emerge as an important water channel for tissues involved in fluid secretion. A comparison between all nine aquaporins is shown in Table IV.

Concluding Remarks

The identification of nine aquaporins with similar as well as distinctive properties suggests that this class of proteins has a variety of functions, including the transport of water. Some appear to be ubiquitous, whereas others are tissue specific and hormone sensitive. Such water channel diversity may help explain the rather different permeability properties seen in transporting epithelia and the ability of some cells to respond to osmotic challenges.

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