



## Review

## Human aquaporins: Regulators of transcellular water flow☆☆



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## ABSTRACT

**Background:** Emerging evidence supports the view that (AQP) aquaporin water channels are regulators of transcellular water flow. Consistent with their expression in most tissues, AQPs are associated with diverse physiological and pathophysiological processes.

**Scope of review:** AQP knockout studies suggest that the regulatory role of AQPs, rather than their action as passive channels, is their critical function. Transport through all AQPs occurs by a common passive mechanism, but their regulation and cellular distribution varies significantly depending on cell and tissue type; the role of AQPs in cell volume regulation (CVR) is particularly notable. This review examines the regulatory role of AQPs in transcellular water flow, especially in CVR. We focus on key systems of the human body, encompassing processes as diverse as urine concentration in the kidney to clearance of brain oedema.

**Major conclusions:** AQPs are crucial for the regulation of water homeostasis, providing selective pores for the rapid movement of water across diverse cell membranes and playing regulatory roles in CVR. Gating mechanisms have been proposed for human AQPs, but have only been reported for plant and microbial AQPs. Consequently, it is likely that the distribution and abundance of AQPs in a particular membrane is the determinant of membrane water permeability and a regulator of transcellular water flow.

**General significance:** Elucidating the mechanisms that regulate transcellular water flow will improve our understanding of the human body in health and disease. The central role of specific AQPs in regulating water homeostasis will provide routes to a range of novel therapies. This article is part of a Special Issue entitled Aquaporins.

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## 1. Introduction: Transcellular water flow: a regulatory role for human

## 1.1. Aquaporins

A striking property of most human tissues is their capacity for extremely rapid fluid transport. This is exemplified by urine concentration in the kidney [1], the rapid formation of a fluid-filled cavity adjacent to the oocyte during ovarian folliculogenesis [2] and secretion of saliva from salivary glands [3]. These processes are essential to human health and rely on the highly-regulated transport of water through tissues [4,5]. Such trans-tissue water flow is possible by two routes: transcellular water flow across both basal and apical membranes, which occurs in response to the osmotic stimuli [4] created by salt transport [6]; or

paracellular flow across cell–cell junctions into intercellular spaces, driven by salt or solute gradients [6]. Paracellular water flow plays an important role in leaky epithelia such as the corneal endothelium [7]. In this review, we focus on the regulatory role of aquaporin (AQP) water channels in mediating transcellular water flow across cell membranes in the major systems of the human body (Fig. 1). The study of the mechanisms of human AQP regulation that mediate transcellular water flow is still in its infancy and therefore this review will also discuss mammalian AQPs as potential models for the regulation of human AQPs (Table 1).

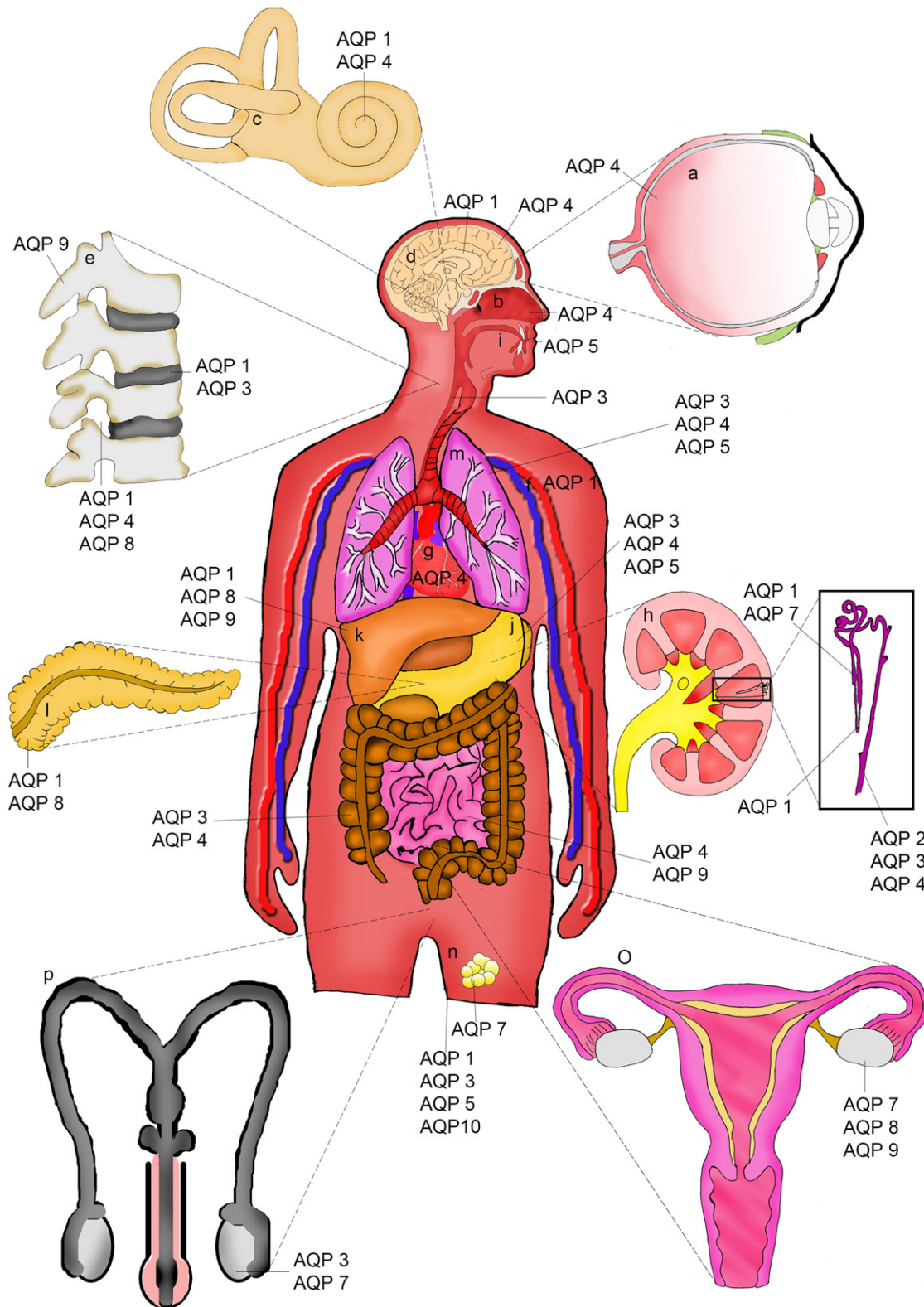
Transcellular water flow is dependent on the permeability of the plasma membrane to water molecules. Water movement by osmosis may be through the lipid bilayer, by passive co-transport with other ions and solutes [8] or through AQP water channels [9]. Many AQP channels are thought to have an exquisite specificity for water and are capable of rapidly transporting it in response to changes in tonicity; evidence suggests that they make a critical contribution to the regulation of transcellular water flow [10].

Since the first AQP was identified by Peter Agre in 1988 [11], thirteen human AQPs have been discovered. Structural results for several family members [12–14] have established that AQP channels share a common

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**Fig. 1.** Aquaporin expression in humans. The figure shows the wide distribution of AQP water channels throughout the human body. Organs are highlighted, starting at top right; within each organ, the major AQPs involved in transcellular water flow are denoted; a) Retina – AQP4, b) Olfactory epithelium – AQP4, c) The inner ear – AQP4 and AQP1, d) Brain – AQP4 in astrocytes and AQP1 in choroid plexus, e) Spinal cord – AQP1, AQP4 and AQP8; Nucleus pulposus cells of the intervertebral disc – AQP1 and AQP3; Osteoclasts – AQP9, f) Blood vessels – AQP1 in endothelial cells, g) Heart – AQP4, h) Kidney (showing the nephron in detail) – AQP1, AQP2, AQP3, AQP4 and AQP7, i) Salivary glands – AQP5, j) Gastrointestinal tract – AQP3, AQP4, AQP5 and AQP9, k) Liver – AQP1, AQP8 and AQP9, l) Pancreas – AQP1 and AQP8, m) Lungs – AQP3, AQP4, AQP5, n) Fat (adipocytes) – AQP7; Skin – AQP1, AQP3, AQP5 and AQP10, o) Female reproductive tract – AQP7, AQP8 and AQP9 in ovaries, and p) Male reproductive system – AQP3 and AQP7 in sperm cells.

**Table 1**

Tissue distribution and roles of AQP in transcellular water flow. AQPs discussed in this review are presented in terms of their localisation and regulatory roles in transcellular water flow within organ tissues.

System	AQP protein localisation	Role of aquaporins in transcellular water flow	References	
Nervous	Retina – AQP4	Suggested role in Muller cell water balance.	[64]	
	Olfactory epithelium – AQP4	Membrane permeability - link to olfaction.	[65]	
	Inner ear – AQP4 (hensons, claudius and Inner Succus cells), AQP1 (fibrocytes)	AQP4 mediated transcellular water flow in to Henson cells exiting via AQP4 on basal membrane of Claudius cells	[66]	
	Brain – AQP4 astrocytes, AQP1 (choroid plexus)	AQP4 at astrocyte end feet for BBB water permeability. AQP1 secretion of CSF, AQP4 absorption of CSF	[62,47]	
Renal (kidney)	Spinal cord – AQP1, AQP4, AQP8	Localisation of AQPs suggests transcellular water flow from perivascular space to interstitium, into central canal	[54]	
	Proximal tubule – AQP1 (apical and basolateral), AQP7 (apical of convoluted and straight)	Water reabsorption, importance of AQP7 unknown	[9,69]	
	Renal collecting duct cells – AQP2 (apical, sub apical vesicles), AQP3 and AQP4 (basolateral)	Urine concentration by AQP2 AVP mediated water absorption - AQP3 and 4 exit pathways into blood	[63,68]	
	Descending thin limb of henle – AQP1	Water reabsorption	[9]	
	Descending vasa recta – AQP1	Water reabsorption	[9]	
Integumentary	Connecting tubule – AQP3	Water homeostasis	[9]	
	Skin – AQP1 (endothelia of dermis), AQP3 + 10 (keratinocytes of epidermis), AQP5 (sweat glands)	Homeostasis, glycerol or water transport for skin hydration, sweat excretion	[96–98]	
Cardiovascular	Fat – AQP7 (adipocytes)	Glycerol transport	[102]	
	Blood vessels – AQP1 strongly expressed in endothelia outside of brain	i.e. Airspace-Capillary osmotic water permeability and heart vasculature	[112]	
Respiratory	Cardiomyocytes – AQP4	Absorption of excess water from interstitial space into to capillaries	[113]	
	Lung alveolar epithelium – AQP5 (apical membrane)	Transcellular water flow route for water absorption and secretion in airway, Role in airway hydration	[117]	
	Airway epithelial lining – AQP3 and AQP4	Possibly provide route for water into capillaries of airway	[87]	
Reproductive	Airway sub-mucosal glands – AQP5 (apical membrane)	Fluid secretions into lumen of submusosal glands for mucous production and hydration	[119]	
	Ovarian granulosa cells – AQP7, AQP8, AQP9	Transcellular water flow in folliculogenesis	[2]	
	Epididymis	Transepithelial water transport and sperm concentration	[129,135,137,138]	
Digestive	Sperm – AQP3, AQP7	CVR to prevent swelling and to aid mobility	[41]	
	Salivary Glands – AQP5 (acinar cells, intercalated duct cells), AQP8 (myoepithelial cells)	Transcellular water transfer in process of primary saliva secretion	[87,147]	
	Oesophagus – AQP3 (stratified epithelia)	Intracellular osmolarity and CVR to water deprived cells	[143]	
	Stomach – AQP3 (stratified epithelia), AQP4 (BLM parietal cells), AQP5 (pyloric gland)	Provide water to cells facing harsh conditions, AQP4 - gastric acid secretion, AQP5 transcellular water secretion for mucous production	[141,143,144]	
	Small intestine – AQP4, AQP9 (goblet cells)	Transcellular colonic fluid transport, AQP9 aids in mucous secretion	[149]	
	Colon – AQP3 (simple + stratified epithelia of distal colon), AQP4 (surface epithelia)	Water absorption from intestine and colonic fluid transport	[144,149]	
	Liver – AQP1 (cholangiocytes), AQP8 (hepatocytes), AQP9 (sinusoidol membrane of hepatocyte)	AQP8 – osmotic driven water transfer and homeostasis, AQP9 – glycerol uptake from blood released by AQP7	[82,152]	
	Pancreas – AQP1 (inter/intralobular ducts), AQP8 (acinar cells)	AQP1 – Transcellular water transfer and pancreatic juice secretion, AQP8 – Pancreatic juice secretion	[146]	
	Musculoskeletal	Muscle fibres – AQP4	Contraction-induced muscle swelling	[160]
		Articular cartilage – AQP1, AQP3	Involved in cell swelling during mechanistic load	[159]
Intervertebral disc – AQP1, AQP3 (nucleus pulposus cells)		AQP1 and 3 involved in NP cell swelling during mechanistic load	[159]	
	Osteoclasts – AQP9	AQP9 osteoclast differentiation and cell fusion – increase in cell volume	[154,156]	

structural architecture. Together with biochemical studies, these structural data [15] have revealed that the functional AQP unit is a homotetramer [15] and that each AQP monomer is composed of six transmembrane (TM)  $\alpha$ -helices connected by alternating intracellular (ICL) and extracellular (ECL) loops. The TM domains form a right-handed bundle around the central pore of each AQP monomer through which water transport occurs [16]. The specificity of the pore for water is a result of direct hydrogen bonding, in a pairwise manner, between a single file of water molecules and the AQP family's signature Asn-Pro-Ala motif at the narrowest part of the pore [16]. Water selectivity is further aided by interactions with the aromatic/arginine constriction site, which physically restricts the pore [16]. While the structural biology of the AQP family is therefore widely accepted, the mechanisms that regulate the physiological function of AQPs are less well established [17].

AQPs are expressed in a wide range of tissues (Fig. 1), often spatially located within a certain region of the cell. This enables them to play a central role in the flow of water through those tissues, which typically triggers cell volume regulation (CVR) mechanisms. Whilst transport through all AQPs utilises a common passive mechanism, their regulation and cellular distribution varies significantly between systems, specifically in constituent tissues and cells [17]. This review examines the regulation of transcellular water flow in the human body by AQP channels with a particular focus on CVR.

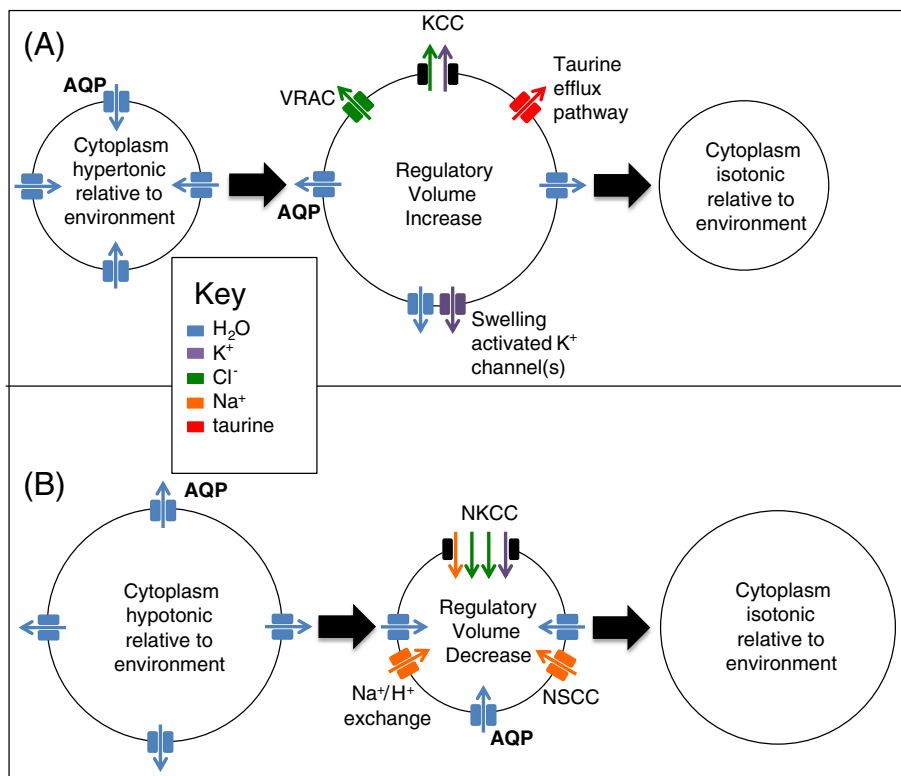
## 2. Transcellular water flow and cell volume regulation

CVR is a necessary mechanistic component of AQP-mediated transcellular water flow (Fig. 2). It comprises regulatory volume decrease (RVD), usually in response to hypotonicity-induced cell swelling, and regulatory volume increase (RVI), usually in response to hypertonicity-induced cell shrinkage. The molecular mechanisms underlying these responses are not yet fully understood, but it is unlikely that there is a

single common mechanism [18]. The signalling pathways associated with CVR appear to be cell-type dependent [18]. Nonetheless, the end results of these varied pathways are similar: RVD relies on osmolyte (potassium chloride and taurine [19]) and water efflux from the cell to reduce cell volume whereas RVI is achieved by osmolyte and associated water influx via import of sodium to the cell. Although the rapid RVI process following cell shrinkage involves inorganic ions, after hours of prolonged hypertonic exposure, animal cells often replace the ions with non-perturbing organic osmolytes. The mechanisms for this include external transport into the cell, down-regulation of organic degradation and up-regulation of organic synthesis [20]. These mechanisms facilitate the homeostasis of osmolality within the cell.

### 2.1. Regulatory volume decrease

In RVD (Fig. 2), the activation of  $K^+$  channels allows efflux of  $K^+$  from the cell and subsequent water loss by osmosis either through AQPs or directly through the lipid bilayer; biophysical data show that AQP expression can increase membrane water permeability by up to ~50 fold [21,22]. This  $K^+$  efflux can be either dependent on intracellular calcium concentration [ $Ca^{2+}$ ] (e.g. in human cervical cancer cells [23]) or [ $Ca^{2+}$ ]<sub>i</sub> independent (e.g. in Ehrlich ascites tumour cells [24]). In most cell types an intact actin cytoskeleton is necessary for hypotonicity-induced  $K^+$  efflux. However, in trigeminal ganglion neurons, cytochalasin D (an actin polymerisation inhibitor) treatment stimulated swelling activation of a  $K^+$  current [25] demonstrating that in these cells an intact actin network not only is unnecessary for RVD, but also appears to be inhibitory. In some cells, protein kinase C (PKC) activation has also been shown to induce an outward  $K^+$  current via the same channels that are activated in RVD [26]. Movement of  $K^+$  out of the cell is favoured by the concentration gradient but to maintain the electrostatic membrane potential, volume-regulated anion channel(s) (VRAC) simultaneously move anions (mainly  $Cl^-$  during RVD)



**Fig. 2.** Solute transport pathways mediating CVR (A) regulatory volume decrease and (B) regulatory volume increase. Water movement by osmosis may be through AQPs or directly through the cell membrane depending on the cellular AQP isoforms and expression levels. KCC: potassium chloride co-transporter; NKCC: sodium potassium chloride co-transporter; VRAC: volume regulated anion channel; NSCC: non-selective cation channel.

out of the cell, probably activated by tyrosine kinases [27,28]. It is also thought that four  $K^+-Cl^-$  co-transporters (KCCs), known to be activated by cell swelling, may be involved [29,30].

## 2.2. Regulatory volume increase

In RVI (Fig. 2), the activation of  $Na^+-H^+$  exchangers and  $Na^+-K^+-2Cl^-$  co-transporters (NKCCs) causes cellular influx of  $Na^+$  and subsequent volume increase by osmotic movement of water [18]. The  $Na^+-H^+$  exchange pump, NHE1, is known to be activated by cell shrinkage [31], which may be mediated by binding of calmodulin to its carboxy-terminus [32]. The co-transporter, NKCC1, is known to be activated by cell shrinkage, potentially through lysine-deficient protein kinase 1 (WNK1) and proline/alanine-rich protein kinase (SPAK) signalling [33]. Amiloride-sensitive non-selective cation channels (NSCCs) may also play a role [34].

## 2.3. The regulatory role of aquaporins

The water permeability of cell membranes may not be the rate-limiting factor in CVR [35], but any rapid change in cell volume is likely to involve AQP. For example, the stretch-activated transient receptor potential vanilloid type 4 (TRPV4) channel is a  $Ca^{2+}$ -biased NSCC that is activated by cell swelling [36] and has been implicated in osmosensing [37]. In some cell types TRPV4 has been shown to provide a  $Ca^{2+}$  signal that is correlated with activation of the  $K^+$  and  $Cl^-$  channels responsible for the decrease in cellular osmolality associated with RVD [38,39]. In human and murine salivary gland cells, TRPV4 has a functional interaction with AQP5: in AQP5 knockout cells, the hypotonicity-induced calcium influx through TRPV4 was attenuated and subsequent RVD was abolished. Hypotonicity also increased cell surface expression of both TRPV4 and AQP5 and increased their co-localisation [40].

In another example, the sperm of AQP3  $-/-$  mice did not undergo their normal RVD process and the mice displayed reduced fertility [41,42]. Upon entry into the female reproductive tract, sperms normally encounter a decrease in extracellular osmolality, which is thought to be the signal that activates sperm motility [41]. However, this hypotonic stress also causes cell swelling which, if left uncorrected by RVD, leads to impaired fertilisation caused by excessive bending of the sperm tail inside the uterus [42]. If AQP3 was simply acting passively as a water channel, RVD would not be abolished in AQP3  $-/-$  mice but rather the timescale on which the cell reaches osmotic equilibrium would be increased. One explanation for reduced fertility and altered RVD in AQP3  $-/-$  mice is therefore that AQP3, either alone or as part of a macromolecular complex, is involved in the signalling pathway that activates RVD in sperm.

When exposed to a hypotonic extracellular solution, cultured renal cortical collecting duct (renal CCD) cells, which do not endogenously express AQP2, swell in proportion to the change in extracellular osmolality but do not exhibit RVD. However, when they are transfected with AQP2, these cells show an RVD of approximately 40%. Shrinkage is mediated by  $Ca^{2+}$  influx through TRPV4, which activates  $Ca^{2+}$ -dependent  $K^+$  and  $Cl^-$  channels and  $Ca^{2+}$ -dependent  $Ca^{2+}$  release from intracellular stores. In renal CCD cells expressing AQP2, hypotonic stress causes translocation of TRPV4 to the plasma membrane, which is absent in AQP2-negative cells. When TRPV4 was translocated to the cell surface prior to hypotonic exposure using 4 $\alpha$ -phorbol 12,13-didecanoate, RVD was recovered in AQP2-null cells, showing that it is not simply the high water permeability of AQP2 that allows RVD. However, there does not appear to be any co-localisation between endogenous TRPV4 and overexpressed AQP2 in this system, either before or after hypotonic shock, indicating a functional rather than physical interaction [43]. These observations suggest that AQP2 (and therefore possibly other members of the AQP family) forms part of a sensory and signalling pathway that results in TRPV4 translocation, possibly via sensing of extracellular osmolality.

Taken together, these examples support the idea of a signalling or sensory role for AQP in RVD mechanisms. We have been unable to find any evidence of AQP playing similar roles in RVI. However, given that a variety of AQP could be involved in the RVD mechanism and that RVI and RVD share a common basic mechanism involving the movement of osmolytes to elicit a volume change, it would not be surprising to discover a link between RVI and AQP that goes beyond a passive water conduction mechanism.

## 3. Regulation of transcellular water flow in the human body

### 3.1. The brain and nervous system

#### 3.1.1. Localisation of AQP

AQP are widely expressed in the central nervous system (CNS); indeed, more studies have been conducted on AQP in the CNS than on AQP in the peripheral and enteric nervous systems (for a detailed review on AQP in the nervous system see Papadopoulos et al. [44]). The major AQP found in the CNS are (i) AQP4 in the glia [45] and neurons [46], (ii) AQP1 in the epithelia of the choroid plexus (which forms the cerebrospinal fluid (CSF)-brain barrier) [47], dorsal root ganglia [48] and oesophageal neurons [49] and (iii) AQP9 in the substantia nigra [50]. Glial cells are not nerve cells but are essential for regulation and homeostasis of the CNS and comprise approximately 90% of cells in the brain [51]. Astrocytes are the most abundant glial cells within the brain [51] and high levels of AQP4 are expressed in their plasma membranes. The distribution of AQP4 is highly polarised to the astrocytic end feet, which contact the blood vessels associated with the blood-brain and brain-fluid interfaces [81]. The astrocytic end feet therefore function as part of the blood-brain-barrier (BBB) water exchange mechanism; this has been established to be AQP4 dependent since deletion of AQP4 in mice resulted in a 31% decrease in brain water uptake measured using the wet/dry mass method to determine brain water content [52].

#### 3.1.2. Regulatory role of AQP

As the main AQP of the CNS, AQP4 is expected to play a major role in the regulation of water flow in the brain, spinal cord [53,54] and interstitial fluid surrounding neurons, thereby maintaining the  $K^+$  concentrations required for neuroexcitation [46,55]. In support of this, when AQP4 was silenced in astrocytes by RNA interference, the apparent diffusion coefficient (which is a measure of water movement within tissues, determined by magnetic resonance imaging) was decreased by 50% in rat brain [56]. It is in this context that low AQP4 expression levels have been associated with epileptic seizures [55].

There is evidence that AQP translocation occurs in astrocytes, which may be responsible for the regulation of AQP abundance in the plasma membrane [57]. Although AQP4 is primarily expressed at the plasma membrane, its surface abundance is increased by AQP4-carrying vesicles in response to hypotonic stimuli in cultured rat cortical astrocytes: a positive correlation between vesicle mobility and AQP4 density at the plasma membrane was observed [58]. As well as AQP4, the stretch-activated TRPV4 channel is also strongly expressed in astrocytic end feet [59]. It has been suggested that AQP4 and TRPV4 are co-expressed and form a molecular complex, interacting at the plasma membrane to control CVR in astrocytes. When mouse primary astrocytes were put under hypotonic stress, an increase in intracellular  $Ca^{2+}$  was followed by an RVD response; this mechanism failed in cells deficient in AQP4 and in cells where RNA interference was used to silence TRPV4, even if they were transfected with AQP1 [59,60]. This suggests that RVD in astrocytes may be AQP4/TRPV4 specific, although the swelling of AQP1-expressing cells in this study was not as marked as for AQP4-expressing cells. Therefore, if total cell volume is the biophysical quantity being detected during the RVD response, it is possible that the loss of RVD simply reflects the fact that a volume threshold has not been met. If

there is an AQP4/TRPV4 interaction, it may be similar to the interaction between AQP2 and TRPV4 in renal cells [43], discussed above.

AQP4 has been largely associated with the pathophysiology of brain oedema [61] of which there are two major types: vasogenic oedema is the accumulation of water in the extracellular space, usually due to impaired function of the BBB; cytotoxic oedema is the swelling of astrocytes whilst maintaining their cellular integrity [44]. Movement of water from the blood across endothelia into astrocytes is mediated by AQP4 channels; reduced cytotoxic oedema was seen in AQP4  $-/-$  mice [62]. AQP4 is also thought to mediate the reabsorption of excess fluid in vasogenic brain oedema by transcellular movement of fluid from the extracellular space at the astrocytic end feet to the vascular and ventricular regions of the brain through the endothelial and epithelial barriers. In support of this, AQP4 null mice show an increase in brain water accumulation due to the reduction of transcellular water transfer to blood or CSF [61].

AQP1 expression is restricted to the choroid plexus region of the brain under normal conditions [47], but it is expressed in microvascular endothelia and reactive astrocytes of brain tumours where it is thought to play a role in the development of vasogenic oedema [63]. Osmotically-induced water transport was reduced 5-fold in AQP1 null mice along with a decrease in intracranial pressure and a 25% decrease in CSF production [47]. The same group also investigated the expression and localisation of AQPs in mouse spinal cord, an area that has not been the focus of many studies. Expression of AQP4 was detected in the astrocytic end feet of the grey matter in contact with capillaries and the fibrous glial processes of the white matter, again surrounding the blood vessels. The distribution of several other AQPs throughout the spinal cord suggests a role in maintenance of water balance via transcellular water flow; fluid moves rapidly from the spinal subarachnoid space into the perivascular space and across the interstitium into the central canal. This rapid water movement may be mediated by AQP4 and AQP9 in the glia limitans and AQP8 across the ependymal layer into the central canal [54].

In addition to its localisation in astrocytes, AQP4 is also expressed in the retina [64], the olfactory epithelium [65] and within Claudius' and Henson's cells of the inner ear [66]. Large  $K^+$  fluxes trigger an AQP4-mediated transcellular water influx that exits via AQP4 channels localised on the basal membrane of Claudius' cells [66]; AQP4 involvement in CVR may be an important mechanistic component of acoustic signal transduction since AQP4 null mice are completely deaf but have no alterations to the morphology of the inner ear [66].

### 3.1.3. Conclusions

Transcellular water flow within the brain is largely associated with the formation and clearance of oedema; the highly-polarised distribution of AQP4 at the end feet of astrocytes is key in the absorption of excess vasogenic fluid for clearance by the capillaries into the bloodstream. However, AQP4 may have an additive effect on water accumulation in cytotoxic oedema as summarised in Fig. 3. The positioning of AQP1 along the epithelial barrier of the CSF-containing choroid plexus indicates a role in supplying water for CSF via a transcellular route. It is also evident that AQPs play an important role in signalling throughout the nervous system's peripheral and sensory organs by their contribution to CVR mechanisms and  $K^+$  concentration maintenance. Understanding these mechanisms will be essential in developing treatments for brain oedema following stroke, trauma and meningitis.

## 3.2. The renal system

### 3.2.1. Localisation of AQPs

The role of AQPs in the kidney is one of the most extensively studied areas in AQP research [67]. The kidney is the major site of arginine vasopressin – (AVP; also called anti-diuretic hormone or ADH) mediated body water homeostasis; in response to osmotic stimuli, water reabsorption occurs through AQP channels enabling urine concentration.

AQPs in the kidney exhibit a highly-polarised and specific localisation pattern (Fig. 1): AQP1 is expressed in proximal tubule (PT) cells; AQP2 is very prominent in the renal collecting duct (renal CD) principal cells and is found at the apical plasma membrane [68] and in sub-apical vesicles [1]; AQP3 and AQP4 are expressed in renal CD cells at the basolateral membrane [69,70]. AQP1 is responsible for most water reabsorption in the kidney [71] while AQP2 fine tunes water reabsorption; it is translocated within the sub-apical vesicles to the apical membrane after AVP activation, increasing membrane water permeability [1]. Once water has entered a renal CD cell, AQP3 and AQP4 provide exit routes to the blood [72].

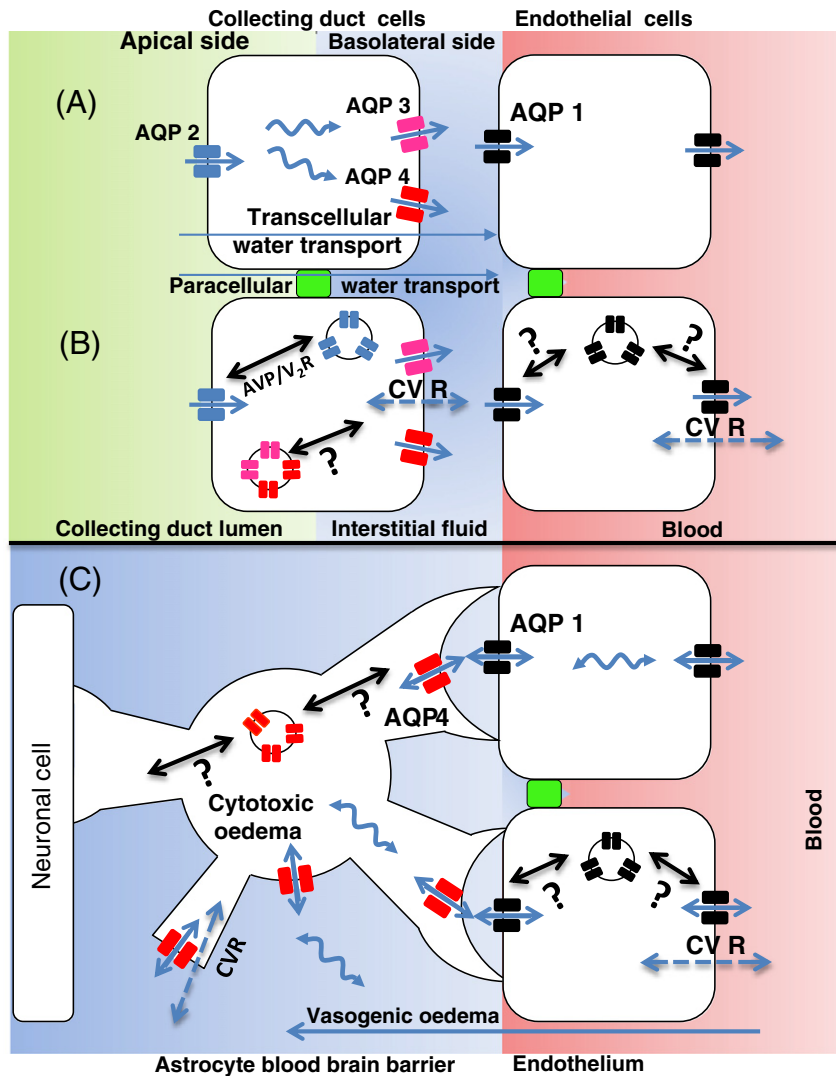
### 3.2.2. Regulatory role of AQPs

AQP2/AVP-activated translocation is induced by a well-studied protein kinase A (PKA)-dependent pathway [73]. Upon recognition of an osmotic stimulus, AVP is released and subsequently binds the G protein-coupled receptor (GPCR), vasopressin  $V_2$  receptor (V2R), on the basolateral membrane of renal CD principal cells. This results in G protein activation of PKA and subsequent phosphorylation of AQP2 at Ser266 within intracellular vesicles; this facilitates vesicle translocation along the microtubule networks to the apical membrane [74] (Fig. 3A, B). The critical role of this mechanism in the fine control of water reabsorption is exemplified by its dysfunction in nephrogenic diabetes insipidus; in this disease, mutations in the vasopressin 2 receptor or in AQP2 itself lead to a decreased ability to concentrate urine [75].

An intracellular  $Ca^{2+}$  influx is needed to generate the necessary RVD response following hypotonic exposure; in the distal kidney, this only occurs in the presence of AQP2 [76]. A functional interaction between the  $Ca^{2+}$ -permeable TRPV4 ion channel and AQP2 has been demonstrated to occur under hypotonic conditions in renal CD cells and is involved in RVD. Notably, the TRPV4 channel is only activated in cells that express AQP2. When TRPV4 is blocked by ruthenium red,  $Ca^{2+}$  entry is abolished along with RVD. This confirms AQP2 and TRPV4 as essential components of the hypotonicity-induced RVD cellular response [43]. It has also been shown that AQP2 can regulate CVR by interacting with tropomyosin 5b (TM5b) and altering the dynamics of the cytoskeleton. Ser256 phosphorylation induces an AQP2-TM5b interaction leading to depolymerisation of actin filaments; this mechanism is reversible [77].

AQP2 is also regulated independently of AVP in response to hypertonic conditions. Hypertonic exposure (600 mOsm/kg) was shown to significantly increase activity of the AQP2 promoter, independent of AVP, in MDCK cells expressing murine AQP2. The responsive element was suggested to reside between  $-6.1$  and  $-4.3$  kb 5' flanking region of the AQP2 gene [78]. Acute hypotonicity has also been shown to induce translocation of AQP2 to the plasma membrane in the absence of AVP: rapid plasma membrane and trans-Golgi network accumulation of AQP2 in rat renal CD cells were shown to be dependent on MAPK, P38 and ERK1/2 activity [79].

Early reports suggested that AQP1 was constitutively expressed in PT cell membranes in the kidney [80], however recent studies have shown that AQP1 is expressed in both the cytoplasm and in the membrane of cultured cells and can be induced to undergo rapid and reversible translocation to the plasma membrane upon hypotonic stimulation mediated by TRP channels, calcium, PKC and microtubules [57,81]. AQP1 has also been shown to undergo translocation to the plasma membrane of cholangiocytes in response to secretin activation of specific G protein-coupled receptors [82]. Further studies have demonstrated that the diuretic effect of acetazolamide involves triggered AQP1 translocation by promoting AQP1 and myosin heavy chain interactions causing AQP1 localisation to proximal tubule cell membranes followed by ubiquitin-mediated AQP1 degradation through the proteasome [83]. AQP2 ubiquitination has also been shown to be involved in AQP2 expression and localisation [84]. Phosphorylation of AQP1 has been shown to be involved in translocation of AQP1 to the plasma membrane in oocytes [85].



**Fig. 3.** Human AQPs are regulators of transcellular water flow. (A) Paracellular water flow and AQP-mediated transcellular water flow in the kidney collecting duct. (B) Mechanisms of AQP regulation in kidney collecting duct via triggered translocation. (C) Involvement of AQPs in transcellular water flow in brain oedema. Blue arrows, H<sub>2</sub>O movement; black arrows, AQP translocation; green box, tight junction; AVP/V<sub>2</sub>R, arginine vasopressin/vasopressin 2 receptor-stimulated AQP2 translocation; ?, Unknown translocation mechanism, and blue dashed arrow: regulatory role of AQPs in CVR (for details see Fig. 2).

### 3.2.3. Conclusions

Kidney AQPs form a highly-organised network that facilitates the maintenance of water homeostasis. The specific localisation of AQPs within renal CD cells provides a transcellular pathway for water to be reabsorbed from the urine through AQP2 channels into renal CD cells and back into the blood via AQP3 and AQP4 on basolateral membranes. The regulation of AQP1 expression and translocation mediates transcellular water flow in PT cells [83].

### 3.3. Specialised secretory tissues

#### 3.3.1. Localisation and regulatory roles of AQPs

Specialised secretory tissues rely on AQP-dependent transcellular water flow to facilitate their fluid homeostasis. In the salivary gland, AQP5 facilitates transcellular water flow in both acinar and parotid salivary cells [86]; the salivary cells isolated from AQP5 <sup>-/-</sup> mice had dramatically reduced membrane water permeability following exposure to hypertonic or hypotonic conditions [3]. The localisation of AQP5 on the luminal membrane is consistent with the membrane's high water permeability and its role in osmotic water transport from the acinar cells to the lumen of the gland used in the production of saliva [87,88].

Immunohistochemistry of human salivary glands (HSG) has demonstrated that AQP3 is present on the basolateral membranes of mucous and serous acinar cells [89], indicating a possible role for AQP3 as well as AQP5 in transcellular water flow during saliva formation [89].

In HSG, AQP5 translocation was shown to occur in a microtubule-dependent manner; elevation of [Ca<sup>2+</sup>]<sub>i</sub> by stimulation with thapsigargin (a Ca<sup>2+</sup>-ATPase inhibitor) and a Ca<sup>2+</sup> ionophore resulted in AQP5 localisation at the plasma membrane, which was inhibited by pre-treatment with the microtubule inhibitors, colchicine and vinblastine [90]. GFP-tagged human AQP5 has been shown to translocate to the plasma membrane of HSG cells upon stimulation with carbachol (a muscarinic type 3 receptor (M<sub>3</sub>R) agonist) [91], but little is known about the molecular mechanisms involved.

AQP5 translocation to the apical membrane of rat parotid gland cells has been shown to occur following stimulation of M<sub>3</sub>R with acetylcholine (ACh) and subsequent [Ca<sup>2+</sup>]<sub>i</sub> elevation. Furthermore, following incubation with a Ca<sup>2+</sup> ionophore translocation was activated without ACh stimulation [92]. Increased levels of AQP5 mRNA and protein were observed when rat submandibular acinar cell lines were exposed to hypotonic conditions causing rapid cell swelling and more efficient RVD. EGTA chelation of intracellular and extracellular Ca<sup>2+</sup> did not

affect CVR in a rat acinar cell line; in fact CVR was found to be  $K^+$ - and  $Cl^-$ -dependent, with mitogen-activated ERK-activating kinase (MEK) and the  $\beta$ -amino acid, taurine, playing important roles [93].

Increasing  $[Ca^{2+}]_i$  has been shown to trigger AQP5 translocation from cytosolic compartments to the plasma membrane while the removal of extracellular  $Ca^{2+}$  has been shown to inhibit translocation of AQP1 in HEK293 cells [57]. Acinar cells from mice lacking the TRPV4 channel or AQP5 display reduced hypotonicity-induced  $Ca^{2+}$  influx and a suppressed RVD response suggesting an important role for TRPV4 and AQP5 interactions in generating the  $Ca^{2+}$  response required for effective RVD after hypotonic cell stress [40]. There is little knowledge on the regulation of AQP5 in sweat glands. Eccrine sweat glands have a high water permeability to support their role in fluid secretion; primary sweat is deposited into the lumen of the eccrine gland before water movements allow sweat to be secreted through the ductal region [94]. Recently, AQP5 has been shown to be expressed in apical membranes, intracellular canaliculi of secretory coils and in basolateral membranes of human eccrine sweat glands. Rapid apical translocation of human AQP5 occurs in stably-transfected MDCK cells after treatment with the  $Ca^{2+}$  ionophore, A23187, suggesting the involvement of  $[Ca^{2+}]_i$  in AQP5 translocation. Rapid translocation of AQP5 to the apical plasma membrane of cells has also been shown in mouse sweat glands during sweating [95]. In humans, ACh activation of muscarinic receptors raises  $[Ca^{2+}]_i$  and induces sweating [94]. It is thought that AQP5 regulation by  $[Ca^{2+}]_i$  contributes to sweat release by increasing apical membrane permeability, and that AQP5 may co-localise with the  $Ca^{2+}$  channel, ANO1, at the plasma membrane [95]. However, the mechanisms behind AQP5 translocation within the sweat gland, including the role of phosphorylation, still require further elucidation.

### 3.3.2. Conclusions

AQP5 is involved in regulating transcellular water flow in the luminal regions of glands. This facilitates the formation of secretions at the necessary concentrations and viscosities required to maintain water homeostasis.

## 3.4. The integumentary system

### 3.4.1. Localisation and regulatory roles of AQPs

The integumentary system comprises the skin, hair and nails, but nerves, certain glands and fat are often also classified as parts of the integument. The skin plays an integral part in water homeostasis and provides a barrier function against excessive water loss. Its water and glycerol content is essential for normal function; this is largely under the control of AQP3, which is expressed mainly in the plasma membranes of the stratum basale of the epidermis, with decreasing expression towards the stratum granulosum and none in the stratum corneum [96]. Sugiyama and colleagues [97] first reported expression of AQPs 3 and 9 in human cultured keratinocytes, although AQP9 was only observed in differentiating cells. AQP3-null mice display impaired barrier function and reduced stratum corneum hydration, which was not corrected by skin occlusion or exposure to high humidity. However, topical or oral administration of glycerol has shown to correct many defective skin functions in AQP3-null mice [98].

AQP1 has been detected in skin biopsies in a study of atopic eczema, although its expression was no different in diseased or control skin, whereas up-regulation of AQP3 was found [99]. Skin diseases that show reduced stratum corneum hydration display altered expression levels of AQP3, dependent upon the disease. In 2011, Voss and colleagues showed that in psoriatic skin, AQP3 was preferentially expressed in the cytoplasm rather than the plasma membrane suggesting that AQP3 may be important for transcellular water and glycerol transport. AQP5 is also thought to be expressed at the plasma membrane in the stratum granulosum and may play a role in transcellular water homeostasis in the skin [100].

AQP7 is the primary glycerol transporter in white (WAT) and brown (BAT) adipose tissues. AQP7 is abundantly expressed in the plasma membrane of adipocytes and fasting has been shown to up-regulate AQP7 mRNA in the adipocytes of rodents [101]. In the model mouse adipocyte cell line, 3T3-L1, increases in AQP7 mRNA expression and glycerol release were correlated during cell differentiation indicating that plasma membrane glycerol permeability may mediate the accumulation of fat in adipocytes [102]. AQP3 and 9 are also expressed in human adipose tissue, with AQP3 predominantly localised in the cytoplasm and AQP9 constitutively expressed in the plasma membrane; a positive correlation between the transcript levels of AQPs 3, 7 and 9 and body weight (BMI) has been suggested [103]. AQP7 gene expression is down-regulated in the WAT of obese human subjects compared with normal controls [104,105], but unchanged in type 2 diabetes [104]. However, the link between human AQPs and obesity is contradictory; a review by Maeda et al. [106] describes some of these studies. More recently AQP10 has been proposed to be an alternative pathway for glycerol efflux in human adipocytes [107].

### 3.4.2. Conclusions

AQP3, 7, 9 and 10 are aquaglyceroporins. It is therefore likely that the roles of these AQPs in the integumentary system primarily involve transport of glycerol, although they may also be involved in the regulation of transcellular water flow; this remains to be determined.

## 3.5. The cardiovascular system

### 3.5.1. Localisation and regulatory roles of AQPs

Cardiac AQPs have not been investigated to the same extent as AQPs in the brain and kidney. In the heart, water moves from the interstitial space, across endothelia and into blood vessels. This process is typically attributed to paracellular water transport through the endothelium of the heart since it is considered to be 'leaky' compared to the endothelium of other organs [108]. Myocardial stunning is the reduced output of the heart, often seen following cardiac surgery such as heart bypass, and has been associated with cell swelling and oedema. The expression of members of the AQP family in the myocardium has been poorly characterised and their role, if any, in the pathology and resolution of cardiomyocyte swelling, oedema and general function within the heart has not been investigated (for a detailed review on AQPs and myocardial water management see Egan [109]).

AQP1 has been detected in the human, rat and mouse heart tissue [110], while AQP4 has only been detected in the mouse heart [110]. Immunofluorescence images revealed that the abundantly-expressed AQP1 is distributed in human and murine cardiac microvasculature at high levels only because of the dense vascularity of the heart muscle [110]. Immunofluorescence also showed that AQP4 was localised in the plasma membranes of the cardiomyocytes of the mouse heart tissue. Functional studies on cardiac membrane vesicles from AQP1 and AQP4 knockout mice found that only AQP1 had a role in water permeability in the heart; vesicles from AQP1-null mice had reduced permeability but deletion of AQP4 produced no reduction in water permeability [110]. A study using rabbit ventricular cardiomyocytes suggested that water movement in the heart is mediated by paracellular water flow and does not occur via AQPs [111]. On the rare occasion that an osmotic gradient is present in the heart (e.g. during cell swelling after cardiac surgery), endothelial AQP1 might mediate the flow of water from the expanded interstitial space into the capillaries [112]; indeed AQP1 is the major AQP expressed in vascular endothelial cells [113].

There is controversy over the details of AQP4 expression in the human heart; some studies have reported mRNA expression but no or very little protein [110]. However, a recent study [114] demonstrated the presence of AQP4 protein in the human heart using Western blotting; it was localised to the plasma membrane with a very weak signal in the cytosol. Immunoblot analyses of cultured mouse cardiomyocytes also showed that AQP4 was present while immuno-gold



electron microscopy images showed AQP4 protein on the plasma membrane of the cardiomyocytes. Cardiac oedema arises when tissue with a reduced blood supply (ischemic) becomes hypertonic, causing water to flow from the capillaries (possibly via AQP1) into cardiomyocytes; this causes cell swelling and reduced cardiac output. Ischemic mice had a decrease in AQP4 mRNA, as measured by RT-PCR. AQP4 knockout mice presented with lower cardiac ischemic injury, measured as infarction, suggesting that AQP4 may be a possible target for treatment of myocardial infarction.

### 3.5.2. Conclusions

The major AQP of the cardiovascular system is AQP1 (Fig. 1), which probably regulates water permeability of the heart's capillary networks by mediating the flow of water through the endothelial layer into the blood. AQP1 may aid the absorption of excess water from the interstitial space into the capillaries, however this is controversial. Further research is therefore required on the role of AQPs in the heart; this might provide evidence for AQP1 and/or AQP4 as drug targets for the treatment of myocardial stunning oedema within the interstitium. AQP4 has only recently been detected at the protein level within human cardiomyocytes, which could lead to research into AQPs as transcellular water transporters for clearance and formation of interstitial oedema and cardiomyocyte swelling.

## 3.6. The airway

### 3.6.1. Localisation of AQPs

Airway hydration, sub-mucosal secretions and alveolar fluid transport all require water permeability of the epithelial and endothelial membranes of the airway [115]. AQPs are consequently expressed in bronchopulmonary tissues (Fig. 1) and are regulated in a way that facilitates transcellular water transport [116,117]. AQP1 is expressed predominantly in the microvascular endothelia throughout the lung and upper airways [117], while AQP3 and AQP4 are present in the basolateral membranes of the airways' epithelial lining [88]. AQP5 is expressed in the apical membrane of type I alveolar epithelial cells of the distal lung and acinar sub-epithelial glandular cells, which provides the major route (along with AQP1) for the osmotically driven flow of water within the entire airway system [118]. AQP5 is also expressed superficial epithelium of the bronchus and, as such, is ideally placed to regulate the hydration of the airway surfaces [119]. Indeed, studies with knockout mice have shown that AQP5 enhances fluid secretion [120]. In addition, AQP5 expression is reduced in inflammatory airway conditions, such as chronic obstructive pulmonary disease (COPD), that associate with mucus hypersecretion [119]. Modulation of AQP5 expression could therefore serve as therapy for inflammatory airway conditions, alleviating symptoms such as the increase in desiccated mucus which potentiates the chronic airways infections typical of cystic fibrosis [121].

### 3.6.2. Regulatory role of AQPs

When AQP expression within the airway was first discovered, several murine AQP knockout studies were published that investigated the hypothesis that water can be transported from the alveolar airspace across the interstitial and capillary compartments via osmotically-driven AQP transport. Initially, osmotic water permeability ( $P_f$ ) of the lungs was measured in AQP1 and AQP4 knockout mice;  $P_f$  was reduced by 10-fold in the endothelia of AQP1 null mice suggesting a transcellular route. However, it was concluded that AQP1 does not provide the route for alveolar fluid absorption and little effect was observed on  $P_f$  in the AQP4 knockout studies [122]. A year later, the same group conducted a very similar study, this time using AQP5 knockout mice; the airspace-capillary  $P_f$  was reduced by 10-fold in the AQP5 knockout and a further 2–3-fold when a double knockout of AQP1/AQP5 was used. The authors concluded that AQP5 and AQP1 are the main routes for transcellular water flow in the airway with the primary role of

AQP5 being water transport across the apical plasma membrane of type I alveolar epithelial cells [123].

TRPV4 was subsequently shown to regulate AQP5 abundance under hypotonic conditions in mouse lung epithelial (MLE) cells [124]. After 2 h in hypotonic medium, a decrease in AQP5 abundance in MLE cells was observed; this result was observable after 30 min but not before 10 min exposure. This decline in AQP5 was blocked in the presence of ruthenium red, which is a TRPV4 inhibitor, and also when the cells were cultured in  $\text{Ca}^{2+}$ -free medium, even when the osmolality was reduced to 127 mOsm (which is hypotonic); these data support a role for extracellular  $\text{Ca}^{2+}$  in the regulation of AQP5 abundance. RT-PCR results showed that mRNA levels were not affected when protein levels decreased. When a lysosomal inhibitor was added, a reduction in AQP5 mRNA levels was not seen suggesting that AQP5 protein is probably undergoing degradation. The same results were observed in TRPV4-expressing and control HEK-293 cells transfected with AQP5; an AQP5 reduction in response to hypotonicity was only seen in cells expressing TRPV4, which was also blocked by ruthenium red. The regulation of membrane permeability by AQP5 abundance was concluded to be tightly controlled by osmolality and mediated by TRPV4 [124].

Further evidence that osmolality regulates AQP5 expression was provided by Hoffert and colleagues [125], who showed that hypertonic stress induces AQP5 expression in MLE cells cultured in hypertonic medium (500 mOsm). AQP5 protein levels increased after 8 h and peaked 24 h post-exposure, returning to baseline after 6 h in isotonic medium. Only relatively impermeable solutes affected AQP5 expression, suggesting that an osmotic gradient between a cell and its environment is involved in the regulation of AQP5 expression. This expression mechanism was demonstrated to require the activation of the extracellular signal-regulated kinase (ERK) pathway since several ERK inhibitors blocked AQP5 expression; however without a hypertonic stimulus, AQP5 expression was inhibited and ERK activators failed to induce expression. In the same study, rats that had been given daily intraperitoneal injections of hypertonic saline had a 2-fold increase in AQP5 expression in the lung compared with control rats, suggesting the physiological relevance of AQP5 regulation mechanisms in vivo [125].

AQP5 expression has also been shown to be regulated by a cyclic AMP/protein kinase A (cAMP/PKA)-dependent pathway. In MLE cells, addition of the cell-permeable cAMP analogue, cpt-cAMP, caused a 4-fold increase in AQP5 mRNA and protein levels in a dose-dependent manner; increased protein synthesis was ablated by the addition of the PKA inhibitor, H89. Immunofluorescence studies using confocal microscopy on MLE cells after a 24 h cpt-cAMP treatment revealed that AQP5 was translocated to the apical plasma membrane. Increasing endogenous cAMP levels by treatment with forskolin and the  $\beta$ -adrenergic agonist, isoproterenol, also induced AQP5 protein expression. The forskolin effect was also seen to work ex vivo in murine lung tissue suggesting that this cAMP-dependent molecular mechanism may occur in vivo [126].

Sidhaye and colleagues [127] looked at the effects of cAMP on the regulation of AQP5 distribution and abundance. Using AQP5-expressing mouse lung epithelial cells, the distribution of AQP5 was observed by immunofluorescence and surface biotinylation. After short-term treatment of cells with cAMP, AQP5 was internalised and there was a reduced abundance of AQP5 at the membrane; long term exposure to cAMP (approximately 8 h) resulted in higher-than-baseline AQP5 abundance at the apical membrane indicating an up-regulation of AQP5 membrane expression. Following short-term exposure, AQP5 abundance was also temporarily decreased followed by a marked increase after an 8 h exposure. When cells were treated with the  $\beta$ -adrenergic agonist, tetrabutaline, which is known to increase intracellular levels of cAMP, identical results to those observed after cAMP addition were seen in terms of distribution and protein abundance. The same effects were observed in vivo using mice injected subcutaneously with the agonist. When the PKA inhibitor, H89, was

added, all cAMP-dependent effects were inhibited, additionally purified AQP5 was phosphorylated by PKA but not PKC or casein, indicating that PKA activation is required for AQP5 regulation in the airways.

Contrasting results were obtained from a study in which human bronchial epithelial cells were stably transfected with wild type (WT) AQP5 and two AQP5 mutants [128]. The first mutant was an alanine substitution of Ser156, the PKA substrate site for phosphorylation of both AQP2 and AQP5, which is involved in AQP2 apical membrane translocation. The second mutation was within the second Asn-Pro-Ala motif, which forms the AQP pore. These experiments were designed to examine the importance of pore formation and PKA phosphorylation on the membrane expression of AQP5. WT-AQP5 was shown to be expressed at the apical plasma membrane and in sub-apical vesicles, while the Ser156Ala mutant also showed membrane expression. This indicated that blocking the PKA binding site did not affect AQP5 translocation as in the case of AQP2. Expression of an Asn185Asp mutant was localised throughout the cytoplasm suggesting that like AQP1, AQP5 requires the Asn-Pro-Ala motif for correct channel formation and that it may have a role in either protein folding or oligomerisation. When the PKA inhibitor, H89, was used, no difference in membrane expression compared to the basal level was seen after 30 min, which was also the case after a 30 min cAMP treatment in all three stable cell types. AQP2 translocation to the apical membrane was induced by the addition of cAMP and this was blocked by pre-treatment with H89. Also when the AQP2 Ser156 was mutated, AQP2 was expressed within the cytoplasm and unable to translocate to the membrane. In WT-AQP5 and Asn185Asp mutant cells, phosphorylation was seen before the addition of cAMP. However, in the Ser156Ala mutant cells, AQP5 was never phosphorylated indicating that Ser156 is the PKA substrate in AQP5, that membrane localisation of the protein is not regulated by PKA and that cAMP stimulation may be a separate event in contrast to the AQP2 mechanism and the previous studies conducted by other groups [128].

### 3.6.3. Conclusions

Direct evidence has been published for the involvement of AQPs in transcellular water flow across the alveolar epithelium via an apical AQP5 route and through AQP1 in the endothelia [118]. This movement of water between the alveolar airspace and capillary compartments is essential for airway hydration, effective airways defences and reabsorption of excess alveolar fluid.

## 3.7. The reproductive system

The permeability of cell membranes to water and hormones in both the male and female reproductive systems is essential for folliculogenesis [2], spermatogenesis [129] and sperm osmoadaptation [41]. There are few studies on the regulation of AQPs in the reproductive system, but expression profiles have been outlined and up-to-date reviews are available [41,129,130]. Emerging evidence suggests physiological roles for AQPs within human reproductive systems and that water shifts are often transcellular and AQP mediated [2]; AQPs have recently been associated with the pathogenesis of some reproductive disorders such as polycystic ovary syndrome (PCOS) [131] and ovarian cancer, where AQP5 and AQP9 up-regulation were observed within the tissue of malignant ovarian tumours [130].

### 3.7.1. The female reproductive system

The role of AQPs in the ovary, specifically the ovarian follicle, has been well studied. During folliculogenesis, the antrum is expanded by a large, rapid influx of water through the granulosa cell (GC) lining; it is unknown whether this water transport is mediated by paracellular mechanisms or by transcellular flow through AQP channels. McConnell and colleagues [2] demonstrated that water movement into the antrum of isolated rat follicles was 3.5-fold greater than that of C-inulin (a complex sugar that moves through tissues via paracellular transfer),

indicating that the influx of water into the antral cavity has a transcellular component. When follicles were pre-treated with HgCl<sub>2</sub> (an AQP inhibitor), the movement of water was reduced to that of inulin. This suggested a role for AQPs in mediating water movement during folliculogenesis, especially in light of the detection of AQP7, AQP8 and AQP9 in GCs by flow cytometry [2]. AQP7 and AQP9 are aquaglyceroporins; their presence within the ovarian follicle suggests that the ability of small neutral solutes to be transported rapidly across the plasma membrane may be a requirement of folliculogenesis.

In a recent study in women with PCOS, immunofluorescence was used to confirm the presence of AQP9 in the nucleus, cytoplasm and plasma membrane of human GCs. In a study of 14 PCOS sufferers and 31 control subjects who were infertile from tubal blockage, GCs and follicular fluid were collected from the participants. Total testosterone (TT) and luteinising hormone (LH) levels were elevated in follicular fluid from PCOS compared with samples from control women; sex hormone binding globulin (SHBG) levels were lower in PCOS patients. RT-PCR results indicated that AQP9 mRNA levels were decreased in PCOS sufferers and that there was a significant correlation between AQP9 mRNA and TT, LH and SHBG levels in PCOS samples, but no correlations in control samples. *In vitro* studies showed that the treatment of GCs with dihydrotestosterone (DHT) had an inhibitory effect on AQP9 mRNA expression and that the addition of LY294002, a phosphatidylinositol 3-kinase (PIK3) inhibitor, attenuated this down-regulation such that AQP9 mRNA levels were raised compared with those treated with DHT alone. The addition of H89 and forskolin did not rectify the DHT-initiated AQP9 mRNA decrease suggesting that PKA and cAMP pathways are not involved in this mechanism. This suggests that hyperandrogenism (excess of androgenic hormones) of the follicular fluid occurs in PCOS and this suppresses AQP9 expression in GCs through a PIK3 pathway affecting follicular development [131]. Further work into the mechanisms behind AQP9 regulation in healthy and pathogenic ovaries may provide insight into possible treatments for diseases where hyperandrogenism is an issue.

The data discussed above suggest that transcellular water flow into the antrum of the ovarian follicle is a key aspect of folliculogenesis and that the movement of water for expansion of the antrum is likely to be AQP mediated.

### 3.7.2. The male reproductive system

There is increasing evidence that AQPs play an important role in sperm cell RVD; this ensures maintenance of the structure and function of sperm and thus male fertility. AQP7 cDNA was first isolated from rat testis and was shown to be expressed abundantly throughout the testis and in the plasma membrane of late stage spermatoids [132]. Aquaglyceroporins, AQP3 and AQP7, have been identified within human sperm [40,41,42,131,132,133] and their roles investigated. AQP3 has been described as the regulator of sperm osmoadaptation during male to female transition, during which sperm are exposed to a hypo-osmotic environment with the potential to harm the sperm by excess swelling and reduced motility [41]. AQP3 is located at the plasma membrane of the sperm flagellum; AQP3 mutant cells show decreased motility, increased swelling and tail bending after entering the hypotonic environment of the uterus therefore hindering the sperm's chances of reaching the oviduct and mediating a fertilisation event. These defects are probably due to ineffective RVD mechanisms and consequent swelling after hypotonic stress [42]. A more recent study has outlined a relationship between AQP7 localisation and sperm characteristics; transmission electron microscopy images showed expression of AQP7 within the pericentriolar region of the neck, equatorial region of the acrosome and a diffuse staining along the tail. Abnormal sperm samples, characterised by malformations of the head, midpiece, or tail, displayed lower intensity and diffuse staining in the cytoplasmic residual bodies, head and tail. A specific correlation between normal sperm AQP7 labelling and sperm

motility and morphology suggested that AQP7 also has a role in regulation of sperm cells and male fertility [134].

Several members of the AQP family are expressed within the epididymis of the male reproductive tract [129]. They are localised to the epithelial layer and are thought to play an important role in trans-epithelial water transport and sperm concentration [129]. AQP9 was the first AQP identified in the epididymis [135] and has been labelled the major apical AQP of the epithelium principal cells; it allows transepithelial flow of solutes such as glycerol, urea, mannitol and sorbitol and is modulated by androgens in male adult rats [136]. AQP3 is localised exclusively to the basal cell membranes of the epididymis and although AQP1 is absent from the epididymis epithelial cells it is expressed within the smooth muscle and endothelium of the vascular channels throughout the epididymis [137], together with AQP10 [138].

AQPs are important in facilitating an RVD response in sperm cells especially upon the introduction to the hypotonic environment of the female reproductive tract, which in AQP3-deficient sperm can cause detrimental swelling and reduced mobility. If the regulation of sperm cells is dependent upon RVD mechanisms, future work into this field should concentrate on elucidating these mechanisms. RVD mechanisms in other tissues often involve complexes with AQPs, such as the AQP4/TRPV4 complex in astrocytes [60] and the AQP2/TM5b interaction in the kidney [77]. It is therefore feasible that AQP3 and/or AQP7 in human sperm might form molecular complexes with ion channels such as the volume sensitive chloride channel CLC-3, which has been identified in mammalian sperm and implicated in RVD [139,140].

### 3.8. The digestive system

#### 3.8.1. Localisation and regulatory roles of AQPs

Secretion and absorption, two of the main functions of the digestive system, both require the transfer of fluid across cellular membranes. Daily secretions in the form of saliva, gastric juices, intestinal mucous, bile and pancreatic juice comprise a total volume of approximately 7.5 L of fluid in the human digestive system; approximately 9 L of fluid is absorbed daily [141]. Several members of the AQP family are expressed throughout the digestive system including AQP1 in the apical and basolateral membranes and the cytoplasm of cholangiocytes, the pancreas and throughout the endothelial cells of capillaries responsible for transendothelial water transfer [142]; the epithelial lining of the gastrointestinal tract contains AQP3 [143] and AQP4 [144,145]. Pancreatic duct cells express AQP8 in the apical plasma membrane [146] and AQP8 [147] and AQP9 [148] are found in the hepatocytes of the liver; in-depth reviews on the expression and localisation of AQPs in the digestive system are available elsewhere [141,149].

In the upper digestive tract, AQP3 is expressed abundantly in stratified epithelia of the oral cavity to the fore-stomach. AQP3 is localised in the basal and intermediate cell membrane becoming less abundant towards the epithelial surface and is thought to provide a supply of water from the sub-epithelial side of these cells which face harsh conditions, such as the low pH of the stomach, to prevent them from dehydration [143]. Immunocytochemistry has shown strong AQP4 expression at the basolateral membrane of the gastric parietal cells in mice and was hypothesised to play a role in gastric acid secretion. Wang and colleagues [144] used several secretory agonists to increase gastric acid output in AQP4 null and control mice; no significant differences were seen in secretion levels suggesting that the deletion of AQP4 did not affect the stomach's ability to secrete gastric acid [144].

In the distal colon and rectum, AQP3 is localised on the basolateral membrane of the epithelial cells lining the lumen [143]. Inhibition of AQP3 by HgCl<sub>2</sub> in rats induced severe diarrhoea, suggesting a role for AQP3 in regulating faecal water content [150], although it should be noted that mercury is a toxic, non-specific inhibitor of AQP3 [151]. AQP3 may mediate the reabsorption of water from faeces by transporting it from the lumen, across the endothelial layer into the blood vessels via AQP1 [113]. The mechanism by which this is

controlled is unknown but further understanding could lead to treatments for over- or under-active bowel problems. For example, the temporary inhibition of AQP3 could have a laxative effect.

AQP8 may have a role in bile secretion in hepatocytes, which are responsible for the formation of bile before it is secreted into the bile duct and modified by cholangiocytes. AQP8 was detected in the cytoplasm and intracellular vesicles of rat hepatocytes by confocal microscopy. Short-term treatment with cAMP induced re-distribution of AQP8 to the plasma membrane and an increase in water permeability within 10 min. The microtubule inhibitor, colchicine, blocked the effects of the cAMP treatment indicating that AQP8 translocation is stimulated by cAMP and is microtubule dependent [152].

#### 3.8.2. Conclusions

The digestive system is a major site of fluid movement and has a wide AQP expression profile within its organ network. A polarised AQP expression pattern suggests that an organised transcellular route for water is an essential role of AQPs in facilitating high secretion and absorption rates.

### 3.9. The musculoskeletal system

#### 3.9.1. Localisation and regulatory roles of AQPs

Articular cartilage and intervertebral disc (IVD) tissue are specialised biomechanical structures that are under constant compressive loads [153,154]. The cells within these avascular tissues are exposed to constantly harsh conditions as the IVD is ~80% water [155] and articular cartilage tissue is around ~70% water [156]. The IVD is composed of three distinct regions: the gelatinous nucleus pulposus (NP), which is encapsulated by the annulus fibrosus and the cartilaginous end plates [153]. The native cells of the NP and cartilage tissue both secrete proteoglycans and type II collagen; the collagen meshwork traps negatively-charged proteoglycans (such as aggrecan) which attract cations (mainly K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>+</sup>) resulting in the influx of water; this process is responsible for the high osmotic potential of these tissues [157,158] enabling them to resist static and dynamic biomechanical loads [154]. Both the NP cells and the chondrocytes must regulate their volume and water content in these rapidly changing osmotic environments; however little is known about the mechanisms which they employ to do this. Recently, studies have taken place to identify which AQPs are expressed in these tissues; AQP1 and low levels of AQP3 have also been identified within the NP cells of the human IVD [159] while AQP1 and AQP3 have been shown to be expressed and co-localised at the membrane of equine articular cartilage chondrocyte cells [154] and chondrocytes of the human knee [156].

AQP1 and AQP4 are expressed in skeletal muscle and a study has shown that cell volume changes that occur during muscle contraction rely on rapid water influx [160]. AQP1 was found in the endothelial cells of capillaries within the muscle tissue and AQP4 on the plasma membrane of muscle fibre cells [160]. The localisation of AQP1 and AQP4 within the muscle tissue suggests a pathway for transcellular water flow through the endothelial cell membrane and the sarcolemma; these AQPs may function together as transporters for water between the blood and myofibrils during mechanical muscle activity.

#### 3.9.2. Conclusions

It is not surprising that the native cells of the NP and articular cartilage express AQPs and it is highly likely that AQPs are responsible for CVR in these highly osmotic environments. More work is required to elucidate the functional roles of aquaporins in these tissues; a number of published studies have suggested roles for AQPs as components of the vital cellular apparatus for maintenance of physiological homeostasis of the musculoskeletal system.

#### 4. Conclusions

The constitutive distribution of AQPs is achieved by AQP gene expression and/or AQP protein degradation on a timescale from hours to days [161]. Further rapid spatial and temporal distribution of AQPs is regulated by stimulated or triggered translocation of AQP-containing vesicles to and from a particular membrane. This is particularly well studied for V2R-mediated AQP2 translocation in kidney collecting duct cells [74] (Fig. 3B). Similar mechanisms of triggered translocation have been demonstrated for other AQPs [57]. We propose that the resulting spatial and temporal distribution of AQPs is crucial for the regulation of transcellular water flow in the major systems of the human body.

For example, reabsorption of water from the lumen of the kidney collecting duct involves transcellular water flow mediated by AQP2 at the apical membrane and AQP3 and AQP4 at the basolateral membrane. Transcellular water flow through endothelial cells of the capillary into the blood is then mediated by AQP1 (Fig. 3A). In another example, AQP4 knockout mice show near normal levels of intracranial pressure and water content but reduced accumulation of water in the brain following cytotoxic oedema caused by ischemic stroke, cerebral injury and meningitis [162,163]. In vasogenic oedema mouse models, AQP4 null mice show greater water accumulation in the brain [61]. These studies suggest that water homeostasis in a non-pathological state may be independent of AQP4-mediated transcellular water flow but that AQP4 regulates transcellular water flow in cerebral oedema (Fig. 3C). In vasogenic oedema, water is eliminated from the extracellular space through AQP4 into the intact astrocytes that make up the BBB. Water then exits the astrocyte through AQP4 in the membrane of astrocytic foot processes that surround the capillary. Transcellular water flow through endothelial capillary cells into the blood is mediated by AQP1. However, in cytotoxic oedema, transcellular water flow from the blood into astrocytes is mediated by AQP4.

In conclusion, it appears that AQPs are crucial for the regulation of water homeostasis, providing selective pores for the rapid movement of water, and other uncharged solutes, across diverse cell membranes and playing regulatory roles in CVR. Gating mechanisms, which allow conformationally distinct open and closed states, have been proposed for human AQPs through molecular dynamic simulations [164], but have only been specifically reported for plant and microbial AQPs [165]. Consequently, it is likely that the distribution and abundance of AQPs in a particular membrane are the determinants of membrane water permeability and a regulator of transcellular water flow.

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#### References

- [1] D. Marples, M.A. Knepper, E.I. Christensen, S. Nielsen, Redistribution of aquaporin-2 water channels induced by vasopressin in rat kidney inner medullary collecting duct, *Am. J. Physiol.* 269 (1995) C655–C664.
- [2] N.A. McConnell, R.S. Yunus, S.A. Gross, K.L. Bost, M.G. Clemens, F.M. Hughes Jr., Water permeability of an ovarian antral follicle is predominantly transcellular and mediated by aquaporins, *Endocrinology* 143 (2002) 2905–2912.
- [3] C.M. Krane, J.E. Melvin, H.V. Nguyen, L. Richardson, J.E. Towne, T. Doetschman, A.G. Menon, Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation, *J. Biol. Chem.* 276 (2001) 23413–23420.
- [4] T. Zeuthen, General models for water transport across leaky epithelia, *Int. Rev. Cytol.* 215 (2002) 285–317.
- [5] S. Tripathi, E.L. Boulpaep, Mechanisms of water transport by epithelial cells, *Q. J. Exp. Physiol.* 74 (1989) 385–417.
- [6] M.J. O'Donnell, S.H. Maddrell, Paracellular and transcellular routes for water and solute movements across insect epithelia, *J. Exp. Biol.* 106 (1983) 231–253.
- [7] J. Fischberg, Fluid transport across leaky epithelia: central role of the tight junction and supporting role of aquaporins, *Physiol. Rev.* 90 (2010) 1271–1290.
- [8] D.D. Loo, T. Zeuthen, G. Chandry, E.M. Wright, Cotransport of water by the Na<sup>+</sup>/glucose cotransporter, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 13367–13370.
- [9] S. Nielsen, B.L. Smith, E.I. Christensen, M.A. Knepper, P. Agre, CHIP28 water channels are localized in constitutively water-permeable segments of the nephron, *J. Cell Biol.* 120 (1993) 371–383.
- [10] O. Carlsson, S. Nielsen, E. Zakaria, B. Rippe, In vivo inhibition of transcellular water channels (aquaporin-1) during acute peritoneal dialysis in rats, *Am. J. Physiol.* 271 (1996) H2254–H2262.
- [11] B.M. Denker, B.L. Smith, F.P. Kuhajda, P. Agre, Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules, *J. Biol. Chem.* 263 (1988) 15634–15642.
- [12] W.E. Harries, D. Akhavan, L.J. Miercke, S. Khademi, R.M. Stroud, The channel architecture of aquaporin 0 at a 2.2-Å resolution, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 14045–14050.
- [13] R. Horsefield, K. Norden, M. Fellert, A. Backmark, S. Tomroth-Horsefield, A.C. Terwisscha van Scheltinga, J. Kvassman, P. Kjellbom, U. Johanson, R. Neutze, High-resolution x-ray structure of human aquaporin 5, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 13327–13332.
- [14] K. Murata, K. Mitsuoka, T. Hirai, T. Walz, P. Agre, J.B. Heymann, A. Engel, Y. Fujiyoshi, Structural determinants of water permeation through aquaporin-1, *Nature* 407 (2000) 599–605.
- [15] S. Tomroth-Horsefield, K. Hedfalk, G. Fischer, K. Lindkvist-Petersson, R. Neutze, Structural insights into eukaryotic aquaporin regulation, *FEBS Lett.* 584 (2010) 2580–2588.
- [16] U. Kosinska Eriksson, G. Fischer, R. Friemann, G. Enkavi, E. Tajkhorshid, R. Neutze, Subangstrom resolution X-ray structure details aquaporin-water interactions, *Science* 340 (2013) 1346–1349.
- [17] A.C. Conner, R.M. Bill, M.T. Conner, An emerging consensus on aquaporin translocation as a regulatory mechanism, *Mol. Membr. Biol.* 30 (2013) 1–12.
- [18] E.K. Hoffmann, I.H. Lambert, S.F. Pedersen, Physiology of cell volume regulation in vertebrates, *Physiol. Rev.* 89 (2009) 193–277.
- [19] R.J. Huxtable, Physiological actions of taurine, *Physiol. Rev.* 72 (1992) 101–163.
- [20] D.H. Evans, *Osmotic and Ionic Regulation: Cells and Animals*, CRC Press, Boca Raton, 2009.
- [21] B. Yang, A.S. Verkman, Water and glycerol permeabilities of aquaporins 1–5 and MIP determined quantitatively by expression of epitope-tagged constructs in *Xenopus* oocytes, *J. Biol. Chem.* 272 (1997) 16140–16146.
- [22] A.N. van Hoek, A.S. Verkman, Functional reconstitution of the isolated erythrocyte water channel CHIP28, *J. Biol. Chem.* 267 (1992) 18267–18269.
- [23] M.R. Shen, C.Y. Chou, J.A. Browning, R.J. Wilkins, J.C. Ellory, Human cervical cancer cells use Ca<sup>2+</sup> signalling, protein tyrosine phosphorylation and MAP kinase in regulatory volume decrease, *J. Physiol.* 537 (2001) 347–362.
- [24] N.K. Jorgensen, S. Christensen, H. Harbak, A.M. Brown, I.H. Lambert, E.K. Hoffmann, L.O. Simonsen, On the role of calcium in the regulatory volume decrease (RVD) response in Ehrlich mouse ascites tumor cells, *J. Membr. Biol.* 157 (1997) 281–299.
- [25] L. Piao, H. Lee, C.K. Park, I.H. Cho, Z.G. Piao, S.J. Jung, S.Y. Choi, S.J. Lee, K. Park, J.S. Kim, S.B. Oh, Mechanosensitivity of voltage-gated K<sup>+</sup> currents in rat trigeminal ganglion neurons, *J. Neurosci.* 83 (2006) 1373–1380.
- [26] W.Z. Lan, P.Y. Wang, C.E. Hill, Modulation of hepatocellular swelling-activated K<sup>+</sup> currents by phosphoinositide pathway-dependent protein kinase C, *Am. J. Physiol. Cell Physiol.* 291 (2006) C93–C103.
- [27] A. Lepple-Wienhues, I. Szabo, T. Laun, N.K. Kaba, E. Gulbins, F. Lang, The tyrosine kinase p56lck mediates activation of swelling-induced chloride channels in lymphocytes, *J. Cell Biol.* 141 (1998) 281–286.
- [28] B.C. Tilly, N. van den Berghe, L.G. Teertoolen, M.J. Edixhoven, H.R. de Jonge, Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances, *J. Biol. Chem.* 268 (1993) 19919–19922.
- [29] H. Guizouarn, R. Motais, Swelling activation of transport pathways in erythrocytes: effects of Cl<sup>-</sup>, ionic strength, and volume changes, *Am. J. Physiol.* 276 (1999) C210–C220.
- [30] G. Gamba, Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters, *Physiol. Rev.* 85 (2005) 423–493.
- [31] A. Kapus, S. Grinstein, S. Wasan, R. Kandasamy, J. Orłowski, Functional characterization of three isoforms of the Na<sup>+</sup>/H<sup>+</sup> exchanger stably expressed in Chinese hamster ovary cells. ATP dependence, osmotic sensitivity, and role in cell proliferation, *J. Biol. Chem.* 269 (1994) 23544–23552.
- [32] S. Wakabayashi, B. Bertrand, T. Ikeda, J. Pouyssegur, M. Shigekawa, Mutation of calmodulin-binding site renders the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE1) highly H<sup>+</sup>-sensitive and Ca<sup>2+</sup> regulation-defective, *J. Biol. Chem.* 269 (1994) 13710–13715.
- [33] K.B. Gagnon, R. England, E. Delpire, Volume sensitivity of cation-Cl<sup>-</sup> cotransporters is modulated by the interaction of two kinases: Ste20-related proline-alanine-rich kinase and WNK4, *Am. J. Physiol. Cell Physiol.* 290 (2006) C134–C142.
- [34] S.B. Ross, C.M. Fuller, J.K. Bubián, D.J. Benos, Amiloride-sensitive Na<sup>+</sup> channels contribute to regulatory volume increases in human glioma cells, *Am. J. Physiol. Cell Physiol.* 293 (2007) C1181–C1185.
- [35] A.S. Verkman, More than just water channels: unexpected cellular roles of aquaporins, *J. Cell Sci.* 118 (2005) 3225–3232.
- [36] B. Nilius, J. Vriens, J. Prenen, G. Droogmans, T. Voets, TRPV4 calcium entry channel: a paradigm for gating diversity, *Am. J. Physiol. Cell Physiol.* 286 (2004) C195–C205.
- [37] W. Liedtke, Transient receptor potential vanilloid channels functioning in transduction of osmotic stimuli, *J. Endocrinol.* 191 (2006) 515–523.
- [38] R.G. O'Neil, S. Heller, The mechanosensitive nature of TRPV channels, *Pflugers Arch.* 451 (2005) 193–203.
- [39] W. Liedtke, C. Kim, Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon! *Cell. Mol. Life Sci.* 62 (2005) 2985–3001.
- [40] X. Liu, B.C. Bandayopadhyay, T. Nakamoto, B. Singh, W. Liedtke, J.E. Melvin, I. Ambudkar, A role for AQP5 in activation of TRPV4 by hypotonicity: concerted involvement of AQP5 and TRPV4 in regulation of cell volume recovery, *J. Biol. Chem.* 281 (2006) 15485–15495.

- [41] Q. Chen, E.K. Duan, Aquaporins in sperm osmoadaptation: an emerging role for volume regulation, *Acta Pharmacol. Sin.* 32 (2011) 721–724.
- [42] Q. Chen, H. Peng, L. Lei, Y. Zhang, H. Kuang, Y. Cao, Q.X. Shi, T. Ma, E. Duan, Aquaporin3 is a sperm water channel essential for postcopulatory sperm osmoadaptation and migration, *Cell Res.* 21 (2011) 922–933.
- [43] L. Galizia, A. Pizzoni, J. Fernandez, V. Rivarola, C. Capurro, P. Ford, Functional interaction between AQP2 and TRPV4 in renal cells, *J. Cell. Biochem.* 113 (2012) 580–589.
- [44] M.C. Papadopoulos, A.S. Verkman, Aquaporin water channels in the nervous system, *Nat. Rev. Neurosci.* 14 (2013) 265–277.
- [45] S. Nielsen, E.A. Nagelhus, M. Amiry-Moghaddam, C. Bourque, P. Agre, O.P. Ottersen, Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain, *J. Neurosci.* 17 (1997) 171–180.
- [46] D.K. Binder, X. Yao, Z. Zador, T.J. Sick, A.S. Verkman, G.T. Manley, Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels, *Glia* 53 (2006) 631–636.
- [47] K. Oshio, H. Watanabe, Y. Song, A.S. Verkman, G.T. Manley, Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1, *FASEB J.* 19 (2005) 76–78.
- [48] S.D. Shields, J. Mazario, K. Skinner, A.I. Basbaum, Anatomical and functional analysis of aquaporin 1, a water channel in primary afferent neurons, *Pain* 131 (2007) 8–20.
- [49] H. Gao, C. He, X. Fang, X. Hou, X. Feng, H. Yang, X. Zhao, T. Ma, Localization of aquaporin-1 water channel in glial cells of the human peripheral nervous system, *Glia* 53 (2006) 783–787.
- [50] J. Badaut, J.M. Petit, J.F. Brunet, P.J. Magistretti, C. Charriat-Marlangue, L. Regli, Distribution of Aquaporin 9 in the adult rat brain: preferential expression in catecholaminergic neurons and in glial cells, *Neuroscience* 128 (2004) 27–38.
- [51] F. He, Y.E. Sun, Glial cells more than support cells? *Int. J. Biochem. Cell Biol.* 39 (2007) 661–665.
- [52] N.N. Haj-Yasein, G.F. Vindedal, M. Eilert-Olsen, G.A. Gundersen, O. Skare, P. Laake, A. Klungland, A.E. Thoren, J.M. Burkhardt, O.P. Ottersen, E.A. Nagelhus, Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood–brain water uptake and confers barrier function on perivascular astrocyte endfeet, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 17815–17820.
- [53] J.E. Rash, T. Yasumura, C.S. Hudson, P. Agre, S. Nielsen, Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 11981–11986.
- [54] K. Oshio, D.K. Binder, B. Yang, S. Schecter, A.S. Verkman, G.T. Manley, Expression of aquaporin water channels in mouse spinal cord, *Neuroscience* 127 (2004) 685–693.
- [55] V. Medici, C. Frassoni, L. Tassi, R. Spreafico, R. Garbelli, Aquaporin 4 expression in control and epileptic human cerebral cortex, *Brain Res.* 1367 (2011) 330–339.
- [56] J. Badaut, S. Ashwal, A. Adami, B. Tone, R. Recker, D. Spagnoli, B. Teron, A. Obenaus, Brain water mobility decreases after astrocytic aquaporin-4 inhibition using RNA interference, *J. Cereb. Blood Flow Metab.* 31 (2011) 819–831.
- [57] M.T. Conner, A.C. Conner, C.E. Bland, L.H. Taylor, J.E. Brown, H.R. Parri, R.M. Bill, Rapid aquaporin translocation regulates cellular water flow: mechanism of hypotonicity-induced subcellular localization of aquaporin 1 water channel, *J. Biol. Chem.* 287 (2012) 11516–11525.
- [58] M. Potokar, M. Stenovec, J. Jorgačevski, T. Holen, M. Kreft, O.P. Ottersen, R. Zorec, Regulation of AQP4 surface expression via vesicle mobility in astrocytes, *Glia* 61 (2013) 917–928.
- [59] V. Benfenati, M. Amiry-Moghaddam, M. Caprini, M.N. Mylonakou, C. Rapisarda, O.P. Ottersen, S. Ferroni, Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes, *Neuroscience* 148 (2007) 876–892.
- [60] V. Benfenati, M. Caprini, M. Dovizio, M.N. Mylonakou, S. Ferroni, O.P. Ottersen, M. Amiry-Moghaddam, An aquaporin-4/transient receptor potential vanilloid 4 (AQP4/TRPV4) complex is essential for cell-volume control in astrocytes, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 2563–2568.
- [61] M.C. Papadopoulos, G.T. Manley, S. Krishna, A.S. Verkman, Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema, *FASEB J.* 18 (2004) 1291–1293.
- [62] S. Saadoun, B.A. Bell, A.S. Verkman, M.C. Papadopoulos, Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice, *Brain* 131 (2008) 1087–1098.
- [63] S. Saadoun, M.C. Papadopoulos, D.C. Davies, B.A. Bell, S. Krishna, Increased aquaporin-1 water channel expression in human brain tumours, *Br. J. Cancer* 87 (2002) 621–623.
- [64] J. Li, R.V. Patil, A.S. Verkman, Mildly abnormal retinal function in transgenic mice without Muller cell aquaporin-4 water channels, *Invest. Ophthalmol. Vis. Sci.* 43 (2002) 573–579.
- [65] D.C. Lu, H. Zhang, Z. Zador, A.S. Verkman, Impaired olfaction in mice lacking aquaporin-4 water channels, *FASEB J.* 22 (2008) 3216–3223.
- [66] J. Li, A.S. Verkman, Impaired hearing in mice lacking aquaporin-4 water channels, *J. Biol. Chem.* 276 (2001) 31233–31237.
- [67] L.N. Nejsum, The renal plumbing system: aquaporin water channels, *Cell. Mol. Life Sci.* 62 (2005) 1692–1706.
- [68] K. Fushimi, S. Uchida, Y. Hara, Y. Hirata, F. Marumo, S. Sasaki, Cloning and expression of apical membrane water channel of rat kidney collecting tubule, *Nature* 361 (1993) 549–552.
- [69] K. Ishibashi, S. Sasaki, K. Fushimi, S. Uchida, M. Kuwahara, H. Saito, T. Furukawa, K. Nakajima, Y. Yamaguchi, T. Gojohori, Molecular cloning and expression of a member of the aquaporin family with permeability to glycerol and urea in addition to water expressed at the basolateral membrane of kidney collecting duct cells, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 6269–6273.
- [70] J. Terris, C.A. Ecelbarger, D. Marples, M.A. Knepper, S. Nielsen, Distribution of aquaporin-4 water channel expression within rat kidney, *Am. J. Physiol.* 269 (1995) F775–F785.
- [71] F. Magni, C. Sarto, D. Ticozzi, M. Soldi, N. Bosso, P. Mocarelli, M.G. Kienle, Proteomic knowledge of human aquaporins, *Proteomics* 6 (2006) 5637–5649.
- [72] T. Ma, Y. Song, B. Yang, A. Gillespie, E.J. Carlson, C.J. Epstein, A.S. Verkman, Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 4386–4391.
- [73] Y. Noda, S. Sasaki, Trafficking mechanism of water channel aquaporin-2, *Biol. Cell.* 97 (2005) 885–892.
- [74] P.I. Nedvetsky, G. Tamma, S. Beulshausen, G. Valenti, W. Rosenthal, E. Klusmann, Regulation of aquaporin-2 trafficking, *Handb. Exp. Pharmacol.* (190) (2009) 133–157(133–157).
- [75] S. Sasaki, Aquaporin 2: from its discovery to molecular structure and medical implications, *Mol. Aspects Med.* 33 (2012) 535–546.
- [76] L. Galizia, M.P. Flamenco, V. Rivarola, C. Capurro, P. Ford, Role of AQP2 in activation of calcium entry by hypotonicity: implications in cell volume regulation, *Am. J. Physiol. Renal Physiol.* 294 (2008) F582–F590.
- [77] Y.H. Li, K. Eto, S. Horikawa, S. Uchida, S. Sasaki, X.J. Li, Y. Noda, Aquaporin-2 regulates cell volume recovery via tropomyosin, *Int. J. Biochem. Cell Biol.* 41 (2009) 2466–2476.
- [78] K. Kasono, T. Saito, T. Saito, H. Tamemoto, C. Yanagida, S. Uchida, M. Kawakami, S. Sasaki, S.E. Ishikawa, Hypertonicity regulates the aquaporin-2 promoter independently of arginine vasopressin, *Nephrol. Dial. Transplant.* 20 (2005) 509–515.
- [79] U. Hasler, P. Nunes, R. Bouley, H.A. Lu, T. Matsuzaki, D. Brown, Acute hypertonicity alters aquaporin-2 trafficking and induces a MAPK-dependent accumulation at the plasma membrane of renal epithelial cells, *J. Biol. Chem.* 283 (2008) 26643–26661.
- [80] M.A. Knepper, J.B. Wade, J. Terris, C.A. Ecelbarger, D. Marples, B. Mandon, C.L. Chou, B.K. Kishore, S. Nielsen, Renal aquaporins, *Kidney Int.* 49 (1996) 1712–1717.
- [81] M.T. Conner, A.C. Conner, J.E. Brown, R.M. Bill, Membrane trafficking of aquaporin 1 is mediated by protein kinase C via microtubules and regulated by tonicity, *Biochemistry* 49 (2010) 821–823.
- [82] R.A. Marinelli, N.F. LaRusso, Aquaporin water channels in liver: their significance in bile formation, *Hepatology* 26 (1997) 1081–1084.
- [83] J. Zhang, Y. An, J. Gao, J. Han, X. Pan, Y. Pan, L. Tie, X. Li, Aquaporin-1 translocation and degradation mediates the water transportation mechanism of acetazolamide, *PLoS One* 7 (2012) e45976.
- [84] E.J. Kamsteeg, P.J. Savelkoul, G. Hendriks, I.B. Konings, N.M. Nivillac, A.K. Lagendijk, P. van der Sluijs, P.M. Deen, Misrouting of the Aquaporin-2 mutant E258K to multivesicular bodies/lysosomes in dominant NDI is associated with its monoubiquitination and increased phosphorylation by PKC but is due to the loss of E258, *Pflügers Arch.* 455 (2008) 1041–1054.
- [85] Z. Han, R.V. Patil, Protein kinase A-dependent phosphorylation of aquaporin-1, *Biochem. Biophys. Res. Commun.* 273 (2000) 328–332.
- [86] S. Raina, G.M. Preston, W.B. Guggino, P. Agre, Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues, *J. Biol. Chem.* 270 (1995) 1908–1912.
- [87] X. He, C.M. Tse, M. Donowitz, S.L. Alper, S.E. Gabriel, B.J. Baum, Polarized distribution of key membrane transport proteins in the rat submandibular gland, *Pflügers Arch.* 433 (1997) 260–268.
- [88] S. Nielsen, L.S. King, B.M. Christensen, P. Agre, Aquaporins in complex tissues. II. Subcellular distribution in respiratory and glandular tissues of rat, *Am. J. Physiol.* 273 (1997) C1549–C1561.
- [89] V. Gresz, T.H. Kwon, P.T. Hurley, G. Varga, T. Zelles, S. Nielsen, R.M. Case, M.C. Steward, Identification and localization of aquaporin water channels in human salivary glands, *Am. J. Physiol. Gastrointest. Liver Physiol.* 281 (2001) G247–G254.
- [90] J. Tada, T. Sawa, N. Yamanaka, M. Shono, T. Akamatsu, K. Tsumura, M.N. Parvin, N. Kanamori, K. Hosoi, Involvement of vesicle-cytoskeleton interaction in AQP5 trafficking in AQP5-gene-transfected HSG cells, *Biochem. Biophys. Res. Commun.* 266 (1999) 443–447.
- [91] B.H. Lee, A.E. Gauna, G. Perez, Y.J. Park, K.M. Pauley, T. Kawai, S. Cha, Autoantibodies against muscarinic type 3 receptor in Sjogren's syndrome inhibit aquaporin 5 trafficking, *PLoS One* 8 (2013) e53113.
- [92] Y. Ishikawa, T. Eguchi, M.T. Skowronski, H. Ishida, Acetylcholine acts on M3 muscarinic receptors and induces the translocation of aquaporin5 water channel via cytosolic Ca<sup>2+</sup> elevation in rat parotid glands, *Biochem. Biophys. Res. Commun.* 245 (1998) 835–840.
- [93] A.K. Hansen, H.K. Galtung, Aquaporin expression and cell volume regulation in the SV40 immortalized rat submandibular acinar cell line, *Pflügers Arch.* 453 (2007) 787–796.
- [94] K. Sato, The physiology, pharmacology, and biochemistry of the eccrine sweat gland, *Rev. Physiol. Biochem. Pharmacol.* 79 (1977) 51–131.
- [95] R. Inoue, E. Sohara, T. Rai, T. Satoh, H. Yokozeki, S. Sasaki, S. Uchida, Immunolocalization and translocation of aquaporin-5 water channel in sweat glands, *J. Dermatol. Sci.* 70 (2013) 26–33.
- [96] R. Sougrat, M. Morand, C. Gondran, P. Barre, R. Gobin, F. Bonte, M. Dumas, J.M. Verbavatz, Functional expression of AQP3 in human skin epidermis and reconstructed epidermis, *J. Invest. Dermatol.* 118 (2002) 678–685.
- [97] Y. Sugiyama, Y. Ota, M. Hara, S. Inoue, Osmotic stress up-regulates aquaporin-3 gene expression in cultured human keratinocytes, *Biochim. Biophys. Acta* 1522 (2001) 82–88.
- [98] M. Hara, A.S. Verkman, Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 7360–7365.

- [99] M. Olsson, A. Broberg, M. Jernas, L. Carlsson, M. Rudemo, M. Suurkula, P.A. Svensson, M. Benson, Increased expression of aquaporin 3 in atopic eczema, *Allergy* 61 (2006) 1132–1137.
- [100] D. Blaydon, L. Lind, V. Plagnol, K. Linton, F.D. Smith, N. Wilson, W.H. McLean, C. Munro, A. South, I. Leigh, E. O'Toole, A. Lundström, D. Kelsell, Mutations in AQP5, encoding a water-channel protein, cause autosomal-dominant diffuse nonepidermolytic palmoplantar keratoderma, *Am. J. Hum. Genet.* 93 (2013) 330–335.
- [101] K. Kishida, I. Shimomura, H. Nishizawa, N. Maeda, H. Kuriyama, H. Kondo, M. Matsuda, H. Nagaretani, N. Ouchi, K. Hotta, S. Kihara, T. Kadowaki, T. Funahashi, Y. Matsuzawa, Enhancement of the aquaporin adipose gene expression by a peroxisome proliferator-activated receptor gamma, *J. Biol. Chem.* 276 (2001) 48572–48579.
- [102] K. Kishida, H. Kuriyama, T. Funahashi, I. Shimomura, S. Kihara, N. Ouchi, M. Nishida, H. Nishizawa, M. Matsuda, M. Takahashi, K. Hotta, T. Nakamura, S. Yamashita, Y. Tochino, Y. Matsuzawa, Aquaporin adipose, a putative glycerol channel in adipocytes, *J. Biol. Chem.* 275 (2000) 20896–20902.
- [103] A. Rodriguez, V. Catalan, J. Gomez-Ambrosi, S. Garcia-Navarro, F. Rotellar, V. Valenti, C. Silva, M.J. Gil, J. Salvador, M.A. Burrell, G. Calamita, M.M. Malagon, G. Fruhbeck, Insulin- and leptin-mediated control of aquaglyceroporins in human adipocytes and hepatocytes is mediated via the PI3K/Akt/mTOR signaling cascade, *J. Clin. Endocrinol. Metab.* 96 (2011) E586–E597.
- [104] V. Ceperuelo-Mallafre, M. Miranda, M.R. Chacon, N. Vilarrasa, A. Megia, C. Gutierrez, J.M. Fernandez-Real, J.M. Gomez, E. Caubet, G. Fruhbeck, J. Vendrell, Adipose tissue expression of the glycerol channel aquaporin-7 gene is altered in severe obesity but not in type 2 diabetes, *J. Clin. Endocrinol. Metab.* 92 (2007) 3640–3645.
- [105] M.P. Marrades, F.I. Milagro, J.A. Martinez, M.J. Moreno-Aliaga, Differential expression of aquaporin 7 in adipose tissue of lean and obese high fat consumers, *Biochem. Biophys. Res. Commun.* 339 (2006) 785–789.
- [106] N. Maeda, Implications of aquaglyceroporins 7 and 9 in glycerol metabolism and metabolic syndrome, *Mol. Aspects Med.* 33 (2012) 665–675.
- [107] U. Laforenza, M.F. Scaffino, G. Gastaldi, Aquaporin-10 represents an alternative pathway for glycerol efflux from human adipocytes, *PLoS One* 8 (2013) e54474.
- [108] U. Mehlhorn, H.J. Geissler, G.A. Laine, S.J. Allen, Myocardial fluid balance, *Eur. J. Cardiothorac. Surg.* 20 (2001) 1220–1230.
- [109] J.R. Egan, T.L. Butler, C.G. Au, Y.M. Tan, K.N. North, D.S. Winlaw, Myocardial water handling and the role of aquaporins, *Biochim. Biophys. Acta Biomembr.* 1758 (2006) 1043–1052.
- [110] T.L. Butler, C.G. Au, B. Yang, J.R. Egan, Y.M. Tan, E.C. Hardeman, K.N. North, A.S. Verkman, D.S. Winlaw, Cardiac aquaporin expression in humans, rats, and mice, *Am. J. Physiol. Heart Circ. Physiol.* 291 (2006) H705–H713.
- [111] M.A. Suleymanian, C.M. Baumgarten, Osmotic gradient-induced water permeation across the sarcolemma of rabbit ventricular myocytes, *J. Gen. Physiol.* 107 (1996) 503–514.
- [112] M.R. Kellen, J.B. Bassingthwaite, Transient transcappillary exchange of water driven by osmotic forces in the heart, *Am. J. Physiol. Heart Circ. Physiol.* 285 (2003) H1317–H1331.
- [113] A.S. Verkman, Aquaporin water channels and endothelial cell function\*, *J. Anat.* 200 (2002) 617–627.
- [114] A. Rutkovskiy, K.O. Stenslokken, L.H. Mariero, B. Skrbic, M. Amiry-Moghaddam, V. Hillestad, G. Valen, M.C. Perreault, O.P. Ottersen, L. Gullestad, C.P. Dahl, J. Vaage, Aquaporin-4 in the heart: reexpression, regulation and functional role in ischemia, *Basic Res. Cardiol.* 107 (2012) 280(012-0280-6. Epub 2012 Jul 10).
- [115] M.A. Matthay, H.G. Folkesson, A.S. Verkman, Salt and water transport across alveolar and distal airway epithelia in the adult lung, *Am. J. Physiol.* 270 (1996) L487–L503.
- [116] A.S. Verkman, M.A. Matthay, Y. Song, Aquaporin water channels and lung physiology, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 278 (2000) L867–L879.
- [117] H.G. Folkesson, M.A. Matthay, H. Hasegawa, F. Kheradmand, A.S. Verkman, Transcellular water transport in lung alveolar epithelium through mercury-sensitive water channels, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 4970–4974.
- [118] Z. Borok, A.S. Verkman, Lung edema clearance: 20 years of progress: invited review: role of aquaporin water channels in fluid transport in lung and airways, *J. Appl. Physiol.* 93 (2002) 2199–2206.
- [119] K. Wang, Y.L. Feng, F.Q. Wen, X.R. Chen, X.M. Ou, D. Xu, J. Yang, Z.P. Deng, Decreased expression of human aquaporin-5 correlated with mucus overproduction in airways of chronic obstructive pulmonary disease, *Acta Pharmacol. Sin.* 28 (2007) 1166–1174.
- [120] Y. Song, A.S. Verkman, Aquaporin-5 dependent fluid secretion in airway submucosal glands, *J. Biol. Chem.* 276 (2001) 41288–41292.
- [121] G. Doring, P. Flume, H. Heijerman, J.S. Elborn, Consensus Study Group, Treatment of lung infection in patients with cystic fibrosis: current and future strategies, *J. Cyst. Fibros.* 11 (2012) 461–479.
- [122] C. Bai, N. Fukuda, Y. Song, T. Ma, M.A. Matthay, A.S. Verkman, Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice, *J. Clin. Invest.* 103 (1999) 555–561.
- [123] T. Ma, N. Fukuda, Y. Song, M.A. Matthay, A.S. Verkman, Lung fluid transport in aquaporin-5 knockout mice, *J. Clin. Invest.* 105 (2000) 93–100.
- [124] V.K. Sidhaye, A.D. Guler, K.S. Schweitzer, F. D'Alessio, M.J. Caterina, L.S. King, Transient receptor potential vanilloid 4 regulates aquaporin-5 abundance under hypotonic conditions, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 4747–4752.
- [125] J.D. Hoffert, V. Leitch, P. Agre, L.S. King, Hypertonic induction of aquaporin-5 expression through an ERK-dependent pathway, *J. Biol. Chem.* 275 (2000) 9070–9077.
- [126] F. Yang, J.D. Kawedia, A.G. Menon, Cyclic AMP regulates aquaporin 5 expression at both transcriptional and post-transcriptional levels through a protein kinase A pathway, *J. Biol. Chem.* 278 (2003) 32173–32180.
- [127] V. Sidhaye, J.D. Hoffert, L.S. King, cAMP has distinct acute and chronic effects on aquaporin-5 in lung epithelial cells, *J. Biol. Chem.* 280 (2005) 3590–3596.
- [128] J. Woo, Y.K. Chae, S.J. Jang, M.S. Kim, J.H. Baek, J.C. Park, B. Trink, E. Ratovitski, T. Lee, B. Park, M. Park, J.H. Kang, J.C. Sorim, J. Lee, J. Califano, D. Sidransky, C. Moon, Membrane trafficking of AQP5 and cAMP dependent phosphorylation in bronchial epithelium, *Biochem. Biophys. Res. Commun.* 366 (2008) 321–327.
- [129] H.F. Huang, R.H. He, C.C. Sun, Y. Zhang, Q.X. Meng, Y.Y. Ma, Function of aquaporins in female and male reproductive systems, *Hum. Reprod. Update* 12 (2006) 785–795.
- [130] J. Frede, S.P. Fraser, G. Oskay-Ozcelik, Y. Hong, E. Ioana Braicu, J. Sehoul, H. Gabra, M.B. Djamgoz, Ovarian cancer: ion channel and aquaporin expression as novel targets of clinical potential, *Eur. J. Cancer* 49 (2013) 2331–2344.
- [131] F. Qu, F.F. Wang, X.E. Lu, M.Y. Dong, J.Z. Sheng, P.P. Lv, G.L. Ding, B.W. Shi, D. Zhang, H.F. Huang, Altered aquaporin expression in women with polycystic ovary syndrome: hyperandrogenism in follicular fluid inhibits aquaporin-9 in granulosa cells through the phosphatidylinositol 3-kinase pathway, *Hum. Reprod.* 25 (2010) 1441–1450.
- [132] K. Ishibashi, M. Kuwahara, Y. Gu, Y. Kageyama, A. Tohsaka, F. Suzuki, F. Marumo, S. Sasaki, Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea, *J. Biol. Chem.* 272 (1997) 20782–20786.
- [133] C.H. Yeung, Aquaporins in spermatozoa and testicular germ cells: identification and potential role, *Asian J. Androl.* 12 (2010) 490–499.
- [134] E. Moretti, G. Terzuoli, L. Mazzi, F. Iacoponi, G. Collodel, Immunolocalization of aquaporin 7 in human sperm and its relationship with semen parameters, *Syst. Biol. Reprod. Med.* 58 (2012) 129–135.
- [135] H. Tsukaguchi, C. Shayakul, U.V. Berger, B. Mackenzie, S. Devidas, W.B. Guggino, A.N. van Hoek, M.A. Hediger, Molecular characterization of a broad selectivity neutral solute channel, *J. Biol. Chem.* 273 (1998) 24737–24743.
- [136] N. Pastor-Soler, C. Isnard-Bagnis, C. Herak-Kramberger, I. Sabolic, A. Van Hoek, D. Brown, S. Bretien, Expression of aquaporin 9 in the adult rat epididymal epithelium is modulated by androgens, *Biol. Reprod.* 66 (2002) 1716–1722.
- [137] H.H. Badran, L.S. Hermo, Expression and regulation of aquaporins 1, 8, and 9 in the testis, efferent ducts, and epididymis of adult rats and during postnatal development, *J. Androl.* 23 (2002) 358–373.
- [138] L. Hermo, D. Krzczunowicz, R. Ruz, Cell specificity of aquaporins 0, 3, and 10 expressed in the testis, efferent ducts, and epididymis of adult rats, *J. Androl.* 25 (2004) 494–505.
- [139] D. Duan, C. Winter, S. Cowley, J.R. Hume, B. Horowitz, Molecular identification of a volume-regulated chloride channel, *Nature* 390 (1997) 417–421.
- [140] C.H. Yeung, J.P. Barfield, T.G. Cooper, Chloride channels in physiological volume regulation of human spermatozoa, *Biol. Reprod.* 73 (2005) 1057–1063.
- [141] T. Matsuzaki, Y. Tajika, A. Ablimit, T. Aoki, H. Hagiwara, K. Takata, Aquaporins in the digestive system, *Med. Electron. Microsc.* 37 (2004) 71–80.
- [142] T. Ma, S. Jayaraman, K.S. Wang, Y. Song, B. Yang, J. Li, J.A. Bastidas, A.S. Verkman, Defective dietary fat processing in transgenic mice lacking aquaporin-1 water channels, *Am. J. Physiol. Cell Physiol.* 280 (2001) C126–C134.
- [143] T. Matsuzaki, T. Suzuki, H. Koyama, S. Tanaka, K. Takata, Water channel protein AQP3 is present in epithelia exposed to the environment of possible water loss, *J. Histochem. Cytochem.* 47 (1999) 1275–1286.
- [144] K.S. Wang, A.R. Komar, T. Ma, F. Filiz, J. McLeroy, K. Hoda, A.S. Verkman, J.A. Bastidas, Gastric acid secretion in aquaporin-4 knockout mice, *Am. J. Physiol. Gastrointest. Liver Physiol.* 279 (2000) G448–G453.
- [145] K.S. Wang, T. Ma, F. Filiz, A.S. Verkman, J.A. Bastidas, Colon water transport in transgenic mice lacking aquaporin-4 water channels, *Am. J. Physiol. Gastrointest. Liver Physiol.* 279 (2000) G463–G470.
- [146] P.T. Hurley, C.J. Ferguson, T.H. Kwon, M.L. Andersen, A.G. Norman, M.C. Steward, S. Nielsen, R.M. Case, Expression and immunolocalization of aquaporin water channels in rat exocrine pancreas, *Am. J. Physiol. Gastrointest. Liver Physiol.* 280 (2001) G701–G709.
- [147] M.L. Elkjaer, L.N. Nejsum, V. Gresz, T.H. Kwon, U.B. Jensen, J. Frokiaer, S. Nielsen, Immunolocalization of aquaporin-8 in rat kidney, gastrointestinal tract, testis, and airways, *Am. J. Physiol. Renal Physiol.* 281 (2001) F1047–F1057.
- [148] K. Nihei, Y. Koyama, T. Tani, E. Yaoita, K. Ohshiro, L.P. Adhikary, I. Kurosaki, Y. Shirai, K. Hatakeyama, T. Yamamoto, Immunolocalization of aquaporin-9 in rat hepatocytes and Leydig cells, *Arch. Histol. Cytol.* 64 (2001) 81–88.
- [149] Y. Koyama, T. Yamamoto, T. Tani, K. Nihei, D. Kondo, H. Funaki, E. Yaoita, K. Kawasaki, N. Sato, K. Hatakeyama, I. Kihara, Expression and localization of aquaporins in rat gastrointestinal tract, *Am. J. Physiol.* 276 (1999) C621–C627.
- [150] N. Ikarashi, R. Kon, T. Iizasa, N. Suzuki, R. Hiruma, K. Suenaga, T. Toda, M. Ishii, M. Hoshino, W. Ochiai, K. Sugiyama, Inhibition of aquaporin-3 water channel in the colon induces diarrhea, *Biol. Pharm. Bull.* 35 (2012) 957–962.
- [151] M. Kuwahara, Y. Gu, K. Ishibashi, F. Marumo, S. Sasaki, Mercury-sensitive residues and pore site in AQP3 water channel, *Biochemistry* 36 (1997) 13973–13978.
- [152] F. Garcia, A. Kierbel, M.C. Larooca, S.A. Gradihone, P. Splinter, N.F. LaRusso, R.A. Marinelli, The water channel aquaporin-8 is mainly intracellular in rat hepatocytes, and its plasma membrane insertion is stimulated by cyclic AMP, *J. Biol. Chem.* 276 (2001) 12147–12152.
- [153] M.D. Humzah, R.W. Soames, Human intervertebral disc: structure and function, *Anat. Rec.* 220 (1988) 337–356.
- [154] A. Mobasher, E. Trujillo, S. Bell, S.D. Carter, P.D. Clegg, P. Martin-Vasallo, D. Marples, Aquaporin water channels AQP1 and AQP3, are expressed in equine articular chondrocytes, *Vet. J.* 168 (2004) 143–150.
- [155] J. Kraemer, D. Kolditz, R. Gowin, Water and electrolyte content of human intervertebral discs under variable load, *Spine* 10 (1985) 69–71 (Phila Pa. 1976).

- [156] K. Hagiwara, T. Shinozaki, T. Matsuzaki, K. Takata, K. Takagishi, Immunolocalization of water channel aquaporins in human knee articular cartilage with intact and early degenerative regions, *Med. Mol. Morphol.* 46 (2013) 104–108.
- [157] J.C. Iatridis, J.J. MacLean, M. O'Brien, I.A. Stokes, Measurements of proteoglycan and water content distribution in human lumbar intervertebral discs, *Spine* 32 (2007) 1493–1497 (Phila Pa.1976).
- [158] A. Maroudas, H. Muir, J. Wingham, The correlation of fixed negative charge with glycosaminoglycan content of human articular cartilage, *Biochim. Biophys. Acta* 177 (1969) 492–500.
- [159] S.M. Richardson, R. Knowles, D. Marples, J.A. Hoyland, A. Mobasheri, Aquaporin expression in the human intervertebral disc, *J. Mol. Histol.* 39 (2008) 303–309.
- [160] A. Frigeri, G.P. Nicchia, R. Balena, B. Nico, M. Svelto, Aquaporins in skeletal muscle: reassessment of the functional role of aquaporin-4, *FASEB J.* 18 (2004) 905–907.
- [161] E. Gunnarson, M. Zelenina, A. Aperia, Regulation of brain aquaporins, *Neuroscience* 129 (2004) 947–955.
- [162] G.T. Manley, M. Fujimura, T. Ma, N. Noshita, F. Filiz, A.W. Bollen, P. Chan, A.S. Verkman, Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke, *Nat. Med.* 6 (2000) 159–163.
- [163] M.C. Papadopoulos, A.S. Verkman, Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis, *J. Biol. Chem.* 280 (2005) 13906–13912.
- [164] L. Janosi, M. Ceccarelli, The gating mechanism of the human aquaporin 5 revealed by molecular dynamics simulations, *PLoS One* 8 (2013) e59897.
- [165] S. Tornroth-Horsefield, Y. Wang, K. Hedfalk, U. Johanson, M. Karlsson, E. Tajkhorshid, R. Neutze, P. Kjellbom, Structural mechanism of plant aquaporin gating, *Nature* 439 (2006) 688–694.