Water Channels (Aquaporins) and Their Role for Postnatal Adaptation

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ABSTRACT

Birth is a transition from an underwater life in the uterus to a terrestrial life in a milieu where supply of water is limited. Rapid adaptation to the new environment is crucial for survival and health of infants. The discovery of a family of molecules—aquaporin (AQP) water channels—that are responsible for regulated water transport across cell membranes has made it possible to identify the molecular mechanisms behind the postnatal homeostatic adaptation and to better understand water imbalance-related disorders in infancy and childhood. Thirteen mammalian AQP isoforms have been identified, most of them having a unique tissue-specific pattern of expression. Most mammalian AQPs can be dynamically regulated, which makes them potential targets for the development of new drugs for diseases associated

with disturbances in water homeostasis. This review deals with AQP in kidney, lung, and brain. Evidence is presented that AQPs are expressed in a specific age-dependent manner and that the timed expression of AQPs may have a crucial role during the early postnatal period. (*Pediatr Res* 57: 47R–53R, 2005)

Abbreviations

AQP, aquaporin
BPD, bronchopulmonary dysplasia
RDS, respiratory distress syndrome
SIADH, syndrome of inappropriate antidiuretic hormone secretion

Water is a prerequisite for life. Water molecules are polar, which allows them to easily form hydrogen bonds with each other and with other molecules. They serve as excellent solvents for a variety of polar substances in the cells. Water provides solvent shells around charged groups of biopolymers. These shells are essential for the biologic activity of proteins (1).

It took hundreds of millions years for ancient vertebrate organisms to adapt from a life with unrestricted water supply to a terrestrial life. This adaptation has involved the development of numerous intricate mechanisms and regulatory systems that allow animals to exist in air environment, where supply of water is more or less limited. Now it takes mammals a few minutes to make the transition from an underwater existence in the uterus to a postnatal terrestrial life. The first immediate task a newborn infant has to deal with is rapid removal of water out of lung air spaces. Maturation of kidney concentrating capacity occurs in humans during the first year of life. The capacity to maintain a proper water balance in the brain and to protect from brain edema may take even longer time.

Water comprises about 60% of our body weight. In newborn full-term infants, total body water fraction is approximately 75%, and in preterm babies approximately 80–85% of their body mass (2). The ratio between extracellular and intracellular water content changes dramatically in the postnatal period (Fig. 1). Shortly after birth, the body water mass rapidly decreases. This decrease is mainly due to a reduction of extracellular water fraction, which decreases from about 45% to 30% of total body mass during the first 3 mo of life.

As late as in 1990, little was known about the molecular mechanism regulating total body water content and the distribution of water between the extracellular and intracellular space. The discovery of integral membrane proteins that function as water channels (3), resulted in a paradigm shift for the understanding of the mechanisms behind transmembrane water transport, water redistribution processes after birth, and many pathologic conditions related to disturbances in water homeostasis.

More than 10 isoforms of water channels, now named AQPs, have been identified in humans to date. Most of them have a common molecular structure, with six transmembrane domains and intracellular NH₂- and COOH-termini. Functionally, AQPs can be divided into two subfamilies—aquaporins, specialized on transport of water, and aquaglyceroporins, which transport not only water but also small nonpolar molecules like glycerol and urea.

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Figure 1. Developmental changes in body water content (estimated from Ref. 2). (A) Total body water content (open bars) and extracellular water content (filled bars). (B) Ratio of water content in extracellular and intracellular compartments.

Water transport *via* AQPs occurs bidirectionally and is driven by osmotic gradients. The osmotic gradient across the plasma membrane is primarily created by energy-dependent cation transport *via* Na^+ , K^+ -ATPase. This ion pump, which is present in all mammalian cells, uses the energy from ATP hydrolysis to transport sodium out of the cell and potassium into the cell with the stoichiometry 3:2. In epithelial cells, Na^+ , K^+ -ATPase is localized in the basolateral plasma membrane. The sodium gradient created by the pump permits sodium to enter the cell *via* channels and co-transporters inserted in the apical membrane. This results in a flux of sodium that drives water flow *via* AQPs in the same direction.

There are, in addition to AQPs, at least three other pathways for water transport in mammalian tissues. These include passive diffusion of water across the plasma membrane lipid bilayer, transport of water *via* co-transporters such as Na^+ -glutamate or Na^+ -glucose co-transporter (4), and paracellular water transport *via* tight junctions.

Water transport *via* AQPs is much faster than diffusion of water *via* lipid bilayer of plasma membrane (Fig. 2A). Water transport *via* most AQPs is also more efficient than *via* co-transporters (5). Water transport *via* co-transporters is, however, the main mechanism for water uptake in intestinal epithelial cells, which, in contrast to most other types of epithelial cells, have a very low expression of AQPs (4). Paracellular water transport may be significant in leaky epithelia (6) but is negligible compared with the water channel-mediated transport in tight epithelia (7).

Water movement *via* AQP allows a more specific regulation of water distribution than water movement *via* other pathways.

Figure 2. Compared with simple diffusion through the lipid bilayer of the plasma membrane (A, left panel), AQPs water channels (A, right panel) provide much more rapid and energy undemanding transport of water across the plasma membrane. The protein nature of the water pores also offers various possibilities for the regulation of water transport: by trafficking of an AQP between the plasma membrane and a pool of intracellular vesicles (B, left panel), or by changing permeability of an AQP residing in the plasma membrane (B, right panel).

Many mammalian AQPs have been shown to be differentially expressed during development and to be regulated by hormones at the level of protein abundance (8–11). The capacity of AQPs to transport water can also be dynamically regulated by hormones and/or extracellular factors such as heavy metals and pH (11–24). Such dynamic/short-term regulation may occur by changing the opening state of AQP residing in the plasma membrane or by insertion of AQP-containing vesicles into the plasma membrane (Fig. 2B). Information about the mechanisms by which AQPs can be regulated will be a prerequisite for future development of novel therapeutic tools for diseases associated with disturbances in water homeostasis.

Almost all tissues in the human body have a unique set of AQPs that can modulate water balance and cell volume control (Fig. 3). Dysfunction or lack of AQPs has been described in a variety of diseases such as nephrogenic diabetes insipidus, early onset cataract, Sjögren's syndrome, wet lung syndrome, congestive heart failure, SIADH, and brain edema (for reviews on the pathophysiology of AQPs, see Refs. 25,26).

KIDNEY AQPs

Under normal physiologic conditions, the adult kidney filters approximately 180 L of plasma fluid a day and excretes only 0.5–2 L of urine. During severe dehydration, <0.2% of the filtrate is excreted; during volume expansion, >7% of the filtrate will be excreted. Thus, the variations of transcellular water transport are huge, depending on fluid intake and water losses *via* skin and lung.

It is now generally recognized that most of the water reabsorption in the nephron occurs transcellularly *via* AQPs (27). Figure 4 illustrates the expression of AQPs in the nephron. The bulk of water from ultrafiltrate is reabsorbed in proximal part

Figure 3. Expression of AQPs isoforms in human tissues.

Figure 4. Distribution of AQPs isoforms in the nephron (27). AQP1 is present both in the apical and in the basolateral plasma membrane of proximal tubular cells and in the descending limb of the loop of Henle. AQP2 is localized in the apical plasma membrane and/or in intracellular vesicles in the principal cells of collecting duct. AQP3 and AQP4 are present in the basolateral membrane of the same cells as AQP2. AQP6 is localized in intracellular vesicles in intercalated cells of the collecting duct. AQP7 is present in the apical membranes of proximal straight tubules (S3 segment).

of the nephron *via* AQP1, which is present both in apical and basolateral plasma membranes of the epithelial cells in proximal tubules and descending limb of loop of Henle. The water reabsorption is driven by osmotic gradients. Ascending limb of Henle's loop and distal convoluted tubule lack AQPs and are water-impermeable. The urine in the distal convoluted tubule is nearly isoosmotic, because sodium is actively reabsorbed in thick ascending limb by Na^+ , K^+ -ATPase. Approximately 15% of the filtrate is delivered to the collecting duct, where water reabsorption occurs in regulated manner in compliance with the needs of the body. The regulation of the water transport in collecting duct can be attributed to the ability of AQPs to be dynamically regulated (12–16,19–21,24). When the kidney is exposed to antidiuretic hormone, AVP, water is reabsorbed *via* AQP2 in the apical plasma membrane and AQP3 and AQP4 in the basolateral plasma membrane. AVP acts by insertion of AQP2 into the apical plasma membrane of

the collecting duct principal cells (see below). Active sodium reabsorption *via* Na^+ , K^+ -ATPase in water-impermeable thick ascending limb, recirculation of urea, and the countercurrent multiplier mechanism creates a highly hypertonic renal medullary interstitium. This hypertonicity acts as a very strong driving force for the water reabsorption in the collecting duct in the presence of AVP.

AQP2, the main renal target for AVP, is of major importance for the urinary concentrating capacity. Defects in AQP2 structure cause approximately 10% of hereditary nephrogenic diabetes insipidus in human population (28). AQP2 abundance is down-regulated in congenital urinary tract obstruction and lithium-induced nephrogenic diabetes insipidus, and is up-regulated in congestive heart failure and hepatic cirrhosis (29–33).

AQP2 is located in collecting duct principal cell apical membrane and/or in subapical intracellular vesicles that represent an instantly recruitable reserve for on-demand increasing the water permeability of the collecting duct. These AQP2-containing vesicles are recruited to the plasma membrane by AVP and other antidiuretic factors, such as oxytocin (12–15). It is generally agreed that, under basal conditions, there is certain constitutive turnover of AQP2 between the plasma membrane and intracellular vesicles. AVP, acting on V2-receptors, increases cAMP production and stimulates protein kinase A dependent phosphorylation of AQP2. Vesicles carrying phosphorylated AQP2 are directed to the apical pole of the cells and fuse with the plasma membrane. The AVP-mediated recruitment of AQP2 to the apical membrane causes a huge increase in the water permeability of the apical plasma membrane. Antidiuretic factors, like prostaglandin E_2 , can decrease AVP-induced water permeability of the principal cells by shifting the balance between AQP2 exo- and endocytosis in the opposite direction (19,24).

The urinary concentration capacity in humans develops gradually postnatally and reaches full maturation at approximately 18 mo (34,35). The low concentrating capacity in infants provides poor protection against extrarenal water losses and can, in older infants, be associated with increased susceptibility to of hypertonic dehydration. The developmental pattern of AQP2 in kidney has been shown to parallel the development of the concentrating capacity (8,36–38). AQP2 mRNA and AQP2 protein levels increase gradually postnatally and reach adult level by the end of weaning. Both AQP2 expression and the development of the urinary concentrating capacity can be prematurely induced by glucocorticoid hormone treatment (8). It should be noted though that low AQP2 expression is not the only limiting factor in the capacity to concentrate urine (14,36,38) and that maturation of this function also involves development of nephron architecture, the capacity to create a cortico-papillary solute gradient, and development of sensitivity of collecting duct cells to AVP (reviewed in Ref. 39).

The use of indomethacin treatment to suppress prostaglandin production in infants with patent ductus arteriosus often leads to water retention (40,41). In the presence of drugs that suppress endogenous prostaglandin production, AQP2 will remain in the plasma membrane (19). This may explain the water retaining effects of indomethacin treatment in infants.

Urinary excretion of AQP2 can serve as indicator of AVP-AQP2 pathway activity (42,43). We have in ongoing studies found that urinary excretion of AQP2 during first 2–3 postnatal weeks in humans is increased with increasing gestational age at birth. In infants with renal insufficiency, excretion of AQP2 is decreased (44).

AQP1, AQP3, and AQP4 have also been shown to play roles for the concentration of urine. AQP1 is the dominant AQP isoform in the proximal nephron. It mediates water permeability of both apical and basolateral plasma membranes of tubular epithelium in this nephron segment. In rat proximal tubules, expression of AQP1 mRNA and protein is not detectable in fetus and is induced shortly after birth (37,45,46). Knockout of AQP1 in mice leads to nephrogenic diabetes insipidus (47). In reported cases of AQP1-null humans, a decreased ability to concentrate urine has been demonstrated after dehydration or in response to AVP treatment (48).

AQP4 is located in the basolateral membranes of collecting duct principal cells. AQP4 can be regulated by hormones, but, unlike AQP2, regulation does not involve a shuttling mechanism (20,21). Activation of protein kinase C leads to phosphorylation and reduces water permeability of AQP4 residing in the plasma membrane. Interestingly, this effect can be triggered by activation of dopamine receptors. AVP and β -adrenergic agonist isoproterenol can enhance the water permeability of plasma membrane AQP4, and this effect can be mimicked by adenylyl cyclase activator forskolin.

Rat renal AQP4 mRNA levels increase gradually after birth, indicating a developmental regulation during postnatal life (10). AQP4 protein expression is detected in rat kidney by immunohistochemistry in the very early postnatal period (49).

AQP3 protein is detected early after birth (37,38). AQP3 has so far not been shown to be regulated by hormones, but it has, as described below, a very distinct sensitivity to extracellular pH (17,22). Acidification of extracellular fluid leads to a decrease of AQP3 water permeability.

LUNG AQPs

After introduction of surfactant in the early 1990s and the growing use of antenatal steroids since that time, the number of term and preterm infants having severe RDS has declined. However, management of babies suffering from RDS still causes much debate and concern, not least because of the recognized high risk of adverse outcome (50). Acute RDS often develops into a chronic lung disease, BPD. One of the most prominent features of RDS and BPD is lung edema that is believed to be caused by inadequate lung water clearance. Close to term, the human fetal lung secretes approximately 0.5 L of fluid a day. At birth, the lung epithelium switches from a secretory to absorptive epithelium. This switch involves increased expression of the epithelial sodium channel and of the sodium pump, and also changes in expression of lung AQPs. At birth, most of the lung fluid is expelled mechanically, but some will remain to be absorbed during the first postnatal days. In premature infants this ability of lung to reabsorb water is often impaired, contributing to the still high incidence of RDS and BPD (51).

In the postnatal lung, a thin layer of liquid covers the airways. The depth and composition of airway surface liquid must be strictly controlled to keep the airways free from infection and to maintain an effective gas exchange. Composition of airway surface liquid components affects efficiency of mucus clearance on airway surface, which is important both for an adequate gas exchange and for defense against infections (52). Acidity of airway surface liquid is of particular importance, inasmuch as it can affect mucus clearance by influencing both mucus rheology and ciliary beat frequency (53). Asthma, bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis are all associated with an acidified airway surface liquid (54–56).

It is likely that water transport *via* AQP is of importance both for lung water clearance at birth and for maintenance of airway surface liquid composition in postnatal life. AQPs are abundantly expressed in the lung. At least four AQP isoforms, AQP1, AQP3, AQP4, and AQP5, are expressed in the lung tissue (Fig. 5). AQP1 is expressed in capillary endothelium; the other AQPs are localized in the airway epithelial cells. The physiologic role of each AQPs remains to be fully clarified. So far, the function of lung AQPs was mainly studied in rodents (57). Transcapillary osmotic water permeability was greatly reduced by AQP1 deletion, and the airspace-to-capillary water permeability was significantly decreased by additional knockout of AQP4 (58). Because rodent lung epithelium appears to be more leaky than the human, the requirements for a regulated water transport should be more stringent in humans (59). Studies in human AQP1-null individuals have established that AQP1 is a determinant of vascular permeability in the lung (60). Furthermore, the distribution of AQPs in human lung has been reported to be different from that in rodents (61).

The level of AQP1 in the lung endothelium is dramatically increased perinatally and can be further up-regulated by maternal corticosteroid treatment (9).

Expression of AQP4 peaks immediately after birth (10,62), suggesting that this water channel may be critically involved in

Figure 5. Distribution of AQPs isoforms in the lung (84). AQP1 is expressed in the plasma membranes of endothelial cells of the lung capillaries. AQP3 is localized basolaterally in epithelial cells lining trachea and bronchi. In humans, AQP3-specific signals were also detected in the apical plasma membrane of proximal and terminal bronchioles and in the basolateral membranes of type II pneumocytes (61). AQP4 is expressed in basolateral membranes of tracheal and bronchial epithelium. In humans, AQP4 was also detected in type I pneumocytes in alveoli. AQP5 is localized in apical membranes of type I pneumocytes and in secretory glands in the upper airways.

lung water clearance. Lung AQP4 mRNA can be prematurely induced in embryonic rats both by glucocorticoids and β -adrenergic agonists (10). The effect of β -adrenergic agonists is particularly interesting, since they are well known to improve lung function in preterm infants. We have in an ongoing study using bronchial lavage aspirates from premature babies found that AQP4 abundance in lung can also be up-regulated by maternal corticosteroid treatment in humans. The material was obtained from two groups of newborn preterm babies that only differed with regard to glucocorticoid exposure.

AQP3, localized apically in human alveolar epithelium (61), may be of special clinical interest, since it is pH-regulated (17,22). Extracellular acidification significantly decreases the water permeability of AQP3 expressed in human bronchial epithelial cell line, but does not influence other AQPs expressed in lung epithelium, AQP4 and AQP5 (22). The effect of extracellular pH on AQP3 is mediated by two histidine residues that are present in the extracellular part of AQP3 molecule. Histidine can bind hydrogen ions at low pH, which may lead to a conformational change in AQP3 molecule resulting in a decreased water permeability of AQP3 water pore. Two other amino acid residues, tyrosine 124 and serine 152, are also involved in the pH sensitivity of AQP3. In conditions of airway surface liquid acidification, the AQP3-mediated water permeability will be inhibited, which may contribute to the poor lung water clearance in asphyxiated and acidotic babies. AQP3 is also inhibited by heavy metals nickel and copper (22,23). Copper is widely used in water distribution systems, and considerable amounts of copper can be present in the tap water (63,64). Drinking copper-containing water can be especially hazardous for infants because of an imbalance between absorption and excretion of copper in early postnatal period (65).

Alveolar AQP5 may also play a role in regulation of airway surface liquid composition. AQP5 protein level gradually increases after birth (62). It has recently been found that AQP5 can be phosphorylated by protein kinase A, and a sustained exposure to β -adrenergic agonist increases AQP5 protein expression and membrane localization in the lung (11). The ability of AQP5 to be dynamically regulated makes it an attractive potential target in treatment of lung edema related disorders.

BRAIN AQP

Three water channels have at the time for this review been reported to be located in the brain: AQP1, AQP4, and AQP9. AQP1 is exclusively expressed in the choroid plexuses (66). AQP4 and AQP9 are both expressed in astrocytes. AQP9 was also detected in a subpopulation of catecholaminergic neurons (67,68). No other water channel has yet been identified in neurons. It has been suggested that water transport in the plasma membrane of neurons might occur *via* co-transporters such as the Na^+ -glutamate co-transporter (5). For a recent review on brain AQPs, see Ref. 69.

AQP1 is located in the apical membrane of epithelial cells in the choroid plexus. Studies in knockout mice suggest that it plays an important role in the formation of cerebrospinal fluid

(70). In rat, AQP1 mRNA is expressed early in fetal life (45). Hypothetically, inhibition of AQP1 might be used as a symptomatic therapeutical tool in hydrocephalus. No pharmacological inhibitor of AQP1 exists yet, but studies on AQP1 expressed in *Xenopus* oocyte expression system and in bile duct epithelial cells have shown that this water channel can be dynamically regulated by hormones such as AVP, atrial natriuretic peptide, and secretin (16,18).

AQP9, which is also permeable for glycerol and urea, was identified in the brain only a few years ago and its physiologic and pathophysiologic roles remain to be determined. It has been hypothesized that AQP9 may be involved in brain energy metabolism as a neutral solute channel (71).

AQP4 is the most extensively studied brain AQPs. It is expressed in astrocytes and ependymal cells. It is strongly enriched in astrocyte membranes at the interface between brain neuropil and extracerebral spaces. AQP4 is also abundant in osmosensory areas (68).

AQP4 mRNA expression was reported to increase postnatally in extracts from whole rat brain (10). *In situ* hybridization, semiquantitative immunoblotting, and immunofluorescence microscopy have also revealed very low levels of AQP4 mRNA and protein in the first postnatal week and a significant increase in the second week (72). Little is known, however, about the development of AQP4 expression in different brain regions at the present time.

Under physiologic conditions, AQP4 plays an important protective role by facilitating potassium clearance that occurs after neuronal stimulation (73,74). Reduced efficiency of potassium clearance after neuronal stimulation should be expected to increase the vulnerability to epileptic seizures. Indeed, in transgenic mice, where astrocyte AQP4 is partially lacking and misplaced, the susceptibility to hyperthermia-induced epileptic seizures is enhanced (74). The question of whether certain forms of epilepsy may be associated with abnormalities of brain AQP4 in humans is currently under investigation.

Several conditions such as ischemia, trauma, tumor, infections, hyponatremia, and fulminant hepatic failure are associated with brain edema. Notably, children have a higher susceptibility to brain edema than adults. The presence of AQP4 appears to favor the development of cytotoxic edema (swelling due to increased intracellular fluid). Studies on mice deficient in AQP4 have indicated that in posthypoxic brain edema the presence of AQP4 is harmful (75). In mice where AQP4 was selectively removed from perivascular plasma membrane of astrocytes by dystrophin or α -syntrophin deletion, the development of edema after water intoxication and acute hyponatremia was significantly delayed (76,77). The mechanism for this apparently harmful effect remains to be elucidated.

Studies on mice deficient in AQP4 have also indicated that in edema associated with impairment of capillary endothelium the presence of AQP4 may actually have a positive effect on brain water clearance (78,79) by providing an effective path for water removal from the brain parenchyma.

Acute lead intoxication is a condition that causes brain damage preceded by brain edema. Recent studies from our laboratory have shown that lead increases the water permeabil-

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ity of AQP4 expressed in astrocytes (80). Lead, which is readily taken up by astrocytes, appears to enhance AQP4 water permeability *via* activation of calcium/calmodulin-dependent protein kinases. AQP4 carries several consensus sites for protein phosphorylation. Taking into account the potentially harmful role of AQP4 in many forms of brain edema, it becomes an important topic to elucidate the short- and long-term regulation of this transporter.

It is generally agreed that the capacity of corticosteroids to enhance protein expression is particularly high in the perinatal period. In fetal rat lung, glucocorticoids increase AQP4 mRNA levels (10). So far, there is no report of the effect of corticosteroid on AQPs expression in perinatal or infant brain. If glucocorticoids would stimulate expression of AQP4 in brain, their use in hypoxia and brain edema might, for the reasons given above, be contraindicated. It is therefore important to examine possible effects of glucocorticoids on brain AQP4 in the perinatal period and to consider their possible adverse effects in posthypoxic infants.

AQP4 has two isoforms that differ in the length of NH₂-terminus, the longer AQP4.M1 and the shorter AQP4.M23 (81,82). Studies in mice have revealed the presence of at least two mRNA that encode the M23 protein isoform, AQP4.M23 and AQP4.M23X mRNA (83). This opens the possibility that the transcriptional regulation of AQP4 protein can be tissue specific. In fact, the AQP4.M23X mRNA, which is predominantly expressed in the brain, was found to be developmentally regulated with higher levels in the adult than in the infant mouse.

CONCLUSION AND FUTURE PERSPECTIVES

AQPs are the main molecular targets for factors regulating water metabolism. Expression of AQPs increases after birth in kidney, lung, and brain. The first water channel was identified less than 15 y ago and the understanding of the physiologic and pathophysiological relevance of the 13 mammalian AQPs that have been identified to date is still incomplete. The significance of renal AQPs for tubular water reabsorption and for urinary concentrating capacity is well established. Emerging evidence suggest important roles for lung and brain AQPs. The physiologic and pathophysiological role of AQPs for lung water clearance and for excessive lung epithelial fluid secretion in situations such as asthma will need to be clarified. The clinical relevance of the pH sensitivity of lung AQP3 should be explored. Future studies should also aim at clarifying the physiologic and pathophysiological role of brain AQPs. Of particular importance are the following questions: Will water transport *via* AQPs enhance astrocyte swelling in hypoxia associated with brain edema and would it therefore be beneficial to down-regulate brain AQPs in these situations? Does AQP dysfunction contribute to certain forms of epilepsy? Can AQPs expressed in the choroids plexus be a target for symptomatic treatment of hydrocephalus? It will be an important task for pediatricians and basic scientists to jointly address these questions.

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