Aquaporins as targets for drug discovery

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Over the past decade, significant advances have been made in understanding how water moves in to and out of cells. Investigators have used molecular and structural biological techniques to show that nature has evolved specialized water-conducting proteins called aquaporins, which traverse biological membranes in the cells of animals, plants and even bacteria. It is becoming increasingly clear that these aquaporins have fundamental roles in normal human physiology and pathophysiology. Consequently, aquaporins are attractive targets for the development of novel drug therapies for disorders that involve aberrant water movement, such as edema and kidney disease.

Water is the major component of cells and tissues throughout the animal and plant kingdoms. In humans, water constitutes ~60% of the body mass, which remains relatively constant despite physiological processes that result in the loss of ~2.5 l each day via sweating, breathing and urine and fecal output. This loss is offset by a typical human consumption of water of over 2 l each day, balanced with formation of water from metabolic processes. To maintain constant levels in humans (and all living organisms), water has to move through tissues and cell compartments that are surrounded by hydrophobic lipid bilayer membranes. Approximately two-thirds of water in humans is located within cells, whereas the remaining third forms the extracellular fluid space. At first glance, a hydrophobic phospholipid bilayer would appear to present a significant energy barrier for water movement in to and out of cells. Indeed, the water permeability of biological lipid membranes is extremely low. However, the concentration of water molecules per unit volume is high (55 M), as is the ratio of surface area of lipid membrane to volume of a cell. Thus, following Fick's First Law (rate of diffusion, or flux, of a species is proportional to the concentration gradient), water can diffuse across lipid bilayer membranes, although the activation energy (E_a) required for this process to occur is greater than diffusion in an entirely aqueous environment (10–20 kcal mol^{-1} versus 5 kcal mol^{-1}) [1].

Early evidence for water channels

Several early studies examining water flux in mammalian red blood cells demonstrated that water permeability in these cells is much higher than would be predicted by water simply diffusing across the lipid plasma membrane [2]. Furthermore, the high permeability of mammalian red blood cells to water was strongly inhibited by mercurial sulfhydryl compounds such as mercuric chloride ($HgCl_2$) [3]. Similarly, early studies in kidney demonstrated that water transport occurred at a high rate that could be regulated hormonally through the action of vasopressin (antidiuretic hormone) and inhibited by mercury-containing compounds [4]. These findings led to early speculation of the presence of protein conduits that could facilitate the passage of water across cell membranes. Confirmation of the existence of such a protein came in the early 1990s with the identification of a 28 kDa membrane protein [channel-like integral membrane protein 28 (CHIP28)]

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TABLE 1

Characteristics of human aquaporins

Aquaporin	Chromosome	Water permeability	Permeable to glycerol	Major tissue distribution
AQP0	12q13	Low	–	Lens (eye)
AQP1	7p14	High	–	Erythrocytes, lung, kidney, brain, eye and vascular endothelium
AQP2	12q13	High	–	Kidney
AQP3	9p13	High	Yes	Skin, kidney, lung, eye and gastrointestinal tract
AQP4	18q22	High	–	Kidney, brain, lung, gastrointestinal tract and muscle
AQP5	12q13	High	–	Salivary, lacrimal and sweat glands, lung and eye
AQP6	12q13	Low	–	Kidney
AQP7	9p13	High	Yes	Adipose tissue, kidney and testis
AQP8	16p12	High	–	Kidney, liver, pancreas, gastrointestinal tract and testis
AQP9	15q22	Low	Yes	Liver, leukocytes, brain and testis
AQP10	1q21	Low	Yes	Gastrointestinal tract
AQP11	11q13	NK	NK	Brain, liver and kidney
AQP12	2q37	NK	NK	NK

Abbreviation: NK, not known.

expressed at high levels in red blood cells and kidney proximal tubules [5]: the gene encoding CHIP28 was successfully cloned and expressed in *Xenopus* oocytes [6]. Expression of CHIP28 led to increased osmotic water permeability, which was exemplified by swelling and bursting in hypoosmotic media that could be reversibly inhibited by $HgCl_2$. Because of its apparent water-transporting role, CHIP28 became the first recognized member of the 'aquaporin' (AQP) family and was renamed AQP1. Subsequent studies have shown that AQP1 belongs to a large family of water-transporting proteins. In humans, 13 AQPs have been identified to date (Table 1), although this number pales in comparison to the >100 related proteins also found in plants and bacteria [7].

Molecular and functional properties of aquaporins

Sequence analysis of AQP1 demonstrated that AQP protein subunits comprise six α-helix transmembrane domains with an inverted symmetry between the first three and last three domains. The two loops between transmembrane helices 2–3 and 5–6 contain an amino acid triplet with the sequence Asn-Pro-Ala (NPA) conserved across members of the AQP family. This predicted topology led to the 'hour-glass' model of AQP structure, in which the six bilayer spanning helices surround an aqueous pore formed from the two NPA-containing loops that fold into the bilayer from opposite sides, overlapping at the location of the NPA motifs [8] (Figure 1a). The hour-glass conformation of AQP structure has been verified using electron and X-ray crystal analysis of AQP1 [9,10] (Figure 1c) and the sequence-related bacterial glycerol facilitator GlpF [11].

Biochemical and freeze fracture studies have indicated that, as with other channel-like membrane proteins, functional AQPs exist as tetramers [12]. However, in contrast to most ion channels, the permeation pathway (pore) does not reside at the axis of symmetry formed by the four constituent subunits, but rather each subunit monomer contains a separate pore [12,13] (Figure 1b). Thus, each fully assembled AQP contains four channels for water permeation (Figure 1b and 1d).

How do AQPs facilitate the passage of water while excluding the movement of protons [in the form of hydronium (H_3O^+)] and other ions (e.g. K^+ and Na^+)? Structural studies have revealed that the hour-glass shaped pore of AQP1 comprises cone-shaped water-filled extracellular and intracellular vestibules that are separated by a 20 Å long channel that constricts to ~2.8 Å at its narrowest point [9–12]. This narrow channel limits the size of molecules that can pass through and forces water molecules to flow in single file. In liquid form, water molecules form hydrogen bonds with each other, which facilitate rapid conduction of protons between molecules. Free hydrogen bonding does occur within the AQP pore, except at its narrowest point, which is delimited by the NPA triplet motif. At this section, water molecules reorientate to transiently form partial hydrogen bonds with the conserved asparagines in the NPA motif. It has been argued that this reorientation of water promotes hydrogen bonding to the channel and not adjacent water molecules, thus preventing the conduction of protons [14]. However, more recent studies suggest that a direct electrostatic barrier across the pore, centered around the NPA region, forms the major impediment to the passage of protons [15,16].

Characterization of other proteins structurally related to AQP1 has demonstrated that AQPs vary in their water permeability, and, in many cases, facilitate the passage of larger solutes. The AQP-like GlpF transports glycerol [11]. The human aquaglyceroporins, AQP3, AQP7, AQP9 and AQP10, are also permeable to glycerol [8]. Studies using radiolabeled ligands have shown that some AQPs, for example, AQP9, facilitate the passage of other solutes, including urea, linear polyols, purines, pyrimidines and nucleosides [17]. AQP7 and AQP9 are also reported to be permeable to heavy metal salts (e.g. arsenite) [18]. As has been reported for plant aquaporins, some mammalian AQPs could facilitate the permeation of dissolved gases such as carbon dioxide and ammonia [19,20]. Such a role is supported by the recent report of specialized ammonia-permeating channels in bacteria [21]. Although it is generally accepted that most AQPs preclude the passage of ions, AQP6 has been reported to be permeable to anions such as nitrate [22] and chloride [23].

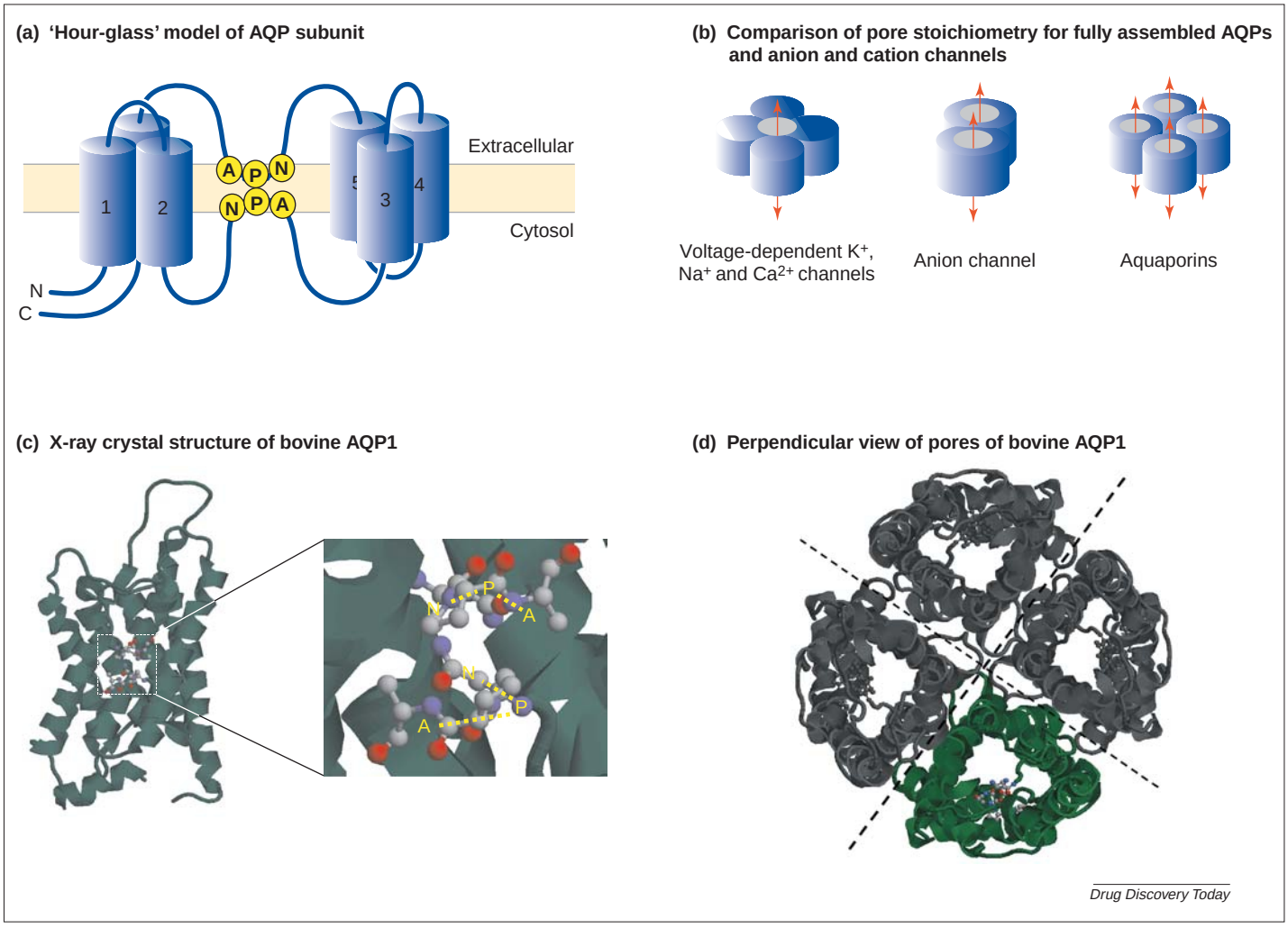

FIGURE 1

Topology of aquaporins. (a) 'Hour-glass' model of AQP subunit topology showing the arrangement of six transmembrane domains (1–6) and the conserved NPA-containing loops that form the selectivity filter of the water-conducting pore. **(b)** Comparison of pore stoichiometry for fully assembled AQPs, cation and anion channels. AQPs comprise four subunits, each with a water-conducting pore. By contrast, anion channels comprise a dimer of subunits, each of which contain a pore, whereas cation channels have a single pore that is formed by the central axis of tetrameric subunits (potassium channels) or four-fold repeats (sodium and calcium channels). **(c)** A ribbon representation of the X-ray crystal structure of a single bovine AQP1 [10] subunit viewed from the side [protein structure Protein Data Bank file (1J4N) rendered with Rasmol]: the location of the conserved NPA loops are shown as an inset to the right. **(d)** A ribbon representation of the X-ray crystal structure of the bovine AQP1 tetramer, viewed from the extracellular side and perpendicular to the pores. The subunit symmetry is indicated by broken lines and a single monomer of the tetramer is illustrated in green.

Although the movement of water through AQPs is primarily driven by osmotic gradients, there is evidence that permeation can be modulated by external factors. AQP3 permeability is reduced at acidic pH, whereas the water and ion permeability of AQP6 is enhanced under similar conditions [22,24]. Furthermore, it has been reported that the water permeability of AQP4 is reduced following protein kinase C activation [25] and that AQP1 could be gated by cyclic GMP [26].

It has long been known that water transport in many cells is sensitive to inhibition by mercury-containing compounds, and the permeability of many human AQPs is also affected by these substances. Early studies demonstrated that AQP1 has a cysteine residue (C189) near the NPA motif in the pore-forming loop that connects transmembrane domains five and six. Mutation of this cysteine to serine results in a loss of inhibition by mercury [27]. Similar pore loop cysteine residues are present in other mercury-sensitive AQPs [28]. By contrast, the mercury insensitive AQP, AQP4, lacks cysteines at any of the known mercury-sensitive sites [29].

Because of its known toxicity and other pharmacological actions, mercury is not the ideal inhibitor to characterize AQP function. Unfortunately, there are only a few other agents that inhibit AQPs: silver salts inhibit AQP1 (as well as plant AQPs) [30]; tetraethyl ammonium, which is widely used as a blocker of potassium channels, blocks AQP1 [31]; and phloretin is a relatively potent inhibitor of several AQPs, including the aquaglyceroporin AQP9 [17]. However, phloretin lacks specificity, exhibiting inhibitory activity against solute transporters, as well as ion channels [32,33].

The absence of good pharmacological modulators has presented a challenge for defining the function of AQPs in physiological and pathophysiological processes. Indeed, much of what is known about AQP function has been determined using non-pharmacological strategies. mRNA and protein expression studies have helped to define potential roles by characterizing tissue, cellular and subcellular AQP localization. The association of mutations of genes encoding AQPs with human hereditary disorders has also aided understanding of AQP function. Some of the most significant advances in defining physiological functions for water-conducting channels have come from the detailed phenotype analysis of transgenic mice lacking genes encoding AQPs. The impact of expression analysis, the study of human genetic disorders and transgenic mice on advancing understanding of the role of AQPs in physiological and disease processes is summarized in the following sections.

Physiology and pathophysiology of aquaporins

Kidney

In humans, the kidney is primarily responsible for filtering and eliminating toxic substances from the blood. This function is achieved by the filtration of blood in specialized units called nephrons, which have important functions in the reabsorption of water, active solute transport and acid–base balance. Adult human kidneys filter >150 l of blood each day. To maintain water balance, much of the water in the filtrate (>99%) must be reabsorbed before it leaves the kidney as urine. Approximately 80% of the water is reabsorbed back in to the blood by the epithelium lining and the proximal tubule and thin descending limb of the nephron. The remaining ~19% is reabsorbed across the epithelia that line the collecting duct. The proximal tubule, thin descending limb and collecting duct of the nephron express the highest levels of AQPs (Figure 2). AQP1 is expressed in the apical and basolateral membranes

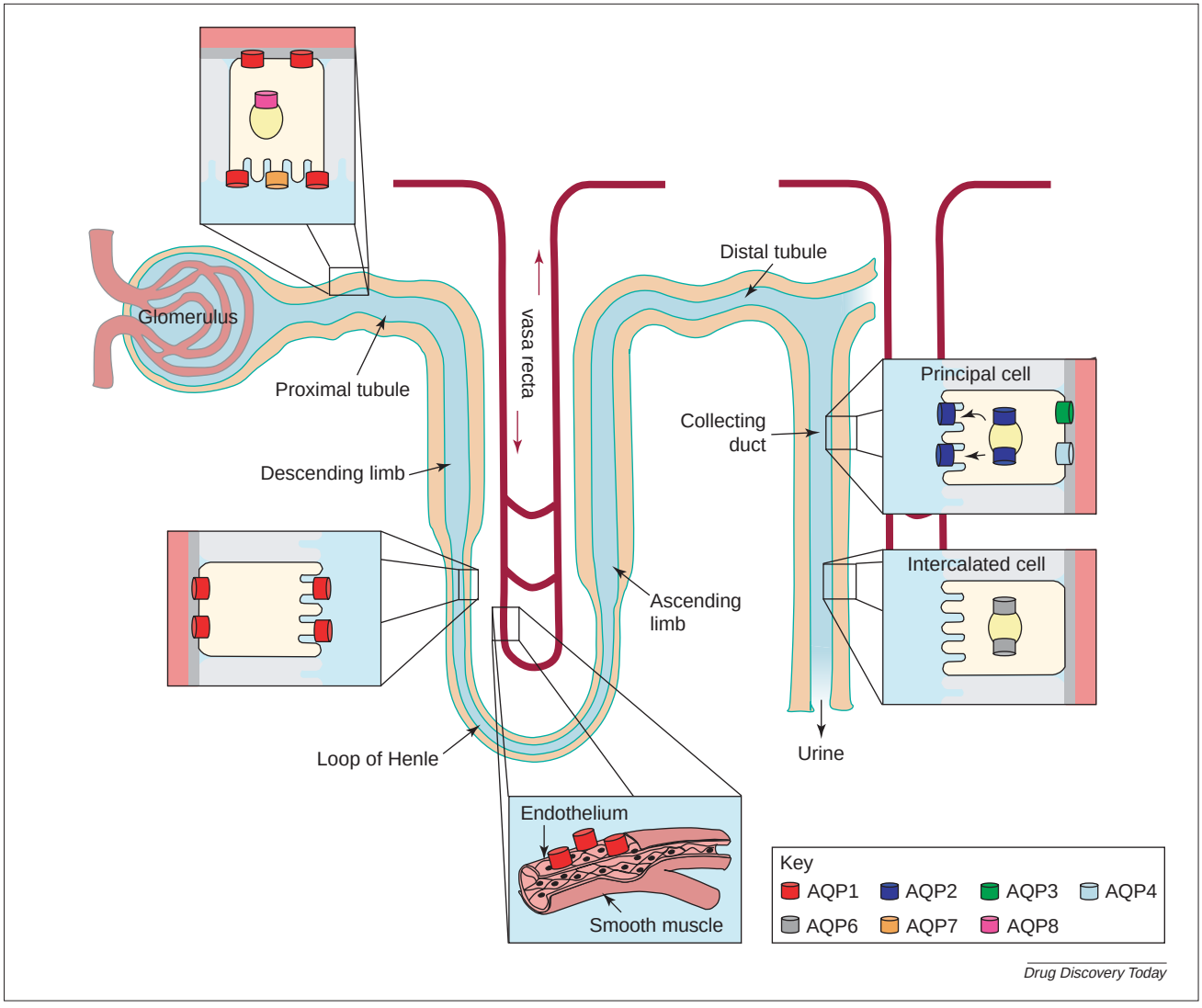

FIGURE 2

Distribution of aquaporins in human kidney. Graphic representation of functional segments of a renal nephron. Blood is filtered by the glomerulus. Of the water in the filtrate, >99% is reabsorbed by epithelia in the proximal tubule, descending limb and collecting ducts (inset figures illustrate the regional distribution of AQPs in the water-transporting epithelia).

of the epithelia lining the proximal tubule and thin descending limb, as well as the endothelium lining the descending vasa recta blood vessel in to which water is reabsorbed [34,35]. The importance of AQP1 in water reabsorption has been demonstrated in mice with a targeted knockout of this AQP. These mice exhibit polyuria (increased urine output) and the inability to concentrate urine [36]. Interestingly, humans lacking functional AQP1 (Colton-null blood antigen) exhibit normal urine output and renal function, except under stress conditions. When deprived of water, individuals lacking AQP1 exhibit a 50% reduction in their ability to concentrate urine [37].

Individuals with the autosomal form of the hereditary genetic disorder nephrogenic diabetes insipidus (NDI) have loss-of-function mutations in *AQP2* and exhibit a profound increase in the production of dilute urine (up to 20 l day^{-1}) [38,39]. In the nephron, AQP2 is expressed in the apical membrane and intracellular vesicles within principal cells of the collecting duct, where the final stages of water absorption and urinary concentration take place [34]. Apical expression of AQP2 is normally controlled by the pituitary antidiuretic hormone vasopressin. When vasopressin binds to its receptor on the basolateral membrane of collecting duct principal cells, intracellular vesicles containing AQP2 fuse with the apical membrane, which increases the apical water permeability and facilitates water reabsorption [40]. Reductions in vasopressin levels in plasma, or loss-of-function or expression of collecting duct principal cell vasopressin 2 (V_2) receptors (the major cause of hereditary NDI), result in a decreased expression of apical AQP2, reduced water reabsorption and consequently increased diuresis. Similarly, the mutations in *AQP2* underlying autosomal dominant NDI result in a trafficking defect that prevents translocation of the AQP from non-endoplasmic reticulum cellular organelles to the collecting duct apical membrane, which, again, leads to diuresis [38,39]. There is evidence that several clinically relevant forms of acquired NDI, including lithium, hypokalemic and cisplatin-induced nephropathy, are also associated with decreased functional expression of AQP2 [34]. In conditions associated with retention of water, such as pregnancy, congestive heart failure and cirrhosis of the liver, significant increases in apical AQP2 expression have been observed [34,41]. As has been demonstrated for V_2 receptor antagonists, development of inhibitors of AQP2 could prove effective in reducing water retention in these conditions [41].

Water transport across the collecting duct principal cells is also facilitated by the basolateral expression of AQP3 and AQP4. The importance of basolateral AQPs is evident from the observation that mice lacking AQP3 exhibit profound polyuria [42]. Interestingly, although AQP4 accounts for 75% of basolateral water transport, mice lacking AQP4 only exhibit a moderately reduced ability to concentrate urine [43].

In addition to facilitating renal water transport, AQPs, such as AQP6, could also be involved in maintaining acid–base balance. AQP6 is coexpressed with H^+-ATPase in intercellular vesicles of acid-secreting cells of the collecting duct [22]. In contrast to other AQPs, AQP6 appears to have a significant permeability to anions and is activated by decreases in pH [22,23].

Respiratory tract

Water transport in the airways of humans and other mammals is an essential component of important physiological processes, including humidification of inspired air and maintenance of the airway surface liquid (ASL) required for exchange of gases, barrier function and clearance of foreign material. To facilitate the transport of water, AQPs are expressed throughout the respiratory tract [44]. In the ciliated epithelia that line the upper airway, AQP5 is expressed in the apical membrane, whereas AQP4 is expressed in the basolateral membrane. AQP5 is also expressed in secretory cells within the submucosal glands, as well as in the apical membrane of type 1 pneumocytes in the alveoli within the lower airway. AQP3 is expressed in basal epithelia in the upper airway, whereas AQP1 is found predominantly in the vascular endothelia within the venule and capillary beds surrounding the airways and alveoli.

Studies of transgenic null mice lacking AQPs have provided evidence for the functional roles of these proteins in lung. Mice lacking AQP5 exhibit reduced secretions from submucosal glands, which contribute to ASL composition, viscosity and volume [45]. Increased viscosity of ASL can impede ciliary clearance of bacteria, which could promote airway infections via a mechanism similar to that occurring in individuals with cystic fibrosis [46]. AQP5-null mice also exhibit a 10–30-fold reduction in osmotic water permeability across the alveolar epithelium [47]. These mice also have comparable decreases in water permeability across lung vascular endothelium. Similar decreased pulmonary vascular water permeability has also been observed in humans lacking functional AQP1 (Colton-null) [48].

Whereas pulmonary fluid overload (edema) is a major cause of human morbidity, the function of AQPs in this case is unclear. Edema formation following adenoviral infection in mice has been associated with a reduction in AQP1 and AQP5 expression [49]. However, development of pulmonary edema in response to inflammation is similar in wild-type mice and mice lacking AQP1 or AQP5 [50].

Despite the extensive expression of AQPs in the respiratory tract, a broad role in airway function remains to be established fully. However, it has been argued that inhibition of AQP5-dependent secretion in airway submucosal glands could provide a therapeutic strategy for reducing fluid secretion in bronchitis and rhinitis, whereas AQP5 activation could potentially reduce viscous fluid secretion in cystic fibrosis [44].

Nervous system

Maintenance of the ionic and osmotic composition and volume of interstitial, glial and neuronal compartments

within the brain and spinal cord is essential for normal function. Small changes in intracellular or extracellular ion or solute composition can dramatically alter neuronal signaling and information processing. Because of the rigid constraints of the cranium and vertebrae, changes in total brain or spinal cord volume can cause devastating neurological damage. Pathophysiological accumulation of water in brain (edema) accounts for much of the morbidity and mortality observed in individuals suffering stroke, traumatic brain injury and brain tumors.

Several recent studies have shown that AQPs are important in central nervous system water homeostasis and neuronal signaling [51,52]. AQP1 is expressed in the apical membrane of the choroid plexus epithelium and could be involved in the production of cerebral spinal fluid (CSF) [53]. Moreover, AQP1 is expressed in the endothelia of brain tumors but not in normal brain endothelium, which suggests the possible involvement of AQP1 in tumor growth [54].

In contrast to AQP1, AQP4 and AQP9 appear to be predominantly expressed in astrocytes in the brain and spinal cord [51]. Astrocytes are star-shaped glial cells that have long processes that radiate to surround nearby blood vessels: the expanded endings of astrocyte processes are known as end-feet. AQP4 expression is localized to the membrane of astrocytic end-feet adjacent to vascular endothelium that forms the blood–brain barrier, suggesting a role in vascular–glial water transport. The observation that cerebral edema caused by traumatic brain injury, brain ischemia or water intoxication in rodents is accompanied by increased expression of AQP4 in astrocytes also implicates AQP4 in brain pathophysiology [51]. Furthermore, transgenic mice lacking functional AQP4 exhibit increased protection from brain edema produced by water intoxication or ischemic injury [55]. In contrast to these findings, AQP4-mediated transcellular water movement has recently been shown to be essential for fluid clearance in vasogenic brain edema (increased permeability of the blood–brain barrier), which occurs during cerebral infections, vascular disease trauma and tumor growth [52,56]. Therefore, inhibitors and activators of AQP4 could potentially provide treatments for cytotoxic and vasogenic brain edema, respectively.

Astroglial water transport mediated by AQP4 is coupled to ion fluxes through ion channels and transporters [57]. Changes in ionic composition of fluids within and around neurons influence neuronal excitability. Mice lacking AQP4 exhibit decreased susceptibility to seizures induced by the chemical convulsant pentylenetetrazole [58], which raises the possibility that AQP inhibitors could also have utility in the treatment of seizure disorders.

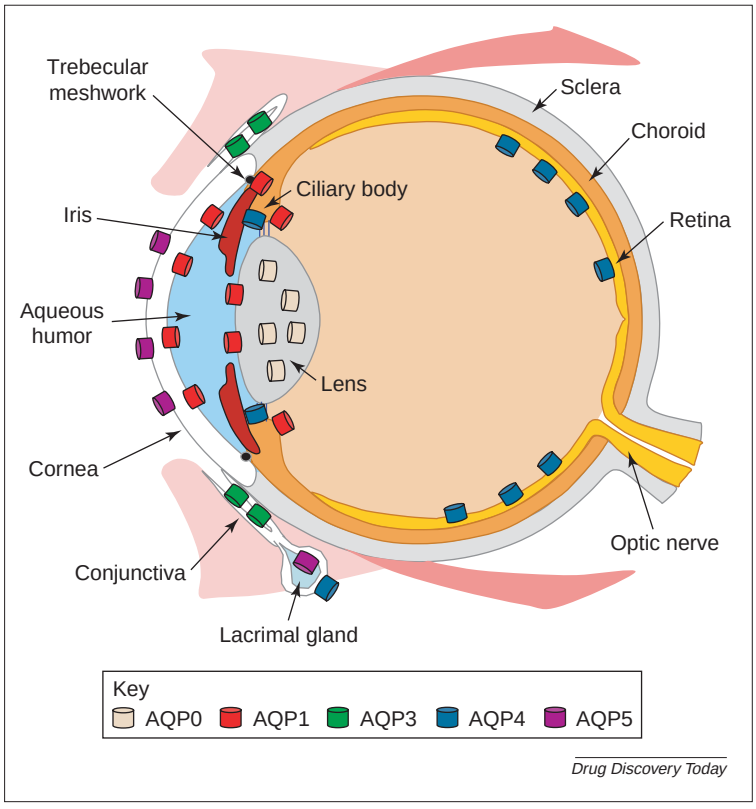

FIGURE 3

Distribution of aquaporins in human eye. AQP0 is expressed in the fiber cells of the lens. AQP1 is expressed in the epithelial cells lining the lens, as well as in the ciliary body and trabecular meshwork, which contribute to the formation and reabsorption of aqueous humor. The epithelium of the conjunctiva expresses AQP3, whereas AQP4 is expressed in glial and Müller cells within the retina, as well as in epithelia in the ciliary body. AQP5 is expressed in apical epithelia of the cornea and in tear-secreting lacrimal glands.

Eye

Water transport is an integral component of eye function [59]. Apart from the obvious production of tears in lacrimal glands, water movement across endothelium and epithelium is essential for maintaining corneal and lens transparency. The continuous formation of aqueous humor in the non-pigmented epithelia of the ciliary body and its drainage through the trabecular meshwork and canals of Schlemm is required to maintain intraocular pressure (IOP). Transport of water across retinal epithelium also helps to maintain retinal adhesion and integrity.

At least five AQPs are expressed in human eye: (i) AQP0 in the lens; (ii) AQP1 in corneal endothelium, ciliary and lens epithelia and trabecular meshwork; (iii) AQP3 in conjunctiva; (iv) AQP4 in ciliary epithelium and retinal Müller cells; and (v) AQP5 in corneal and lacrimal gland epithelia [59] (Figure 3). The importance of AQPs in ocular physiology and pathophysiology is evident from the finding that genetic defects in AQP0 are associated with hereditary cataracts (increased opacity of the lens) [60,61]. Functional knockout of AQP1 and AQP4 in mice results in the reduction of IOP, a finding that led to the speculation that pharmacological inhibitors of these AQPs could have utility in the treatment of glaucoma [62]. AQP1- and AQP5-null mice exhibit changes in corneal thickness, which, in the case of the AQP1 knockout, is also associated with alterations in corneal transparency [63]. Impaired cellular expression of AQP5 in lacrimal glands of humans with Sjogren's syndrome could also contribute to the reduced tear production often observed with this disease [64].

TABLE 2

Clinical conditions associated with known or proposed alterations in aquaporin function or expression

Disorder or disease	Aquaporin involved
Congenital cataracts[a]	AQP0
Colton-null blood antigen transfusion incompatibility[a]	AQP1
Glaucoma	AQP1 and AQP4
Hereditary NDI[a]	AQP2
Chemotherapy-induced polyuric acute renal failure	AQP2
Congestive heart failure	AQP2
Water retention associated with liver cirrhosis	AQP2
Water retention during pregnancy	AQP2
Brain edema (caused by traumatic injury or tumors)	AQP4
Seizures	AQP4
Dry eyes (Sjogren's Syndrome)	AQP5
Dry mouth (Sjogren's Syndrome)	AQP5
Deficient plasma glycerol during exercise[a]	AQP7[b]
Hyperinsulinemia	AQP7 and AQP9

[a]Diseases caused by genetic defects in human aquaporins. [b]G264V mutation in AQP7 is responsible for this condition [68].

Additional roles for aquaporins

In addition to an obvious water-transporting role, it is becoming increasingly clear that the transport of other solutes contributes to the physiological importance of AQPs. The aquaglyceroporins AQP9 and AQP7 are expressed in liver and adipose tissue, respectively [65–67]. Glycerol (produced by lipolysis) is transferred from adipose tissue to the plasma by AQP7 [66,67]. Plasma glycerol is taken up by liver via AQP9 to provide a substrate for hepatic glucose synthesis (gluconeogenesis) during prolonged fasting [65,67]. Coordinated upregulation of AQP7 and AQP9 mRNA expression in rodents during fasting, diabetic insulin deficiency and insulin-resistant hyperinsulinemia indicates the importance of aquaglyceroporins in physiological and pathophysiological glucose metabolism [67]. In humans, loss-of-function genetic defects of *AQP7* are associated with an inability to elevate plasma glycerol during exercise [68].

Glycerol transport through the aquaglyceroporin AQP3 has an important function in skin hydration and barrier function. Mice lacking AQP3 exhibit a decreased hydration of the stratum corneal layer of the skin, which can be resolved by either topical or oral administration of glycerol [69].

In addition to the liver, AQP9 is also highly expressed in leukocytes, where it is thought to be involved in water movement required for cell migration [70]. Furthermore, AQP9, together with other AQP subtypes, is also postulated to have a significant role in reproductive physiology, particularly spermatogenesis and uterine implantation of blastocysts [71]. Auditory physiology could involve water transport through AQPs – mice lacking functional AQP4 exhibit hearing deficits, which in some strains result in complete deafness [72]. Although AQPs are also expressed in many tissues along the gastrointestinal tract, their functions in absorption and secretory processes in this system remain to be elucidated fully [73].

Future prospects: aquaporins as drug development targets

Water transport is a fundamental process contributing to human physiology and pathophysiology. The importance of AQPs in the physiology of water and solute movement is now clear [74]. Therefore, targeted pharmacological modulation of water and solute transport using AQPs would appear to provide novel opportunities for therapeutic interventions in a variety of human disorders (Table 2). Currently, there are few pharmacological modulators of AQPs available, and those that are known lack specificity or are toxic. Furthermore, the broad tissue expression of AQP subtypes in humans will probably necessitate the identification and development of subtype-selective AQP modulators. Discovering new AQP modulators presents a challenge because screening strategies commonly used in the pharmaceutical industry to identify modulators of ion channels, G-protein-coupled receptors and enzymes might not be appropriate for water channels. Moreover, there are no known specific high-affinity ligands for AQPs that can be used in radioligand binding assays. Although direct measurement of water movement through AQPs is probably not feasible in the context of drug discovery assays, measurement of fluxes of radiolabeled AQP permeable solutes, such as glycerol and urea [17,65], is more promising. Technologies employed to evaluate cellular water transport can potentially be used to measure AQP function indirectly [75]. For example, water movement-induced variations in cell volume are associated with changes in cell light-scattering properties. More quantitative measurements of changes in volume can also be achieved using confocal and internal reflectance fluorescence microscopy. Osmotic water permeability in cells has been successfully measured using cell volume-sensitive fluorescent indicators [75,76]. The known modulation of several cell membrane chloride and potassium permeable ion channels by changes in cell volume could also potentially be used to measure AQP function indirectly [77]. However, it remains to be determined if any of these methodologies will prove amenable to conversion to the high-throughput formats required to screen the large compound libraries available at pharmaceutical and biotechnology companies.

Although the development of drugs that target modulation of AQP function is at an early stage, success in such an endeavor could open the door to treatments of life-threatening disorders, for example, congestive heart failure, stroke, traumatic injury and tumor-induced brain swelling, as well as other conditions such as glaucoma and cystic fibrosis, which currently lack adequate medical treatment.

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