A Brief Survey of Aquaporins and Their Implications for Renal Physiology

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Aquaporins (AQPs) are an important family of proteins that efficiently channel water through the cell membranes. Although water can diffuse across biological membranes at measurable rates, physiologists had long predicted the existence of channels to facilitate rapid reabsorption of water by renal tubular cells. With AQPs present, water can "gush" through the membrane at the extraordinary rate of three billion water molecules per second per aquaporin channel. In their absence, water only trickles across the hydrophobic lipid bilayers of cell membranes.

Aquaporins have fascinated researchers over the last decade, culminating in the 2003 Nobel Prize for Chemistry given to their discoverer, Dr. Peter Agre. During the 1990s, scientists identified and characterized members of the mammalian aquaporin family, now designated as AQP0 through AQP10. AQPs are also found in many plant and bacterial species. However, their relevance to the clinical laboratory is only recently emerging. Dr. Agre's Nobel symposium address provides an excellent mini-review of aquaporins in medicine.[1]

Our understanding of renal physiology and pathophysiology has advanced greatly as we account for the subtle implications of various AQP systems. For example, nephrogenic diabetes insipidus (NDI), the inability to produce concentrated urine, can result from several different malfunctions in the AQP2 system controlled by anti-diuretic hormone (ADH).

Virtually all mammalian cells incorporate aquaporins into their cell membranes, and many cells produce multiple aquaporins, each with a specific function. It is therefore not surprising that malfunctions have important clinical conditions. The present article discusses the implications of

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aquaporins for renal physiology, while the accompanying article is focused on the clinical aspects of aquaporins.

ABBREVIATIONS: ADH = anti-diuretic hormone; AQP = aquaporin; AMP = adenosine monophosphate; cAMP = cyclic AMP; CD = collecting duct; cDNA = complementary DNA; CHF = congestive heart failure; DCT = distal convoluted tube; NDI = nephrogenic diabetes insipidus; PCT = proximal convoluted tubule; RBC = red blood cells; RT-PCR = reverse transcription - polymerase chain reaction.

INDEX TERMS: aquaporins; diabetes insipidus; nephrogenic diabetes insipidus; renal physiology.

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HISTORICAL PERSPECTIVE

Diffusion is defined as the passive movement of molecules from a high concentration to an area of lower concentration, and osmosis specifically indicates the movement of water across a semipermeable membrane.[2] For decades, the mechanism of rapid osmosis in renal cell membranes had puzzled scientists, with predictions that water channels must be involved as early as the 1950s.[1,3] Predictions were based on calculated energy requirements for unaided osmosis versus osmosis through channels, as shown in Figure 1. In the 1970s, the fluid mosaic model of membranes provided insights into the membrane environment,[4] but also underscored the difficulty hydrophilic molecules would encounter when crossing the hydrophobic bilayer.[5] By the 1980s, detailed studies of many transport proteins had added depth to our understanding of membrane structure and organization. However, until

the early 1990s, the mechanism of rapid osmosis was still not adequately explained. Since their discovery in 1988,[1,6] the family of known aquaporins has grown to include AQP0-AQP10. More than 200 similar water channels are known to exist in microorganisms as well as plant and animal cells.[7-17] The scientific importance of aquaporins was further validated when Peter Agre, credited with their discovery, was awarded the 2003 Nobel Prize for Chemistry.[1]

In the decade following their discovery, scores of researchers have made remarkable progress toward defining the molecular mechanisms of the aquaporin family and demonstrating their association with a wide range of clinical conditions.[7-17] The accompanying article describes the clinical relevance of a wide range of diseases associated with dysfunction of various aquaporin systems.

Passive diffusion and osmosis
Water is small enough to allow passive diffusion (without energy expenditure) across cell membranes, driven solely by

Figure 1. A generalized representation of water diffusing across a membrane
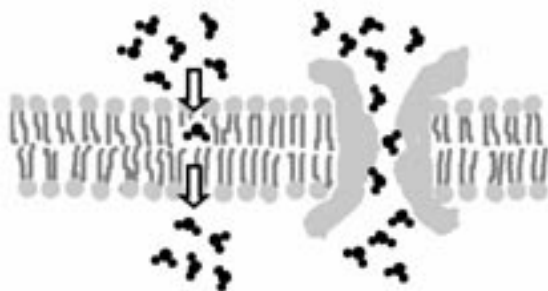
The below illustrates two possible modes of water diffusing across the membrane barrier. On the right, a water molecule is shown crossing the membrane by simple unaided diffusion. In order to accomplish this, the water molecule must temporarily break numerous hydrogen bonds that can form in solution, so that it can cross the hydrophobic membrane. Calculations show that the activation energy required for this unassisted diffusion is > 10 kcal/mole. On the left, water molecules are seen crossing through an aquaporin channel, with this passage requiring < 5 kcal/mole of activation energy. This reduction in activation energy enables water to cross the membrane more than 100 times faster when aquaporins are present.

a concentration gradient. A gradient, or osmotic pressure, results from unequal solute concentrations on the two sides of a membrane.[3] Whenever a gradient in solutes exists, water responds by diffusing toward the side with higher solute concentrations. Thus, water's "urge to dilute" reduces the osmotic pressure until the concentration gradient is eliminated.

Kidney reabsorption illustrates the concept that, when given the opportunity, osmosis can effectively eliminate concentration gradients (Figure 2). As the urine filtrate passes through the proximal convoluted tubule (PCT), two thirds of the electrolytes and greater than 99% of most metabolites are reabsorbed. Yet the osmolality remains virtually unchanged because water follows the reabsorbed ions and metabolites.[18] Thus, as shown in Figure 2, the filtrate volume is reduced approximately 70%, with minimal change in osmolality. For example, with a normal filtration rate of 100 mL/min, the PCT cells must reabsorb 70 mL/min. The transient solute gradients can drive unaided osmosis, but this model simply could not account for the speed of renal reabsorption.

Unaided osmosis left questions
The observed speed of water reabsorption in the PCT was one of several questions. Can water possibly diffuse through hydrophobic membranes fast enough to account for the PCT's reabsorption rates? Also, as the filtrate descends through the loop of Henle into the hyperosmotic medulla, water is reabsorbed, but what prevents a flow reversal as the filtrate turns and ascends toward the renal cortex? If water diffuses through descending loop membranes so freely, how do the membranes in the ascending loop prevent diffusion? Finally, how can the collecting duct (CD) cells vary their water permeability, depending on hormonal control?

The hormone exerting control in the CD has multiple names. Clinical practitioners most frequently use anti-diuretic hormone (ADH). However, most researchers refer to this hormone as vasopressin, or arginine-vasopressin (AVP). For the purposes of this article, we will use ADH.

Evidence suggested the existence of aquaporins
Although water diffuses at an appreciable rate through biological or artificial membranes,[1,4,5,7-17] calculations suggested that the rapid osmosis observed in the PCT would require a transporter molecule.[3] For example, Chandy and coworkers had estimated that an activation energy of >10 kcal/mol is required for unaided osmosis across a hydrophobic lipid bilayer.[5] Such an energy requirement would preclude the rapid transport seen in the renal tubules. The activation

energy for diffusion across red blood cell membranes was calculated to be <5 kcal/mol, or roughly the equivalent of water diffusion within a solution.[1,3] This suggested that water channels must lower the energy requirement for osmosis through the hydrophobic membrane, as shown in Figure 1. However, proving the existence of transporters was complicated by the background of unaided diffusion that exists even without aquaporins. For comparison, unaided osmosis occurs at a rate approximately 10^3 times faster than glycerol or urea and 10^5 times faster than glucose.[4] The observed reversible inhibition of osmosis by $HgCl_2$ further suggested involvement of a transport protein that requires an active cysteine residue. Also, increases in water permeability were shown to correspond with the appearance of membrane proteins, with no observed changes in lipid composition.[7,19]

TECHNIQUES AND METHODOLOGIES USED IN AQUAPORIN RESEARCH

Aquaporins discovered while investigating Rh antigens

Despite their postulation years earlier, the discovery of the first aquaporin actually occurred during a study of the Rh antigens of the red blood cell (RBC) membranes in Peter Agre's laboratory at Johns Hopkins.[1,6,17] Indeed, the article describing the original purification of CHIP28, an integral protein of 28 kDa molecular weight, postulated a role in the membrane skeleton,[6] but did not anticipate that it represented the elusive water channel. The late Dr. John C. Parker of North Carolina is credited with suggesting that CHIP28 was a water channel, based on its occurrence in renal tubule cells as well as RBCs.[1]

Demonstration of AQP1's role as a water channel

Karl Windhager proposed an invaluable strategy for confirming the aquaporin role through insertion of CHIP28 into *Xenopus laevis* oocytes.[1] The complementary DNA (cDNA) for CHIP28 was obtained,[20] and CHIP28 was incorporated into the oocyte membranes.[7] Native oocytes are normally unresponsive to osmotic changes; however, once their membranes contained CHIP28, a dramatic 100-fold increase in water permeability caused cells to swell rapidly and burst. Reversible inhibition by $HgCl_2$ helped confirm that CHIP28 was indeed a water channel and the source of the new osmotic responsiveness.[7] Similarly, embryonic rat cells lack osmotic responsiveness until they begin inserting aquaporins after birth.[19] Additional studies using synthetic liposomes and reconstitution

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Figure 2. A "stretched-out" nephron is shown indicating water reabsorption and osmolality in various portions

As the filtrate moves through the PCT, approximately 70% of Na+ and water and 99% of metabolites are reabsorbed. As the remaining filtrate descends into the hyperosmolar medulla, more water is removed without Na+ reabsorption. In both of these segments AQP1 is primarily responsible for water reabsorption. As the filtrate ascends toward the renal cortex, NaCl is reabsorbed without water reabsorption, because the ascending loop cells lack AQPs. In the DCT and DC, reabsorption of Na+ and water are under hormonal control. Sodium reabsorption is controlled in the DCT by the Renin-Angiotensin-Aldosterone system, while water is controlled by ADH in the CD. ADH signals for AQP2 insertion into CD cell membranes and facilitation of water reabsorption as the filtrate exits through the hyperosmolar medulla.

"Stretched-out" nephron
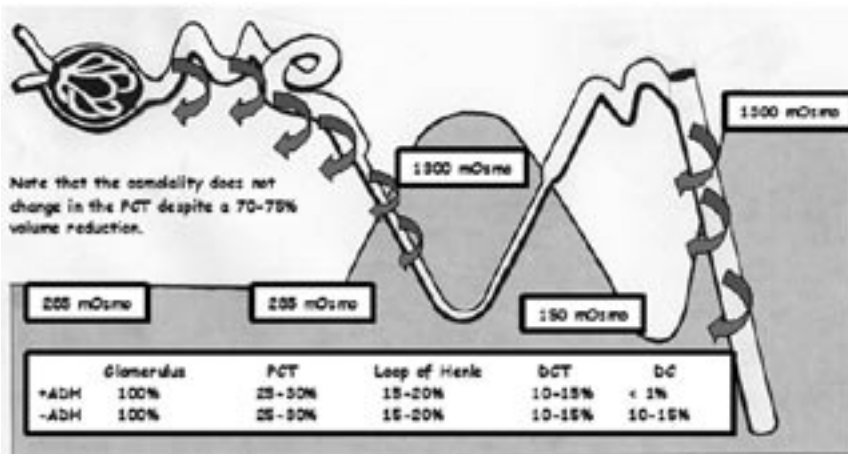
The gray area represents the filtrate osmolality in each segment and arrows represent water reabsorption. The chart at the bottom indicates the percentage of filtrate remaining at the end of each portion, with and without ADH present.

of CHIP28 from RBCs demonstrated that insertion of the protein led specifically to increased water permeability.[21,22] Once CHIP28 was confirmed as a water channel, and several similar channels had been described, the aquaporin terminology was adopted, and CHIP28 was renamed AQP1.

Sequence homologies helped identify new aquaporins

Once their existence and functional role were demonstrated, researchers surveyed various cells for related aquaporin genes.[1,8-16] Probes of cDNA from known aquaporins were used to search for similar genes with sequence homologies.[16] The aquaporin family of proteins contains a pair of unique and highly conserved sequences in the gene, which helped researchers construct valuable cDNA probes.[16] Using this strategy to survey the human genome, the aquaporin family grew rapidly and now includes AQPs 0-10.[12,17]

Molecular techniques used in aquaporin research

Since the isolation of cDNA to CHIP28,[20] cloning of each of the AQP genes has been undertaken using a multitude of molecular techniques. These techniques include: cloning the DNA into a variety of vectors, PCR, reverse transcription-PCR (RT-PCR), electrophoresis followed by all three common forms of blots (Southern, Northern, Western), immunohistochemical analysis, *in vitro* transcription and translation, differential centrifugations, enzyme immunoassay, and many other methods.[23,24] A concise summary of these techniques is available,[4] and a more detailed compilation of these techniques is also available.[25]

Assessment of gene expression

Since all somatic cells contain each aquaporin gene, the expression of a specific aquaporin in a particular tissue helps to determine its functional role. Gene expression or the presence of messenger RNA is demonstrated through the use of RT-PCR.[16,26-28] The appearance of the protein products is then confirmed using immunochemical methods and is related to AQP function. Once gene expression is demonstrated in a tissue, its developmental timing and the impact of physiological conditions can yield insights into its functional role.[26-28] Such studies have implicated the increased expression of AQP2 in the pathogenesis of several conditions of volume overload, including congestive heart failure (CHF), pulmonary edema, and liver cirrhosis.[11,12] In addition to ADH effects, conditions that alter AQP2 expression include: lithium treatments, hypokalemia, hypercalcemia, chronic renal failure, ischemic renal failure, cirrhosis, mephitic syndrome, and seemingly unrelated conditions such as a low protein diet or exposure to high altitudes.[11,12]

Physiologic conditions such as exercise, fasting, or starvation are known to alter the expression of AQP7 and AQP9.[27,28] Correlating the changes in AQP expression in response to physiological or pharmacological conditions often suggests the AQP functional role.[1,8-16] Altered AQP expression following drug treatments also helps differentiate between strategies likely to have beneficial or detrimental results. For example, lithium treatment causes a downregulation of AQP2 expression in CD cells,[26] and explains why many patients on lithium develop transient NDI. Such knowledge may suggest corrective measures for future treatments.

Patient with AQP deficiencies and "knockout mice"

Much valuable information can be gained from observing the pathophysiologic results when either patients or animal models lack functional AQPs.[29-32] Ma and coworkers have provided a good summary of their protocol for inserting defective genes to produce "knockout mice".[29] Patients with an inability to make AQP1 have been extensively studied.[31] Given the importance of AQP1 in the PCT reabsorption of water, it was initially surprising that these patients had only mildly defective urine concentrating ability.[31] However, further research has suggested that other AQPs are present in the PCT. More dramatic forms of NDI have been seen in patients with deficiencies in AQP2 or the ADH-receptors.[30,34] These two conditions are more thoroughly described in the accompanying article.

The effect of a defective AQP4 gene was observed in "knockout mice" to gain information about the functional role of the gene product.[29] AQP4 is expressed in the CD cells, and facilitates water's exit from the basolateral membranes after AQP2 has facilitated uptake into the cells. These mice had a four-fold decrease in ADH-stimulated reabsorption.[29] However, AQP4 is most strongly expressed in the brain, and a major role in osmoregulation had been proposed. In this study, the AQP4-deficient mice demonstrated no gross neuromuscular abnormalities or obvious problems with osmoregulation.[29]

Immunochemical methods establish cellular localizations

The cellular or subcellular location of aquaporins also leads to understanding their physiological role. Most often, immunoassays use microscopic visualization of fluorescent labeled anti-AQP antibodies in tissues or specific membranes.[24,27,30,32,33] Figure 3 shows the specific locations of various AQPs in renal cells that were established using microscopy and immunoassays. Traditional cellular fractionations based on centrifugation have been useful to quantify which cell fractions contain the AQP.

Complex questions require multiple techniques

As research questions become more sophisticated, many answers require a combination of multiple techniques to detect gene expression, cellular locations, and regulation of gene expression. In hormonally regulated systems, a pathologic condition may result from complications with several factors of the multi-step process. For example, NDI can result from AQP2 gene mutations, malfunction of ADH receptors, changes in gene expression, or trafficking malfunctions (the process of folding and transporting of newly synthesized AQP to the cell membrane).[30] Sorting through these complex physiological systems requires a combination of the above techniques to separate individual parts of the system.

AQUAPORINS SOLVE MANY BIOCHEMICAL PUZZLES

As with any quantum leap in science, the discovery of aquaporins not only offers simplified explanations of basic phenomena, such as cellular swelling in hypoosmotic environments, but it also helps explain more complex phenomena. Such phenomena include the hormonally regulated reabsorption in the renal CD, stimulation of transient secretions in salivary and lacrimal glands, the pathophysiology of NDI, development of various forms of edema, and possibly the regulation of plasma osmolality.[1,7-17]

Water and the central puzzle

Prior to the discovery of aquaporins, scientists were perplexed by how easily water permeated the hydrophobic barrier of a lipid bilayer.[1,10,12] Why should water, the very molecule used to define our concept of hydrophobicity, be the primary exception to the rule that hydrophilic molecules can not cross hydrophobic membranes without help?

Many cells have modest osmotic needs. If not involved with reabsorption or secretion, they can rely on unaided osmosis. However, even steep osmotic gradients are simply insufficient to drive water over the large energy hill required for unaided osmosis with the reabsorption rates observed in the PCT (Figure 1). Thus, renal physiologists were left to struggle with several complex puzzles. If membranes of the PCT and descending loop of Henle reabsorb 80% of the urine filtrate, at "gushing" rates of 80 mL to 120 mL per minute, why are neighboring ascending loop membranes almost impermeable to water? On the molecular level, how can one membrane allow rapid osmosis, while others block this seemingly inevitable flow? And finally, what on/off switch enables CD cells to regulate osmosis based on the presence or absence of ADH?


Figure 3. Distribution of aquaporins (AQP1, AQP2, and AQP3) in membranes of renal tubules

Note that AQP1 is present in large quantities in both the apical and basal membranes of the cells of the proximal tubules and descending loop of Henle (panel 3A). Similarly, AQP3 is present in the basal and lateral membranes of the principle cells in the collecting duct (panel 3B). Both AQP1 and AQP3 channels are always present in these membranes. However, AQP2 channels are only inserted into the apical membrane of the collecting duct cells following a signal from ADH.

3A
Proximal tubules, descending thin limbs
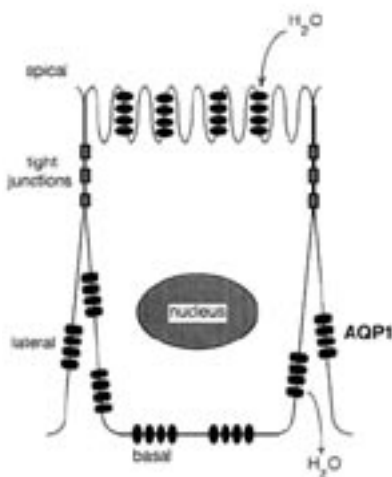
3B
Collecting duct, principal cells
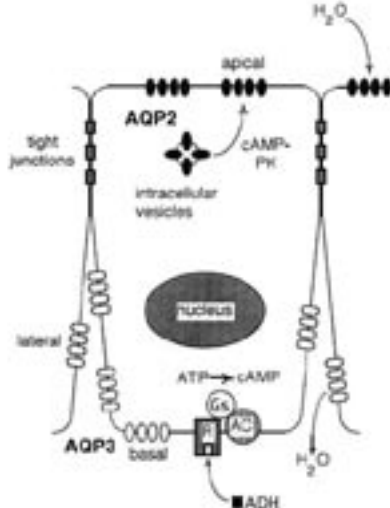
Reprinted from King and others. Mol. Med. Today 2000;(6):60-5

Renal physiology revisited, accounting for aquaporins

The original renal puzzle was unanswerable as previously stated: If most membranes freely allow osmosis, then what prevents osmosis through ascending loop cell membranes? The answer became obvious with the realization that membranes normally restrict osmosis, unless aquaporins are present.

Renal reabsorption of water and NaCl is illustrated in Figure 2. Approximately 70% of both water and Na$^+$ are reabsorbed in the PCT, without changing the osmolality. As the filtrate descends into the medullary region in the descending loop of Henle, another ten percent of original filtrate volume is reabsorbed as the hyperosmolality of the medulla provides a steep concentration gradient and "sucks" water out. Such rapid reabsorption in the PCT and descending loop of Henle is facilitated by a high density of AQP1 and AQP3 (Figure 3A).[1,11,12,15] Permeability studies with AQP1 present demonstrate that water can "gush" at the phenomenal rate of 10^9 water molecules/second/channel.[21,23]

However, as the filtrate ascends toward the cortex, the filtrate is hyperosmolar compared to the decreasing solute concentrations during the ascent toward the renal cortex. Logically, water flow should reverse and re-enter the tubule. Why does flow reversal not happen? If AQP1-mediated osmosis explains the remarkable reabsorption rates in the PCT membranes, its absence also explains the impermeability of the ascending loop membranes. The membranes of ascending loop cells simply lack any AQPs, meaning that no magical bilayer reconstruction or lipid reformulations are required to reduce osmosis to a trickle.

Regulated transport systems

Two basic mechanisms of regulated passive transport have been described. First, gated systems use transporters that open and close, as commonly seen with ion transporters of the neuronal or neuromuscular systems. Second, sequestered transporters remain in intracellular vesicles until a specific signal promotes transport. Insulin-based regulation of glucose transport in muscle and fat cells illustrates the second mechanism.[2,4] In muscle or fat cells, an intracellular pool of glucose transporters is unavailable, except following the binding of insulin to its receptor.[2,4] Thus, glucose uptake by these cells only occurs after a meal and subsequent to the secretion of insulin.

The ADH-controlled reabsorption in the CD is analogous to this second mechanism, with a intracellular pool of pre-synthesized AQP2. In the absence of ADH, these AQP2 channels reside in intracellular vesicles and do not facilitate water reabsorption. When ADH is present, AQP2 channels are inserted into the cell's luminal surface, and water enters the CD cells (as illustrated in Figure 3B), driven by the concentration gradient in the hypertonic medulla. Other AQP channels, designated AQP3 and AQP4, are always present (constitutive) on the plasma side of the CD cells and facilitate the unregulated exodus of water from these cells (Figure 3B). Reabsorption occurs only when ADH binds its receptor, signals for AQP2 insertion, and water to be allows water into the cell. Finally, AQP3 and AQP4 allow water to be drawn out of the opposite cell membrane.[1,8-17] Figure 2 illustrates the difference in osmolality and volume of urine produced with and without ADH present. Thus, both diabetes mellitus and diabetes insipidus relate to a malfunction of two analogous transport systems.

Summary of renal reabsorption

Note that knowledge of the existence of AQPs does not change the basic observations of renal reabsorption as described in introductory classes.[18,35] However, the molecular-level explanations of the phenomena are infinitely more satisfying. Furthermore, diagnostic assessment and therapies will no doubt be altered by our newer, more sophisticated model of renal physiology. Examples of the pathophysiology of several diseases and clinical applications are described in the accompanying article.

Molecular mechanism of aquaporins

The primary questions concerning the aquaporin mechanism were: 1) What causes the pore's impressive selectivity, while allowing incredibly rapid diffusion of water? 2) How are H$_3$O$^+$ ions excluded, while allowing very similar H$_2$O molecules rapid transit? 3) How is the observed HgCl$_2$ inhibition explained?

Molecular details of the common mechanism of the aquaporin family are elucidated in several excellent reviews.[36-44] The folded protein contains four identical subunits that form funnel-shaped entrances from both sides of the membrane with a narrow central constriction that restricts all but the smallest molecules.[1,36-42] Small ions, such as sodium, cannot shed their shell of water molecules and so are excluded from the pore. The H$_3$O$^+$ ions, are excluded by positively charged amino acids in the heart of the pore. Finally, the water molecules must successfully re-orient themselves in different directions as they traverse the aquaporin "gauntlet".[37-44]

In most (but not all) AQPs, a critical cysteine residue reacts with mercury ions and thus accounts for the reversible inhi-

bition of aquaporins by heavy metal ions. The Hg-sensitive cysteine residue is near the constriction, and reaction with mercury effectively blocks the pore.[37-44] Interestingly, before the advent of loop diuretics, mercuric compounds were used to produce a profound diuresis,[1] presumably by specific inhibition of the renal AQPs.

Table 1. Characteristics of the family of aquaporins

Aquaporin	Cellular location	Characteristics and/or role
AQP0	Eye lenses, also called MIP (major intrinsic protein)	Comprises 50% of lens membrane proteins, removes excess water from lens
AQP1	Many cells, including RBCs, renal cells, eye cells, respiratory tissues	Major water channel of renal reabsorption, control of pulmonary edema
AQP2	Renal collecting duct cells (luminal surface)	Hormonally-regulated (ADH) reabsorption in kidney, Intracellular until cAMP-induced relocalization
AQP3	RBCs, renal cells, eyes, and brain cells	Unregulated kidney reabsorption, also transports glycerol
AQP4	Many parts of brain (center of osmoregulation), eye, and respiratory tissues cerebro- and spinal fluids	Possibly involved with osmoregulation and release retinal cells of ADH, control
AQP5	Eyes (lacrimal glands), salivary glands, other secretory glands, and respiratory tissues	Regulated water transport like AQP2, secretion of water from respiratory epithelial and gland cells
AQP6	Intercalated cells of renal collecting duct	Also a gated-ion channel, internalized in cells, possibly involved with plasma pH regulation
AQP7	Adipose tissue	Transports glycerol in addition to water, release of glycerol during metabolism of triglycerides
AQP8	Hepatocytes and pancreatic ducts	Localized in intracellular vesicles, cAMP-induced relocalization may control water in bile secretions
AQP9	Hepatocytes	Transports glycerol in addition to water, uptake of glycerol for use in gluconeo-genesis
AQP10	Reported in kidney	Function unknown

Survey of variations within the aquaporin family

Presently, the human AQP family contains AQP0-AQP10, with different cellular locations and specificities as illustrated in Table 1. Logically, each of these AQP variations probably serves a unique physiologic function. However, the significance of many of the subtle differences is currently unknown and investigations continue.

Aquaporins as blood group antigens

As mentioned above, the discovery of CHIP28 (AQP1) occurred during a study of Rh antigens of RBCs.[6] Although AQP1 has been shown to contain glycosylation corresponding to the ABO system, an AQP1 deficiency does not affect a person's ABO type.[41] ABO blood groups result from the presence or absence of two transglycosidases that modify the terminal residues of "glycosylation trees" on various molecules.[2,4]

The Colton blood group antigen corresponds to a unique amino acid sequence near the N-terminous of AQP1.[1,48] A mutation at residue number 45 subsitutes an alanine for a valine and causes a non-functional AQP1. Patients with the mutation will produce antibodies to the normal Colton sequence following exposure to this antigen from a transfusion or pregnancy. An interesting case described in accompanying article involves a female patient whose AQP1-deficiency was only discovered when a prenatal screen detected anti-Colton (AQP1) antibodies during her second pregnancy.[31] Her primary symptom, aside from the complications of the Colton blood group, was a subclinical NDI that was only obvious following stress.[31]

Aquaglyceroporins and metabolism

Three members of the aquaporin family, AQP3, AQP7, and AQP9, allow efficient diffusion of glycerol, in addition to water.[1,10,13,44-46] This seemingly curious specificity correlates well with their apparent physiological function, since fat and liver cells, known to import and export glycerol, express these glycerol-transporting aquaporins.[27,28,44-46] AQP9 facilitates glycerol uptake by hepatocytes, where glycerol contributes carbons for gluconeogenesis.[1,4,44-46] Glycerol, lactate, and amino acids are used for gluconeogenesis, which is essential for the liver's maintenance of blood glucose levels during fasting or starvation. Fat cells use AQP7 to export glycerol produced during mobilization of triglycerides.[45-46] Thus, both tissues depend on aquaglyceroporins during fasting or starvation conditions, and it is not surprising that both AQP7 and AQP9 are up-regulated during these conditions.[27,28] Although AQP3 has similar ability to promote glycerol diffusion, its expression in numerous cells not involved with gluconeogenesis suggests that its primary role involves osmosis.

Conditions affecting glycerol metabolism also alter expression of AQP 7 and AQP9.[1,27,28] For example, fasting, starvation, uncontrolled diabetes, and exercise all cause a marked increases in AQP9 expression.[27,28,46] As obesity reaches epidemic proportions in the US population and Type II diabetes, insulin-resistance, and metabolic syndrome become popular "buzz words", many unanswered questions remain concerning this newly appreciated aspect of our metabolism. For example, what consequences would result from AQP7 or AQP9 malfunctions? Can medications which alter their expression be useful in controlling these common metabolic conditions?

Intracellular AQP6 and the renal H$^+$ ATPase

Transport of H_3O^+ ions is uniquely characteristic of AQP6, which associates with the H$^+$-specific ATPase pump known to acidify urine in the CD.[47] In addition, AQP6 apparently resides exclusively in intracellular vesicles of the intercalated cells in the CD.[47] Molecular details and physiologic significance of AQP6's odd specificity for transport of acids are not yet established.

Regulated osmosis and AQP2 and AQP5

Most osmosis is not directly regulated and is always driven by concentration gradients, even when facilitated by AQPs. However, the body must regulate osmosis in certain situations. As mentioned above, ADH regulates water reabsorption by controlling the insertion of AQP2 into the CD cell membranes. Receptor binding of ADH causes an increase in cyclic AMP (cAMP) and activates a protein kinase, which phosphorylates a specific serine residue on AQP2.[10,49] Phosphorylation ultimately leads to AQP2 insertion into CD cell membranes.[10,49]

Secretory glands and lung tissues apparently control fluid secretion through analogous regulation of AQP5. Lungs tissues, secretory glands such as the salivary and lacrimal, and ducts of the pancreas and bile are all known to contain AQP5. See Figure 2 in the accompanying article. Salivation, crying, and many other secretions are neither constant nor random, but are closely controlled by nerve stimulation. Interestingly, AQP5 resides in intracellular vesicles and contains a potential phosphorylation site homologous to the control site on AQP2, suggesting that these secretions are controlled by a similar mechanism.[10,49]

CONCLUSION AND SUMMARY

Aquaporins are a family of channel proteins that facilitate osmosis, or the rapid movement of water, across virtually every cell membrane in our bodies. Although water slowly crosses hydrophobic membranes without aquaporins, many physiological systems demand much more rapid osmosis. In the renal PCT and descending loop of Henle, where 80% of renal filtrate is reabsorbed, osmosis occurs at rates approaching 100 mL per minute. AQP1 was first isolated from RBCs and the PCT,[6] and facilitates reabsorption by increasing membrane water permeability by 100-fold. In fact, calculations place the rate of diffusion through these membrane channels at a phenomenal rate of 10^9 water molecules/second/channel.[21,22] Despite this amazing rate, most AQPs are extremely water-specific, effectively excluding ions or small neutral molecules such as glucose.

Two AQPs help control water transport in response to hormonal or neuronal signals. In the renal CD, pre-synthesized AQP2 remains internalized in vesicles until ADH signals for the vesicles to fuse with the cell membrane, which inserts AQP2 and increases reabsorption. A similar mechanism apparently controls AQP5 function in lung epithelial cells and various secretory glands. Another interesting subclass of AQPs involves AQP 3, AQP7, and AQP9, called aquaglyceroporins, because they transport both glycerol and water. These AQPs are found in fat cells and hepatocytes and are responsive to insulin and such physiological conditions as exercise and fasting, suggesting that their primary physiological role involves glycerol transport and control of gluconeogenesis.

Aquaporins are implicated, either as the primary lesion or secondarily, in numerous diseases. For example, NDI results from an ineffective AQP2 response to ADH signals aimed at stimulating water reabsorption. AQP2 is also indirectly involved in edematous conditions such as CHF, cirrhosis, pulmonary edemas, while AQP4 may be involved with cerebral edema.[1,10,11,13] Additional examples of clinical implications are described in the accompanying article.

The use of probes to screen the genome for homologous sequences has allowed researchers to identify the likely members of the aquaporin family. However, investigations into the expression of various AQPs remain an active and fertile area of research. Once the expression of a particular AQP is established, and its precise cellular location is determined, investigations focus on regulators of its expression to help elucidate its physiological role. Pathophysiologic observations of animals and patients with defined mutations to AQP genes are also pointing to new directions for clinical research. Clinical applications are continually expanding with increased understanding of disease processes and exploration of possible therapeutic interventions.

As science gains insights into the AQP family and their clinical correlations, several commercialized version of research assays may soon arrive for routine clinical use. For example, elevated urinary AQP2 levels, as detected by EIA procedures,[48] are associated with such conditions as diabetes insipidus, CHF, and liver cirrhosis. These procedures will likely be commercialized once the clinical significance has been established. Numerous immunoassays using antibodies to variant AQPs and nucleic acid probes to normal and variant AQP genes also have exciting potential for development into clinical markers.[1,12,33,34,45-49]

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