Published in final edited form as:

Expert Rev Mol Med. ; 10: e13. doi:10.1017/S1462399408000690.

Mammalian aquaporins: diverse physiological roles and potential clinical significance

A. S. Verkman

Departments of Medicine and Physiology, Cardiovascular Research Institute, 1246 Health Sciences East Tower, University of California, San Francisco, CA 94143-0521, USA. Tel: +1 415 476 8530; Fax: +1 415-665-3847

A. S. Verkman: alan.verkman@ucsf.edu

Abstract

Aquaporins have multiple distinct roles in mammalian physiology. Phenotype analysis of aquaporin-knockout mice has confirmed the predicted role of aquaporins in osmotically driven transepithelial fluid transport, as occurs in the urinary concentrating mechanism and glandular fluid secretion. Aquaporins also facilitate water movement into and out of the brain in various pathologies such as stroke, tumour, infection and hydrocephalus. A major, unexpected cellular role of aquaporins was revealed by analysis of knockout mice: aquaporins facilitate cell migration, as occurs in angiogenesis, tumour metastasis, wound healing, and glial scar formation. Another unexpected role of aquaporins is in neural function – in sensory signalling and seizure activity. The water-transporting function of aquaporins is likely responsible for these roles. A subset of aquaporins that transport both water and glycerol, the ‘aquaglyceroporins’, regulate glycerol content in epidermal, fat and other tissues. Mice lacking various aquaglyceroporins have several interesting phenotypes, including dry skin, resistance to skin carcinogenesis, impaired cell proliferation, and altered fat metabolism. The various roles of aquaporins might be exploited clinically by development of drugs to alter aquaporin expression or function, which could serve as diuretics, and in the treatment of brain swelling, glaucoma, epilepsy, obesity and cancer.

The aquaporins (AQPs) are a family of small, hydrophobic, integral membrane proteins (~30 kDa/monomer) that are expressed widely in the animal and plant kingdoms, with 13 members identified to date in mammals. AQPs are expressed in many epithelia and endothelia involved in fluid transport, such as kidney tubules, glandular epithelia and choroid plexus, as well as in cell types that do not carry out significant fluid transport, such as skin and fat cells. In most cell types the AQPs reside constitutively at the cell plasma membrane, with the notable exception of AQP2 in kidney collecting duct, where vasopressin regulates AQP2 trafficking between endosomes and the cell plasma membrane.

High-resolution structures have been obtained for several AQPs, and show the assembly of AQP monomers in tetramers, with individual monomers containing six tilted α -helical domains forming a barrel-like structure in which the first three and last three helices exhibit inverted symmetry (Refs 1, 2). Molecular dynamics simulations suggest tortuous, single-file passage of water through a narrow <0.3 nm pore, in which steric and electrostatic factors prevent transport of protons and other small molecules (Ref. 3). AQPs 1, 2, 4, 5 and 8 are primarily water selective, whereas AQPs 3, 7 and 9 (called ‘aquaglyceroporins’) also transport glycerol and possibly other small solutes. Water transport by some AQPs is inhibited by nonspecific, cysteine-sulphydryl-reactive compounds such as mercuric chloride (HgCl₂). There is considerable interest, although little reported progress, in the identification of nontoxic AQP-selective inhibitors, which could serve as valuable research tools and clinical therapies.

Tissue distribution and regulation studies have provided indirect evidence for the involvement of AQPs in a variety of physiological processes. In the case of AQP2, nephrogenic diabetes insipidus in subjects with *AQP2* mutations indicated the requirement of AQP2 for the formation of a concentrated urine (Ref. 4). Much of the knowledge of AQP functions in mammalian physiology has come from phenotype analysis of mice lacking the various mammalian AQPs. One paradigm that has emerged is that tissue-specific AQP expression does not mandate AQP involvement in a physiologically important process, as was found for several AQPs in lung (Ref. 5) and intestine (Ref. 6), AQP4 in skeletal muscle (Ref. 7), AQP5 in sweat gland (Ref. 8), and AQP8 in multiple tissues (Ref. 9). Functional analysis of cells and tissues from knockout mice has also tested proposed roles of AQPs in gas transport and intracellular organellar function, as well as in AQP protein–protein interactions. Although there is evidence that some AQPs may allow transport of CO₂ and NH₃ (Refs 10, 11), physiological studies in mice and transport measurement in isolated tissues have provided evidence against a physiologically significant role of AQPs in gas transport (Refs 12, 13, 14). Negative data were also found for the proposed involvement of AQPs in mitochondrial function (Ref. 15) and in a key AQP protein–protein interaction in the central nervous system – a proposed interaction between AQP4 and the inwardly rectifying K⁺ channel Kir4.1 responsible for K⁺ ion uptake by glial cells during neuroexcitation (Refs 16, 17).

Balancing the many negative phenotype studies, data from knockout mice implicate important roles of AQPs in kidney, brain, eye, skin, fat and exocrine glands, suggesting their involvement in major organ functions and disease processes, including urinary concentrating, brain swelling, epilepsy, glaucoma, cancer and obesity. This reviews focuses on these AQP roles and their significance in normal organ physiology and disease. Figure 1 provides a schematic summary of the various AQP roles in mammalian physiology, and is referred to in the various sections below.

Epithelial fluid transport

Active fluid secretion and absorption

AQPs are expressed in many epithelia, such as kidney tubules, glands, choroid plexus, ciliary body and alveoli, where they increase transepithelial osmotic water permeability. One consequence of increased transepithelial water permeability is active (also referred to as ‘facilitated’ or ‘near-isosmolar’) fluid secretion and absorption. Active fluid transport involves the creation of an osmotic gradient (generally quite small) across an epithelium by active ion/solute transport, which drives water transport through highly water-permeable epithelial cell membranes (Fig. 1a). Reduced epithelial cell osmotic water permeability can consequently impair active fluid transport and osmotic water equilibration, resulting in the secretion (or absorption) of a reduced volume of inappropriately hypertonic fluid. This prediction has been confirmed in AQP5-knockout mice in salivary gland (Refs 18, 19) and airway submucosal gland (Ref. 20). Defective fluid secretion has also been found in AQP1-knockout mice in choroid plexus (Ref. 21), which produces cerebrospinal fluid, and in ciliary epithelium (Ref. 22), which produces ocular aqueous fluid. In each of these systems the rate of transepithelial fluid secretion, normalised to epithelial surface area, is very high, such that the reduced but nonzero water permeability in AQP deficiency impacts on transepithelial osmotic equilibration. Of note, because of the substantial intrinsic water permeability of lipid bilayers, AQP deficiency is generally not associated with more than a five- to tenfold reduction in transepithelial osmotic water permeability. Very rapid, active transepithelial fluid absorption occurs in kidney proximal tubule, where the majority of fluid filtered by the glomerulus is reabsorbed. Proximal-tubule fluid absorption is impaired in mice lacking the proximal-tubule water channel AQP1 (Ref. 23), resulting in inappropriately hypertonic absorbed fluid (Ref. 24).

By contrast to these examples of AQP-dependent transepithelial fluid secretion and absorption, there are examples where AQP deletion does not affect active fluid secretion or absorption. In lung alveolus, although deletion of AQP1 or AQP5 each reduces airspace-capillary water permeability by about tenfold, active fluid absorption is not impaired under normal physiological conditions (Refs 25, 26), including during rapid airspace fluid absorption in the neonatal lung and during stresses such as lung injury (Ref. 27). Similarly, deletion of AQPs does not impair active fluid absorption in the airways (Ref. 28), peritoneal cavity (Ref. 29) and pleural cavity (Ref. 30), or fluid secretion by the sweat gland (Ref. 8). The common feature of these examples is that the area-normalised rates of transepithelial fluid transport are substantially lower than those where AQP deletion impairs fluid transport. Thus, as predicted from the considerations in Fig. 1a, whether AQPs are required to facilitate transepithelial fluid transport depends on the rate of fluid transport.

Osmotic equilibration across kidney tubules and the urinary concentrating mechanism

Another anticipated role of AQPs is in water transport across kidney tubules and microvessels (vasa recta), which is required for the formation of a concentrated urine. The major AQPs expressed in the kidney are AQPs 1, 2, 3, 4 and 7 (Fig. 2a). Deletion of the genes for AQP1 and/or AQP3 in mice results in marked polyuria, as seen in 24 h urine collections (Fig. 2b) (Refs 31, 32). Measurement of urine osmolalities in mice before and after 36 h water deprivation (Fig. 2c) shows that urinary osmolality in AQP1-null mice is low and does not increase with water deprivation, resulting in severe dehydration. AQP3-null mice are able to generate a partially concentrated urine in response to water deprivation, whereas AQP4-null mice manifest only a mild defect in maximum urinary concentrating ability (Ref. 33).

AQP1 deletion produces polyuria and unresponsiveness to water deprivation by two distinct mechanisms: impaired near-isosmolar water reabsorption in the proximal tubule, as described above, and reduced medullary hypertonicity resulting from impaired countercurrent multiplication and exchange (a consequence of low water permeability in the thin descending limb of Henle and outer medullary descending vasa recta). Transepithelial osmotic water permeability in the isolated microperfused S2 segment of proximal tubule was reduced by about fivefold in AQP1-knockout mice (Ref. 23), indicating that most water transport in the proximal tubule is transcellular and AQP1 dependent. AQP1 also provides the major route for transepithelial water permeability in the thin descending limb of Henle and outer medullary descending vasa recta (Refs 34, 35). These results support the conclusions that AQP1 is the principal water channel in these segments, and that AQP1 plays a key role in the antidiuretic kidney in the generation of the hypertonicity of the medullary interstitium by countercurrent multiplication and exchange. The aquaglyceroporin AQP7 is expressed in a small distal segment (S3 segment) of the proximal tubule; however, its deletion in mice is not associated with significant impairment in urinary concentrating ability, but rather with an impairment of glycerol clearance, whose significance remains unclear (Ref. 36).

AQP3 and AQP4 are expressed at the basolateral membrane of collecting duct epithelium, with relatively greater expression of AQP3 in cortical and outer medullary collecting duct, and AQP4 in inner medullary collecting duct. In contrast to AQP1 deficiency, countercurrent multiplication and exchange mechanisms in AQP3/AQP4-null mice are basically intact. The polyuria in AQP3-null mice results from reduced osmotic water permeability of cortical collecting duct basolateral membrane (Ref. 32). Reduced transepithelial osmosis across the collecting duct epithelium interferes with osmotic water extraction from fluid flowing through the tubule lumen (as shown in Fig. 1b), resulting in the excretion of an inappropriately large volume of dilute urine. AQP4-null mice manifest only a mild impairment in maximal urinary concentrating ability (Ref. 33), despite a

fourfold reduced water permeability in microperfused inner medullary collecting duct (Ref. 37), in part because of the relatively greater quantity of fluid absorbed by cortical versus inner medullary segments of the collecting duct. Several mouse models of *AQP2* gene deletion and mutation (reviewed in Ref. 38) support the conclusion from humans with nephrogenic diabetes insipidus that AQP2 is the major vasopressin-regulated water channel whose apical membrane targeting in collecting duct during antidiuresis is crucial for the formation of a concentrated urine. Because transepithelial water transport in collecting duct is transcellular, the impairment in urinary concentration resulting from reduced water permeability (Fig. 1b) can result from reduced water permeability of the serial apical (AQP2-containing) or basolateral (AQP3/AQP4-containing) membrane barriers.

Brain swelling

Another major AQP role related to its water transport function is in brain water balance. AQP4 is expressed in glial cells (astrocytes) throughout the brain and spinal cord, particularly at sites of fluid transport at blood–brain and brain–cerebrospinal-fluid (CSF) interfaces. AQP4 expression is polarised to glial cell foot processes in contact with blood vessels, and in the dense glial cell processes that form the glia limitans lining the CSF-bathed pial and ependymal surfaces in the subarachnoid space and the ventricles. AQP4 provides the major route for water transport across glial cell membranes. Osmotic water permeability in glial cells cultured from AQP4-null mice was sevenfold lower than that from wild-type mice (Ref. 39). Also, greatly slowed accumulation of brain water was found in AQP4-null mice in response to serum hypo-osmolality, as monitored by a noninvasive near-infrared optical method (Ref. 40), or by brain wet-to-dry weight ratios (Ref. 41).

Classification of brain oedema

According to the Klatzo classification (Ref. 42), brain oedema can be classified as cytotoxic (cell swelling) oedema or vasogenic (leaky vessel) oedema (Fig. 3a). In cytotoxic oedema, excess water moves from the vasculature into the brain parenchyma through an intact blood–brain barrier. The forces driving water flow to form cytotoxic oedema are osmotic, generated in water intoxication by reduced plasma osmolality, and in ischaemia and other pathologies by impaired Na^+/K^+ -ATPase pump function with consequent Na^+ and water accumulation in brain cells. When the blood–brain barrier becomes disrupted (as in brain tumour or abscess), water is driven by hydrostatic forces from the vasculature into the extracellular space of the brain in an AQP4-independent manner to form vasogenic oedema. Excess brain water is eliminated primarily through the glia limiting membrane into the CSF, and to a lesser extent back through the blood–brain barrier into the blood. The blood–brain barrier may become an important route for water elimination in obstructive hydrocephalus when other routes or water exit are impaired.

Cytotoxic brain oedema

Phenotype analysis of AQP4-null mice has provided compelling evidence for AQP4-facilitated brain water accumulation in cytotoxic oedema and for AQP4-facilitated brain water elimination in vasogenic oedema. Water intoxication, produced experimentally in mice by intraperitoneal water injection, is an example of pure cytotoxic oedema in which water is driven osmotically into the brain through an intact blood–brain barrier. AQP4-null mice have remarkably improved survival following water intoxication compared with wild-type mice (Fig. 3b), with reduced brain water accumulation and glial cell foot-process swelling (Ref. 43). Reduced brain swelling and improved clinical outcome was also found in AQP4-null mice in a model of ischaemic stroke produced by transient middle cerebral artery occlusion (Ref. 43) and in a model of bacterial meningitis produced by intracisternal streptococcus injection (Ref. 41). Reduced brain swelling in water intoxication was also

reported in α -syntrophin-null mice, which secondarily manifest disrupted brain AQP4 expression (Ref. 44). AQP4 inhibition may thus provide a new approach to reduce brain swelling in cytotoxic oedema, which would complement the currently available therapies, including decompressive craniectomy and intravenous mannitol administration – techniques that have changed little over the last century. Recently, greatly improved outcome with neuronal preservation was found in AQP4-null mice in a model of spinal cord compression injury (Ref. 45), which is likely a consequence of reduced water entry into the spinal cord in AQP4 deficiency by a cytotoxic-like mechanism.

Vasogenic brain oedema and hydrocephalus

By contrast to these examples of cytotoxic oedema, AQP4 deletion in mice increases brain water accumulation and worsens outcome in vasogenic brain oedema and hydrocephalus. In a model of pure vasogenic oedema produced by continuous intraparenchymal fluid infusion, there was increased brain water accumulation with greater elevation in intracranial pressure in AQP4 deficiency (Ref. 46) (Fig. 3c). Similar findings were obtained in other examples of vasogenic oedema, including brain tumour, brain abscess and focal cortical freeze injury (Refs 46, 47), supporting the conclusion that in vasogenic oedema fluid is eliminated primarily by an AQP4-dependent route. Finally, in a kaolin-injection model of obstructive hydrocephalus, producing what has been called 'interstitial oedema', AQP4-null mice develop marked ventricular enlargement (Fig. 3d), probably due to reduced transependymal water clearance (Ref. 48).

AQP4 is thus a major determinant of fluid movement into and out of the brain. Many brain pathologies, such as impact injury and toxic encephalopathies, produce brain oedema by a combination of cytotoxic and vasogenic mechanisms, each with a different time course and severity, so it is difficult a priori to predict whether and when AQP4 inhibition would be beneficial or detrimental.

Swelling of ocular tissues

As in the brain, AQPs in the eye are likely to be important in fluid balance and pathology in some ocular tissues. The eye expresses several AQPs at putative sites of fluid transport. The expression of MIP (major intrinsic protein, also referred to as AQP0) in lens fibre has been known for many years. Mutations in *AQP0* in humans are associated with congenital cataracts (Ref. 49), and recent data suggest the involvement of AQP0 in lens fibre cell adhesion (Ref. 1). AQP1 is expressed in corneal endothelium, and at sites of aqueous fluid production (ciliary epithelium) and outflow (trabecular meshwork). AQP3 is expressed in the conjunctival epithelium. AQP4 is expressed in Müller cells in retina, and is coexpressed with AQP1 in nonpigmented ciliary epithelium. AQP5 is expressed in corneal epithelia. This expression pattern provides indirect evidence for AQP involvement in intraocular pressure regulation (AQP1 and AQP4) (Ref. 22), corneal and lens transparency (AQP0, AQP1 and AQP5) (Refs 50, 51), visual signal transduction (AQP4) (Ref. 52), tear film homeostasis (AQP3 and AQP5) (Ref. 53), and conjunctival barrier function (AQP3) (Ref. 53). These possibilities have been examined systematically by phenotype analysis of AQP-knockout mice. We focus here on AQP involvement in ocular tissue swelling.

Cornea and lens swelling

In cornea, endothelial cell AQP1 and epithelial cell AQP5 are involved in corneal stromal water balance, and thus maintenance of corneal transparency. Corneal thickness is reduced compared with normal in AQP1-null mice and increased in AQP5-null mice (Ref. 51). In an experimental model of corneal swelling produced by exposure of the ocular surface to hypo-osmolar fluid, the recovery of corneal transparency and thickness after hypotonic swelling

was greatly delayed in AQP1-null mice. AQP1 is also expressed in the epithelial cell layer surrounding the lens, where it plays a role in lens water balance (Ref. 50). Although AQP1 deletion did not alter baseline lens morphology or transparency, loss of lens transparency was greatly increased in an in vitro model of cataractogenesis produced by incubation of lenses in high-glucose solutions. Cataract formation was also greatly accelerated in AQP1-null mice in an in vivo model of cataractogenesis produced by acetaminophen toxicity. Notwithstanding lack of a clear-cut mechanism for these observations, the results suggest the interesting possibility of reducing corneal and lens oedema by AQP1 upregulation.

Retinal swelling

There is also evidence implicating AQP4 in retinal swelling. AQP4 is expressed in Müller cells in retina, where it is involved in light signal transduction (see section 'Neural signal transduction' below). Based on the protection against cytotoxic brain oedema conferred by *AQP4* gene deletion, the possibility was tested that *AQP4* deletion protects the retina in a transient ischaemia-reperfusion model produced by 45–60 min elevation in intraocular pressure to 120 mmHg (Ref. 54). Retinal structure and cell number were remarkably preserved in AQP4-null mice, particularly in the inner nuclear and plexiform layers of retina where Müller cells are concentrated. Retinal function and cell survival were also improved in AQP4-null mice, with electroretinographic evidence of significant attenuation of the reduction in b-wave amplitudes. Whether the neuroprotective effects of AQP4 deletion in retina can be exploited in the therapy of human ocular disease remains to be explored.

Cell migration

Impaired angiogenesis in AQP1 deficiency

Phenotype analysis of AQP1-null mice led to the discovery of AQP involvement in cell migration. Given the expression of AQP1 in tumour microvessels (Ref. 55), the involvement of AQP1 in tumour angiogenesis was tested (Ref. 56). AQP1 deletion in mice reduced tumour growth following subcutaneous injection of melanoma cells (Fig. 4a), which was associated with increased tumour necrosis and reduced blood-vessel formation within the tumour bed. In experiments to elucidate the mechanism of defective tumour angiogenesis in AQP1 deficiency, it was found that cultured aortic endothelial cells from AQP1-null mice migrated several-fold slower towards a chemotactic stimulus than AQP1-expressing endothelial cells. Other processes involved in angiogenesis, including endothelial cell proliferation and adhesion, were not impaired in AQP1 deficiency. Transfection of AQP1 or other AQPs into cells that do not express AQPs increased their migration, suggesting the involvement of AQP-facilitated cell membrane water permeability in cell migration. In the migrating cells, AQP1 becomes polarised to the front end of cells (Fig. 4b), and is associated with increased turnover of cell membrane protrusions (lamellipodia), suggesting that AQPs at the leading edge of migrating cells facilitate their migration.

Reduced tumour spread, glial scarring and wound healing in AQP deficiency

Follow-up experiments showed that AQPs facilitate cell migration independent of AQP and cell type. AQP4 facilitates astrocyte cell migration (Refs 57, 58), AQP3 facilitates migration of corneal epithelial cells (Ref. 59) and epidermal cells (Ref. 60), and AQP1 facilitates the migration of cultured renal proximal tubule cells (Ref. 61), B16F10 melanoma and 4T1 breast cancer cells (Ref. 62). These studies also demonstrated that AQP-facilitated cell migration participates not only in angiogenesis but also in other processes including tumour cell spread, glial scar formation, and wound healing. AQP1 expression in tumour cells increases their migration across endothelial barriers, local invasiveness and metastatic potential (Ref. 62). AQP4 deletion in glial cells reduces their migration toward a stab wound in vivo (Ref. 58) and the rate of glial scar formation (Ref. 57). AQP3 deletion impairs

closure of cutaneous wounds (Ref. 60) and corneal wounds (Ref. 59). Although not yet tested, AQPs may also be involved in organ regeneration and immune cell chemotaxis.

Mechanisms of AQP-facilitated cell migration

The enhanced cell migration found for multiple structurally different AQPs, independent of their modulation method (transfection, knockout, RNA inhibition), suggests that AQP-facilitated water transport is the responsible mechanism. AQPs might accelerate cell migration by facilitating rapid changes in cell volume that accompany changes in cell shape as cells squeeze through the narrow extracellular space. Water flow across the cell membrane may also allow migrating cells to generate hydrostatic forces to push apart adjacent stationary cells. This mechanism, however, does not account for the polarisation of AQPs to the front end of migrating cells or for AQP enhancement of lamellipodial dynamics, which support a role for water movement across the leading edge of migrating cells, as was proposed previously (Ref. 63). According to this hypothesis, actin depolymerisation and ion influx increase cytoplasmic osmolality at the front end of the migrating cell, driving water influx across the plasma membrane (Fig. 1c). Water influx would thus expand the adjacent plasma membrane by increased local hydrostatic pressure, followed by actin repolymerisation to stabilise the cell membrane protrusion. There is evidence that regional hydrostatic pressure changes within cells do not equilibrate throughout the cytoplasm on scales of 10 μm and 10 s (Ref. 64), and could thus contribute to the formation of localised cell membrane protrusions. Further studies, including direct measurements of water flow across the leading edge of migrating cells, are needed to validate these ideas.

Neural signal transduction

Impaired neural signal transduction in AQP4 deficiency

AQP4 appears to play an unexpected role in neural function. AQP4 is expressed in supportive cells adjacent to electrically excitable cells, as in glia versus neurons in brain and spinal cord, Müller versus bipolar cells in retina, and supportive versus hair cells in the inner ear. Electrophysiological measurements indicated impaired auditory and visual signal transduction in AQP4-null mice, seen as increased auditory brainstem response thresholds (Refs 65, 66) and reduced electroretinographic potentials (Ref. 52). In brain, seizure susceptibility in response to the convulsant pentylenetetrazol was reduced in AQP4-null mice (Ref. 67). In freely moving mice, electrically induced seizures following hippocampal stimulation, as measured by electroencephalography, showed greater threshold and remarkably longer duration in AQP4-null mice (Ref. 68). In agreement with these findings, α -syntrophin-deficient mice developed more-severe behavioural seizures than wild-type mice following hyperthermia (Ref. 69). Recently, defective olfaction was found in AQP4-null mice as demonstrated in behavioural studies and odorant-induced electro-olfactogram responses (Ref. 70).

Possible mechanisms of impaired neuroexcitation in AQP4 deficiency

The mechanisms for altered neuroexcitation in AQP4 deficiency are unclear at present (Fig. 1d). Delayed K^+ uptake from brain extracellular space (ECS) in AQP4 deficiency has been suggested, which may account for the prolonged seizure phenotype. Measurements of $[\text{K}^+]$ in brain cortex in living mice using K^+ -sensitive microelectrodes showed significant slowing of K^+ clearance following electrical stimulation (Ref. 68). Using a K^+ -sensitive fluorescent dye applied directly to the brain in living mice following craniectomy, altered K^+ wave dynamics were found in a cortical spreading depression model of neuroexcitation, again with delayed K^+ clearance (Ref. 71). How delayed K^+ reuptake from the ECS is related to AQP4 deficiency is not known. It has been proposed that AQP4 associates in a functionally

significant manner with Kir4.1, such that that reduced K⁺-channel function in AQP4 deficiency might account for the delay in K⁺ clearance. However, recent patch-clamp studies in astroglia (Ref. 16) and Müller cells (Ref. 17) provide evidence against this mechanism. Another possible mechanism involves ECS expansion in AQP4 deficiency, which may account in part for reduced seizure susceptibility and prolonged seizure duration in AQP4-null mice. An expanded ECS would provide a larger aqueous volume to dilute K⁺ released into the ECS during neuroexcitation, thereby slowing changes in ECS K⁺ concentration. There is evidence for an expanded ECS in AQP4 deficiency from cortical surface photobleaching (Ref. 72) and microfiberoptic photobleaching (Ref. 73) measurements of the diffusion of fluorescently labelled macromolecules in mouse brain. It remains unclear, however, whether ECS expansion in AQP4 deficiency could account fully for the altered ECS K⁺ dynamics. Perhaps reduced water permeability in AQP4 deficiency may be responsible for defective neuroexcitation function by a mechanism involving impaired cell volume responses. Alternative possible mechanisms include AQP4 interaction with key ion channels, perhaps through PDZ-domain interactions, and maladaptive regulation in AQP4 deficiency of other transporters involved in neuroexcitation.

Glycerol transport by the aquaglyceroporins

For many years the physiological significance of glycerol transport by the aquaglyceroporins was unclear. Phenotype studies of mice lacking aquaglyceroporins have produced a number of remarkable findings for the involvement of AQP3 in epidermal biology and cell proliferation, and of AQP7 in adipocyte metabolism. A recent report on AQP9-null mice showed a subtle phenotype suggestive of impaired hepatic glycerol uptake (Ref. 74), although the mechanism remains to be established as does its proposed significance to diabetes.

AQP3 and skin function

The stratum corneum (SC) is the most superficial layer of skin, consisting of a lamellar lipid layer and terminally differentiated keratinocytes that originate from actively proliferating keratinocytes in lower epidermis. SC hydration is an important determinant of skin appearance and physical properties, and depends on several factors including the external humidity, and SC structure, lipid/protein composition, barrier properties, and concentration of water-retaining osmolytes.

AQP3 is expressed strongly in the basal layer of keratinocytes (Fig. 5a). SC hydration is reduced in AQP3-null mice as measured by high-frequency skin conductance (Ref. 75) (Fig. 5b), which is a linear index of SC water content. Exposure of mice to high humidity or occlusion increased SC hydration in wild-type, but not AQP3-null mice, indicating that water transport through AQP3 is not a rate-limiting factor in transepidermal water loss. If reduced SC hydration is related to a balance between evaporative water loss from the SC and water replacement through AQP3-containing basal keratinocytes, then preventing water loss by high humidity or occlusion should have corrected the defect in SC hydration in AQP3-null mice, which it did not. Skin phenotype analysis also indicated delayed barrier recovery after SC removal by tape-stripping in AQP3-null mice, as well as decreased skin elasticity and delayed wound healing (Ref. 75).

A systematic analysis of SC and epidermal ultrastructure and composition revealed reduced glycerol content in SC and epidermis (Fig. 5c), with normal glycerol in dermis and serum, suggesting reduced glycerol transport from blood into the epidermis in AQP3 deficiency through the basal keratinocytes (Fig. 1e). No significant differences in wild-type versus AQP3-null mice were found in SC structure, cell turnover, lipid profile, protein content, and the concentrations of amino acids, ions and other small solutes (Ref. 76). These observations

suggest that reduced epidermal and SC glycerol content is responsible for the abnormal skin phenotype in AQP3-null mice. Because glycerol is a water-retaining osmolyte, or 'natural moisturising factor', reduced SC glycerol reduces SC hydration and skin elasticity; furthermore, because of its biosynthetic role in the epidermis (see next section), reduced epidermal glycerol is predicted to delay barrier recovery function and wound healing. In support of this hypothesis, glycerol replacement by topical or systemic routes corrected the phenotype abnormalities in AQP3-null mice, and SC glycerol content correlated well with SC water content (Ref. 77). These findings indicate an important role for AQP3 and glycerol in epidermal function, providing a rational scientific basis for the long-standing practice of including glycerol in cosmetic and skin medicinal preparations.

AQP3 and cell proliferation

Recent data support the unexpected involvement of AQP3 in cell proliferation in certain cell types. Remarkably, mice lacking AQP3 failed to produce cutaneous papillomas in an inducer-promoter model of skin cancer, whereas wild-type mice produced multiple tumours (Fig. 5d) (Ref. 78). The motivation for studying AQP3 and skin tumours was the strong expression of AQP3 in basal cells in human skin squamous cell carcinomas, and data showing AQP3-facilitated cell proliferation in several cell types. Wound healing and corneal epithelial cell proliferation are impaired in AQP3 deficiency (Ref. 59), as is the healing of cutaneous wounds (Ref. 60) and regeneration of the colonic epithelium in experimental colitis (Ref. 79).

Experiments to establish the cellular mechanisms responsible for the impaired tumourigenesis phenotype showed impaired promoter-induced cell proliferation in AQP3-null or -knockdown keratinocyte cell cultures. AQP3-deficient keratinocytes had reduced content of glycerol, its metabolite glycerol-3-phosphate, and ATP, without impairment of mitochondrial function. Glycerol supplementation or *AQP3* adenoviral infection (but not *AQP1* adenoviral infection) corrected the defects in keratinocyte proliferation and increased ATP. Further studies revealed correlations between cell proliferation, and ATP and glycerol content. It was proposed that AQP3-facilitated glycerol transport is an important determinant of epidermal cell proliferation and tumourigenesis by a mechanism in which glycerol is a key regulator of cellular ATP energy (Fig. 1f). The mechanism also shows glycerol biosynthetic incorporation into lipids, and positive feedback in which cell proliferation increases AQP3 expression. These findings have potential implications in the prevention and therapy of skin and other cancers, and raise concerns in the use of cosmetics containing ingredients that increase epidermal AQP3 expression whose goal is to improve skin moisture and appearance (Ref. 80).

AQP7 and fat metabolism

AQP7 is expressed in the plasma membrane of adipocytes. Although wild-type and AQP7-null mice grow at similar rates as assessed by mouse weight, over time AQP7-null mice develop significantly greater fat mass compared with wild-type mice (Ref. 81). Adipocytes from adult AQP3-null mice are several-fold larger than those from wild-type mice, suggesting that the greater fat mass in the AQP7-null mice is a consequence of adipocyte hypertrophy. Concentrations of glycerol and triglycerides in serum were unaffected by AQP7 deletion, but adipocyte glycerol and triglyceride concentrations were significantly elevated in AQP7-null mice, suggesting a mechanism for the progressive adipocyte hypertrophy in AQP7 deficiency. Plasma membrane glycerol permeability was reduced significantly in adipocytes of AQP7-null mice, as was glycerol release. However, lipolysis, as measured by free fatty acid release from isolated adipocytes, was similar in wild-type and AQP7-deficient mice, as was lipogenesis, as assayed from the incorporation of [¹⁴C]glucose into triglycerides. From these results, we proposed a simple mechanism for progressive

triglyceride accumulation in AQP7-deficient adipocytes (Fig. 1g), in which reduced plasma membrane glycerol permeability in AQP7 deficiency produces an increased glycerol concentration in adipocyte cytoplasm, resulting in increased glycerol-3-phosphate and triglyceride biosynthesis. Similar conclusions about fat metabolism in AQP7 deficiency were reported independently (Ref. 82), although with some relatively minor differences in phenotype findings compared with our results. It was speculated that AQP7 plays an important role in the pathogenesis of human obesity (reviewed in Ref. 83), although whether this is the case remains to be determined.

Clinical implications/applications

Notwithstanding differences in mouse versus human physiology, the involvement of AQPs in major physiological processes in mouse models likely has a number of clinical implications. The requirement of AQPs for the formation of a concentrated urine suggests that AQP inhibitors, or 'AQP-aquaretics', would act as unique diuretics with potential utility in diuretic-refractory oedematous states such as severe congestive heart failure. Inhibitors of various AQPs are also predicted to have potential efficacy in reducing water entry into the brain in cytotoxic oedema, in improving the outcome following spinal cord injury, in reducing aqueous fluid production in glaucoma, in inhibiting glial scar formation, in reducing angiogenesis and tumour spread, in reducing cell proliferation in certain cancers, and in increasing seizure threshold in epilepsy. Drugs that increase AQP function, acting for example by increasing AQP expression, are predicted to have potential efficacy in reducing fat mass in obesity, in accelerating brain water clearance in vasogenic oedema, and in promoting wound healing and tissue regeneration following injury. Validation of these predictions will require the development of appropriate AQP-specific modulators. Another AQP-related clinical application is in disease diagnosis, as demonstrated for serum AQP4 autoantibodies in diagnosing the optic-spinal form of multiple sclerosis (Ref. 84), and for urinary AQP2 protein in distinguishing among various aetiologies of nephrogenic diabetes insipidus (Ref. 85). Other AQP-related disease markers are likely to be identified. The possibility of AQP polymorphisms contributing to human disease is largely unexplored. It would be worthwhile, for example, to investigate polymorphisms in AQP4 in brain diseases such as hydrocephalus, in AQP3 in skin diseases, and in various AQPs in cancer. Last, the modulation of AQP expression in disease states may be clinically important. In some cases altered AQP expression appears to be a maladaptive response, as in the case of reduced renal AQP2 expression in various forms of polyuria, which further impairs urinary concentrating ability (Ref. 86), and AQP4 upregulation in various aetiologies of brain swelling, which may exacerbate brain water accumulation. There is an expanding literature on altered AQP expression in human diseases, with evidence for altered AQP3 expression in skin diseases (Ref. 87), AQP4 expression in epilepsy (Ref. 88), and AQP7 expression in obesity and metabolic diseases (Ref. 89). In most cases, however, it will likely be difficult to establish the cellular mechanisms and clinical significance of such observations.

Research in progress and outstanding research questions

There remain many unanswered questions about the roles of AQPs in mammalian physiology, as well as exciting opportunities for clinical applications. Although water transport has been studied for many decades and AQP proteins were identified in the early 1990s, many of the new AQP cellular functions were recognised only in the past few years, so it is likely that additional new AQP functions will be discovered. Much work remains in the precise elucidation of cellular mechanisms responsible for AQP involvement in cell migration, neural signal transduction and in vasogenic brain oedema, and in the precise role of the aquaglyceroporins in cellular metabolism and proliferation. Finally, as mentioned in the previous section, identification of chemical AQP-selective modulators is a high priority

in ongoing research, as small-molecule AQP inhibitors and upregulators have the potential to serve as new tools to study AQP function and as potential therapies for major human diseases.

Acknowledgments

Acknowledgements and funding

The contributions of many collaborators and trainees who shaped the ideas presented here are greatly acknowledged: Drs D. Binder, M. Hara-Chikuma, T. Ma, G. Manley, M. Papadopoulos, S. Saadoun, Y. Song, J. Thiagarajah, B. Yang and many others. The AQP mechanism and mouse phenotype studies were supported primarily by the National Institutes of Health, through awards R37 DK35124, R37 EB00415, R01 EY13574, R01 HL59198, R01 HL73856, and P30 DK72517.

References

1. Gonen T, Walz T. The structure of aquaporins. *Q Rev Biophys.* 2006; 39:361–396. [PubMed: 17156589]
2. Fujiyoshi Y, et al. Structure and function of water channels. *Curr Opin Struct Biol.* 2002; 12:509–515. [PubMed: 12163075]
3. Hub JS, de Groot BL. Mechanism of selectivity of aquaporins and aquaglyceroporins. *Proc Natl Acad Sci U S A.* 2008; 105:1198–1203. [PubMed: 18202181]
4. Deen PM, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science.* 1994; 264:92–95. [PubMed: 8140421]
5. Verkman AS. Role of aquaporins in lung fluid physiology. *Resp Physiol Neurobiol.* 2007; 159:324–330.
6. Verkman, AS.; Thiagarajah, JR. Physiology of water transport in the gastrointestinal tract. In: Johnson, LR., et al., editors. *Physiology of the Gastrointestinal Tract.* Vol. 4. New York: Academic Press; 2006. p. 1827–1845.
7. Yang B, et al. Skeletal muscle function and water permeability in aquaporin-4 deficient mice. *Am J Physiol.* 2000; 278:C1108–1115.
8. Song Y, Sonawane N, Verkman AS. Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice. *J Physiol.* 2002; 541:561–568. [PubMed: 12042359]
9. Yang B, et al. Phenotype analysis of aquaporin-8 null mice. *Am J Physiol.* 2005; 288:C1161–C1170.
10. Holm LM, et al. NH_3 and NH_4^+ permeability in aquaporin-expressing *Xenopus* oocytes. *Pflügers Arch.* 2005; 450:415–248. [PubMed: 15988592]
11. Cooper GJ, Boron WF. Effect of PCMBs on CO_2 permeability of *Xenopus* oocytes expressing aquaporin 1 or its C189S mutant. *Am J Physiol.* 1998; 275:C1481–1486. [PubMed: 9843709]
12. Yang B, et al. Carbon dioxide permeability of aquaporin-1 measured in erythrocytes and lung of aquaporin-1 null mice and in reconstituted proteoliposomes. *J Biol Chem.* 2000; 275:2686–2692. [PubMed: 10644730]
13. Fang X, et al. Evidence against aquaporin dependent CO_2 permeability in lung and kidney. *J Physiol (London).* 2002; 543:63–69. [PubMed: 12096051]
14. Yang B, et al. Evidence from knockout mice against physiologically significant aquaporin-8 facilitated ammonia transport. *Am J Physiol.* 2006; 291:C417–C423.
15. Yang B, Zhao D, Verkman AS. Evidence against functionally significant aquaporin expression in mitochondria. *J Biol Chem.* 2006; 281:16202–16206. [PubMed: 16624821]
16. Zhang H, Verkman AS. Aquaporin-4 independent Kir4.1 K^+ channel function in brain glial cells. *Mol Cell Neurosci.* 2008; 37:3–10.
17. Ruiz-Ederra J, Zhang H, Verkman AS. Evidence against functional interaction between aquaporin-4 water channels and Kir4.1 K^+ channels in retinal Müller cells. *J Biol Chem.* 2007; 282:21866–21872. [PubMed: 17525153]
18. Ma T, et al. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem.* 1999; 274:20071–20074. [PubMed: 10400615]

19. Krane CM, et al. Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. *J Biol Chem.* 2001; 27:23413–23420. [PubMed: 11290736]
20. Song Y, Verkman AS. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J Biol Chem.* 2001; 276:41288–41292. [PubMed: 11514581]
21. Oshio K, et al. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel aquaporin-1. *FASEB J.* 2005; 19:76–78. [PubMed: 15533949]
22. Zhang D, Vetrivel L, Verkman AS. Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. *J Gen Physiol.* 2002; 119:561–569. [PubMed: 12034763]
23. Schnermann J, et al. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci U S A.* 1998; 95:9660–9664. [PubMed: 9689137]
24. Vallon V, Verkman AS, Schnermann J. Luminal hypotonicity in proximal tubules of aquaporin-1 knockout mice. *Am J Physiol.* 2000; 278:F1030–F1033.
25. Bai C, et al. Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J Clin Invest.* 1999; 103:555–561. [PubMed: 10021464]
26. Ma T, et al. Lung fluid transport in aquaporin-5 knockout mice. *J Clin Invest.* 2000; 105:93–100. [PubMed: 10619865]
27. Song Y, et al. Role of aquaporins in alveolar fluid clearance in neonatal and adult lung, and in edema formation following acute lung injury, studies in transgenic aquaporin null mice. *J Physiol.* 2000; 525:771–779. [PubMed: 10856128]
28. Song Y, et al. Role of aquaporin water channels in airway fluid transport, humidification, and surface liquid hydration. *J Gen Physiol.* 2001; 117:573–582. [PubMed: 11382807]
29. Yang B, et al. Reduced osmotic water permeability of the peritoneal barrier in aquaporin-1 knockout mice. *Am J Physiol.* 1999; 276:C76–81. [PubMed: 9886922]
30. Song Y, et al. Role of aquaporin water channels in pleural fluid dynamics. *Am J Physiol.* 2000; 279:C1744–1750.
31. Ma T, et al. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J Biol Chem.* 1998; 273:4296–4299. [PubMed: 9468475]
32. Ma T, et al. Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci U S A.* 2000; 97:4386–4391. [PubMed: 10737773]
33. Ma T, et al. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J Clin Invest.* 1997; 100:957–962. [PubMed: 9276712]
34. Chou CL, et al. Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. *J Clin Invest.* 1999; 103:491–496. [PubMed: 10021457]
35. Pallone TL, et al. Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. *J Clin Invest.* 2000; 105:215–222. [PubMed: 10642600]
36. Sohara E, et al. Defective water and glycerol transport in the proximal tubules of AQP7 knockout mice. *Am J Physiol.* 2005; 289:F1195–F1200.
37. Chou CL, et al. Fourfold reduction of water permeability in inner medullary collecting duct of aquaporin-4 knockout mice. *Am J Physiol.* 1998; 274:C549–554. [PubMed: 9486146]
38. Verkman AS. Dissecting the role of aquaporins in renal pathophysiology using transgenic mice. *Semin Nephrol.* (in press).
39. Solenov E, et al. Sevenfold-reduced osmotic water permeability in primary astrocyte cultures from AQP-4-deficient mice, measured by a fluorescence quenching method. *Am J Physiol.* 2004; 286:C426–432.
40. Thiagarajah JR, Papadopoulos MC, Verkman AS. Non-invasive early detection of brain edema in mice by near-infrared light scattering. *J Neurosci Res.* 2005; 80:293–299. [PubMed: 15765520]
41. Papadopoulos MC, Verkman AS. Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. *J Biol Chem.* 2005; 280:13906–13912. [PubMed: 15695511]
42. Klatzo I. Evolution of brain edema concepts. *Acta Neurochir Suppl (Wien).* 1994; 60:3–6. [PubMed: 7976571]

43. Manley GT, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nature Med.* 2000; 6:159–163. [PubMed: 10655103]
44. Amiry-Moghaddam M, et al. An alpha-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc Natl Acad Sci U S A.* 2003; 100:2106–2111. [PubMed: 12578959]
45. Saadoun S, et al. Greatly improved neurological outcome in AQP4-deficient mice after spinal cord compression injury. *Brain.* 131:1087–1098. [PubMed: 18267965]
46. Papadopoulos MC, et al. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J.* 2004; 18:1291–1293. [PubMed: 15208268]
47. Bloch O, et al. Aquaporin-4 gene deletion in mice increases focal edema associated with brain abscess. *J Neurochem.* 2005; 95:254–262. [PubMed: 16181429]
48. Bloch O, Manley GT, Verkman AS. Accelerated progression of kaolin-induced hydrocephalus in aquaporin-4 deficient mice. *J Cereb Blood Flow Metab.* 2006; 26:1527–1537. [PubMed: 16552421]
49. Berry V, et al. Missense mutations in MIP underlie autosomal dominant ‘polymorphic’ and lamellar cataracts linked to 12q. *Nat Genet.* 2000; 25:15–17. [PubMed: 10802646]
50. Ruiz-Ederra J, Verkman AS. Accelerated cataract formation and reduced lens epithelial water permeability in aquaporin-1 deficient mice. *Invest Ophthalmol Vis Sci.* 2006; 47:3960–3967.
51. Thiagarajah JR, Verkman AS. Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling. *J Biol Chem.* 2002; 277:19139–19144. [PubMed: 11891232]
52. Li J, Patil RV, Verkman AS. Mildly abnormal retinal function in transgenic mice without Muller cell aquaporin-4 water channels. *Invest Ophthalmol Vis Sci.* 2002; 43:573–579. [PubMed: 11818406]
53. Levin MH, Verkman AS. Aquaporin-dependent water permeation at the mouse ocular surface: in vivo microfluorimetric measurements in cornea and conjunctiva. *Invest Ophthalmol Vis Sci.* 2004; 45:4423–4432. [PubMed: 15557451]
54. Da T, Verkman AS. Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. *Invest Ophthalmol Vis Sci.* 2004; 45:4477–4483. [PubMed: 15557457]
55. Endo M, et al. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. *Microvasc Res.* 1999; 58:89–98. [PubMed: 10458924]
56. Saadoun S, et al. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature.* 2005; 434:786–792. [PubMed: 15815633]
57. Saadoun S, et al. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J Cell Sci.* 2005; 118:5691–5698. [PubMed: 16303850]
58. Auguste KI, et al. Greatly impaired migration of implanted aquaporin-4-deficient astroglial cells in mouse brain toward a site of injury. *FASEB J.* 2007; 21:108–116. [PubMed: 17135365]
59. Levin MH, Verkman AS. Aquaporin-3-dependent cell migration and proliferation during corneal re-epithelialization. *Invest Ophthalmol Vis Sci.* 2006; 47:4365–4372. [PubMed: 17003427]
60. Hara-Chikuma M, Verkman AS. Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *J Mol Med.* 2008; 86:221–231. [PubMed: 17968524]
61. Hara-Chikuma M, Verkman AS. Aquaporin-1 facilitates epithelial cell migration in kidney proximal tubule. *J Am Soc Nephrol.* 2006; 17:39–45. [PubMed: 16319186]
62. Hu J, Verkman AS. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. *Faseb J.* 2006; 20:1892–1894. [PubMed: 16818469]
63. Condeelis J. Life at the leading edge: the formation of cell protrusions. *Annu Rev Cell Biol.* 1993; 9:411–444. [PubMed: 8280467]
64. Charras GT, et al. Non-equilibration of hydrostatic pressure in blebbing cells. *Nature.* 2005; 435:365–369. [PubMed: 15902261]
65. Li J, Verkman AS. Impaired hearing in mice lacking aquaporin-4 water channels. *J Biol Chem.* 2001; 276:31233–31237. [PubMed: 11406631]

66. Mhatre AN, et al. Aquaporin 4 expression in the mammalian inner ear and its role in hearing. *Biochem Biophys Res Commun*. 2002; 297:987–996. [PubMed: 12359252]
67. Binder DK, et al. Increased seizure threshold in mice lacking aquaporin-4 water channels. *Neuroreport*. 2004; 15:259–262. [PubMed: 15076748]
68. Binder DK, et al. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia*. 2006; 53:631–636. [PubMed: 16470808]
69. Amiry-Moghaddam M, et al. Delayed K⁺ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. *Proc Natl Acad Sci U S A*. 2003; 100:13615–13620. [PubMed: 14597704]
70. Lu D, et al. Impaired olfaction in mice lacking aquaporin-4 water channels. *FASEB J*. 2008 (in press).
71. Padmawar P, Yao X, Bloch O, et al. K⁺ waves in brain cortex visualized using a long-wavelength K⁺-sensing fluorescent indicator. *Nat Methods*. 2005; 2:825–827. [PubMed: 16278651]
72. Binder DK, et al. In vivo measurement of brain extracellular space diffusion by cortical surface photobleaching. *J Neurosci*. 2004; 24:8049–8056. [PubMed: 15371505]
73. Zador Z, et al. Microfiberoptic fluorescence photobleaching reveals size-dependent macromolecule diffusion in extracellular space deep in brain. *FASEB J*. 22:870–879. [PubMed: 17965267]
74. Rojek AM, et al. Defective glycerol metabolism in aquaporin 9 (AQP9) knockout mice. *Proc Natl Acad Sci U S A*. 2007; 104:3609–3614. [PubMed: 17360690]
75. Ma T, et al. Impaired stratum corneum hydration in mice lacking epidermal water channel aquaporin-3. *J Biol Chem*. 2002; 277:17147–17153. [PubMed: 11880378]
76. Hara M, Ma T, Verkman AS. Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery. *J Biol Chem*. 2002; 277:46616–46621. [PubMed: 12270942]
77. Hara M, Verkman AS. Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *Proc Natl Acad Sci U S A*. 2003; 100:7360–7365. [PubMed: 12771381]
78. Hara-Chikuma M, Verkman AS. Prevention of skin tumorigenesis and impairment of epidermal cell proliferation by targeted aquaporin-3 gene disruption. *Mol Cell Biol*. 2008; 28:326–332. [PubMed: 17967887]
79. Thiagarajah JR, Zhao D, Verkman AS. Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. *Gut*. 2007; 56:1529–1535. [PubMed: 17573386]
80. Verkman AS. A cautionary note on cosmetics containing ingredients that increase aquaporin-3 expression. *Exp Dermatol*. (in press).
81. Hara-Chikuma M, et al. Progressive adipocyte hypertrophy in aquaporin-7 deficient mice: adipocyte glycerol permeability as a novel regulator of fat accumulation. *J Biol Chem*. 2005; 280:15493–15496. [PubMed: 15746100]
82. Hibuse T, et al. Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. *Proc Natl Acad Sci U S A*. 2005; 102:10993–10998. [PubMed: 16009937]
83. Funahashi T, et al. Impact of glycerol gateway molecule in adipocytes. *Cell Mol Biol (Noisy-le-grand)*. 2006; 52:40–45. [PubMed: 17543220]
84. Lennon VA, et al. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med*. 2005; 202:473–477. [PubMed: 16087714]
85. Rai T, et al. Urinary excretion of aquaporin-2 water channel protein in human and rat. *J Am Soc Nephrol*. 1997; 8:1357–1362. [PubMed: 9294826]
86. Kwon TH, et al. Physiology and pathophysiology of renal aquaporins. *Semin Nephrol*. 2001; 21:231–238. [PubMed: 11320486]
87. Hara-Chikuma M, Verkman AS. Roles of aquaporin-3 in epidermis. *J Invest Dermatol*. (in press).
88. Lee TS, et al. Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathol*. 2004; 108:493–502. [PubMed: 15517312]

89. Ceperuelo-Mallafre V, et al. 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.* 2007; 92:3640–3645. [PubMed: 17566090]

Figure 1. Aquaporin functions in mammalian physiology

Water-transporting functions of aquaporins (AQPs) are shown in the top half of the figure; glycerol-transporting functions are shown in the lower half of the figure. (a) AQP facilitates rapid, near-isomolar transepithelial fluid secretion: AQP deficiency in an epithelium such as salivary gland slows osmotic water transport into the acinar lumen, resulting in the secretion of a reduced volume of a hypertonic fluid. Numbers represent hypothetical fluid osmolalities. (b) Expression of AQPs 2, 3 and 4 in the kidney collecting duct facilitates the production of a concentrated urine: AQP deficiency reduces transepithelial water permeability, preventing osmotic equilibration of luminal fluid and impairing urinary concentrating ability. Numbers represent hypothetical fluid osmolalities. (c) Proposed mechanism of AQP-facilitated cell migration, showing water entry into protruding lamellipodia in migrating cells. (d) AQP4-dependent neuroexcitation, showing AQP4-facilitated water transport in glial cells, which communicate with neurons through changes in extracellular space volume and K^+ concentration. (e) AQP3 facilitates glycerol entry in the epidermis, allowing water retention (glycerol functions as an osmolyte) and biosynthesis: in AQP deficiency the steady-state glycerol content in epidermis and stratum corneum in skin is reduced, accounting for reduced skin hydration. (f) Proposed mechanism of AQP3-facilitated cell proliferation involving increased cellular glycerol and consequent increased ATP energy and biosynthesis. (g) AQP7 facilitates glycerol escape from adipocytes: adipocyte hypertrophy is seen in AQP7 deficiency, possibly as a result of

impaired AQP7-dependent glycerol escape from adipocytes, resulting in cellular glycerol accumulation and increased triglyceride content. See text for further explanations.

Abbreviations: G3P, glycerol-3-phosphate; TG, triglyceride.

Figure 2. Impaired urinary concentrating function in aquaporin deficiency

Deletion of aquaporins (AQPs) in kidney results in increased urinary output and reduced urinary osmolality. (a) Sites of AQP expression in kidney, showing AQP1 expression in the proximal tubule, thin descending limb of Henle (TDLH) and outer medullary descending vasa recta, AQP2 expression at the lumen membrane and endosomes in collecting duct, and AQP3 and AQP4 at the basolateral membrane in collecting duct. (b) 24 h urine collections showing polyuria in mice lacking AQP1 and AQP3, individually and together. (c) Urine osmolalities before and after 36 h water deprivation (standard error shown). Data summarised from Refs 31, 32 and 33. (The nomenclature style *Aqp* is used to denote mouse aquaporin genes here, but the style AQP is used throughout the main text of this article to denote mammalian AQP proteins in general.)

Figure 3. Aquaporin 4 deficiency slows brain water accumulation in cytotoxic oedema, and brain water elimination in vasogenic oedema

(a) Routes of water movement in brain during oedema formation and elimination. Oedema formation involves water movement through the blood–brain barrier, which is intact in cytotoxic oedema and disrupted in vasogenic oedema. Oedema elimination involves water movement across the glia limitans, ependyma and blood–brain barrier. Aquaporin 4 (AQP4) expression in glial cells is shown as blue circles. Oedema elimination schematics adapted from Ref. 46 (& 2004 FASEB), with permission. (b) Water intoxication model of cytotoxic oedema. AQP4-null mice show improved survival after acute water intoxication produced by intraperitoneal water injection. Adapted from Ref. 43. (c) Increased elevation in intracranial pressure in AQP4-null mice during continuous intraparenchymal infusion of artificial cerebrospinal fluid (0.5 μ l/min). The recordings show intracranial pressure with fluid infusion begun at the arrows. Adapted from Ref. 46 (& 2004 FASEB), with permission. (d) Accelerated progression of hydrocephalus in AQP4 deficiency, as shown by the larger size of lateral ventricles in AQP4-null mice at 5 days after kaolin injection. Adapted from Ref. 48.

Figure 4. Impairment in tumour growth and endothelial cell migration in aquaporin 1 deficiency
 Aquaporin 1 (AQP1) deletion impairs tumour angiogenesis because, in part, of reduced migration of endothelial cells. (a, left) Reduced tumour size in AQP1-null mouse, two weeks after subcutaneous injection of one million B16F10 melanoma cells. (a, right) Tumour growth data (ten mice per group). (b) AQP1 protein (green) polarisation to lamellipodia (arrows) in a migrating CHO cell. Graph in part a and image in part b reprinted from Ref. 56.

Figure 5. Aquaporin 3 deficiency reduces skin hydration and prevents skin tumour formation
 Aquaporin 3 (AQP3) is expressed in the basal layer of keratinocytes in normal skin, and its deletion in mice produces dry skin because of reduced skin glycerol content, and resistance to tumourigenesis. (a) Immunofluorescence showing AQP3 expression (yellow/green) in basal layer of epidermis in mice. Abbreviations: D, dermis; E, epidermis; SC, stratum corneum. Image reproduced from Ref. 75 (&2008 The American Society for Biochemistry and Molecular Biology), with permission. (b) Reduced statum corneum water content in AQP3-null mice, measured by high-frequency skin surface conductance (five mice per group, $*P < 0.01$). Skin conductance was measured after 24 h exposure to relative humidity of 10, 40 or 90%; ‘occluded’ indicates a plastic occlusion dressing that prevents evaporative water loss. Reprinted from Ref. 75 (& 2008 The American Society for Biochemistry and Molecular Biology), with permission. (c) Reduced glycerol content in stratum corneum and epidermis of AQP3-null mice ($*P < 0.01$). Adapted from Ref. 76 (& 2008 The American Society for Biochemistry and Molecular Biology), with permission. (d, left) Absence of cutaneous papillomas in AQP3-null mice treated with an initiator (once) and twice-weekly applications of a promoter for 20 weeks. Arrows point to papillomas. (d, right) Percentage of mice with papillomas after initiator treatment. Part d adapted from Ref. 78 (& 2008 American Society for Microbiology), with permission.