The Journal of Experimental Biology 212, 1707-1715
Published by The Company of Biologists 2009
doi:10.1242/jeb.024125

Review

Aquaporins: translating bench research to human disease

A. S. Verkman

Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, CA 94143, USA

e-mail: alan.verkman@ucsf.edu

Accepted 16 December 2008

Summary

There is considerable potential for translating knowledge of aquaporin structure, function and physiology to the clinic. One area is in aquaporin-based diagnostics. The discovery of AQP4 autoantibodies as a marker of the neuromyelitis optica form of multiple sclerosis has allowed precise diagnosis of this disease. Other aquaporin-based diagnostics are possible. Another area is in aquaporin-based genetics. Genetic diseases caused by loss-of-function mutations in aquaporins include nephrogenic diabetes insipidus and cataracts, and functionally significant aquaporin polymorphisms are beginning to be explored. Perhaps of greatest translational potential is aquaporin-based therapeutics. Information largely from aquaporin knockout mice has implicated key roles of aquaporin-facilitated water transport in transepithelial fluid transport (urinary concentrating, gland fluid secretion), water movement into and out of the brain, cell migration (angiogenesis, tumor metastasis, wound healing) and neural function (sensory signaling, seizures). A subset of aquaporins that transport both water and glycerol, the 'aquaglyceroporins', regulate glycerol content in epidermal, fat and other tissues, and are involved in skin hydration, cell proliferation, carcinogenesis and fat metabolism. Aquaporin-based modulator drugs are predicted to be of broad potential utility in the treatment of edematous states, cancer, obesity, wound healing, epilepsy and glaucoma. These exciting possibilities and their associated challenges are reviewed.

Key words: aquaporin, AQP, water transport, cell migration, angiogenesis, cancer, diuretic epidermis, brain swelling.

Introduction

There are 13 mammalian aquaporins (AQPs), constituting a family of small, hydrophobic, membrane proteins. There is a considerable body of information about AQP structure from electron and x-ray crystallography (reviewed by Fujiyoshi et al., 2002), showing AQP monomers (~30 kDa) containing six membrane-spanning helical domains surrounding a narrow aqueous pore. AQP monomers are super-assembled in membranes as tetramers. While the primary function of most AQPs is in facilitating water movement across cell membranes in response to osmotic gradients, a subset of AQPs, called aquaglyceroporins, also transport glycerol and possibly other small polar molecules. There is controversial evidence that some AQPs may transport gases and ions across membranes. The mammalian AQPs are expressed in various epithelia and endothelia involved in fluid transport, such as kidney tubules and glandular epithelia, as well as in other cell types such as brain glial cells, epidermis and adipocytes. Much of our understanding of AQP functions in mammalian physiology has come from phenotype analysis of mice lacking each of the AQPs (reviewed by Verkman, 2005). Mouse phenotype studies have confirmed the anticipated involvement of AQPs in the urinary-concentrating mechanism and glandular fluid secretion, and led to the discovery of unanticipated roles of AQPs in brain water balance, cell migration, cell proliferation, neural activity, epidermal hydration and ocular function.

This review focuses on translational aspects of AQP research. What AQPs do and don't do in mammalian physiology is reviewed, followed by consideration of AQP-based diagnostics, genetics and therapeutics.

Functions of aquaporins in cell and organ physiology

Anticipated roles of AQPs in urinary-concentrating function and gland fluid secretion

Water transport across kidney tubules and microvessels is important for reabsorption of water filtered by the glomerulus and for the formation of a concentrated urine, which involves countercurrent multiplication and exchange mechanisms and vasopressin-regulated water permeability in the collecting duct. AQP1 is expressed at cell plasma membranes in proximal tubule and thin descending limb of Henle epithelia, and in descending vasa recta endothelia (reviewed by Verkman, 2008). AQP2, the vasopressin-regulated water channel, is expressed in collecting duct apical membrane and intracellular vesicles, and AQPs 3 and 4 are expressed constitutively at the basolateral membrane of collecting duct epithelia. As anticipated, defective urinary-concentrating function was found in mice lacking AQPs 1–4 (Ma et al., 1997; Ma et al., 1998; Ma et al., 2000b; Yang et al., 2001) and in humans with mutations in AQP1 (King et al., 2001) or AQP2 (Deen et al., 1994). Transepithelial water permeability and near-isosmolar fluid absorption in proximal tubule are impaired in mice lacking AQP1 (Schnermann et al., 1998). AQP1 deletion also reduces water permeability in thin descending limb of Henle (Chou et al., 1999) and vasa recta microvessels (Pallone et al., 2000), impairing the generation of a hyperosmolar medullary interstitium. Deletion or mutation of AQPs 2–4 reduces collecting duct water permeability, impairing osmotic equilibration between urinary fluid in the collecting duct lumen and the renal interstitium, as illustrated in Fig. 1A. AQP inhibitors are thus predicted to have 'aquaretic' activity, producing a water>salt diuresis.

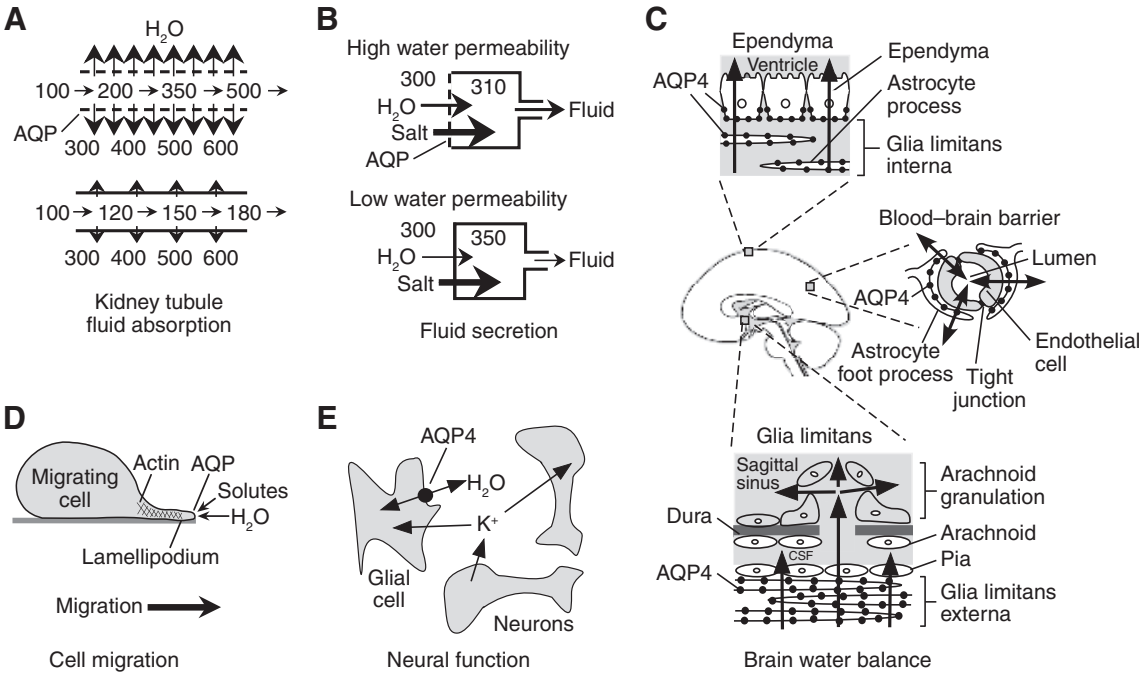

Fig. 1. Roles of aquaporins (AQPs) in mammalian physiology based on their water transport function. (A) Reduced transepithelial water permeability in kidney collecting duct impairs urinary-concentrating ability by impairing osmotic equilibration of luminal fluid. Numbers represent hypothetical fluid osmolalities. (B) Reduced water permeability in an epithelium, such as salivary gland, impairs active, near-isosmolar fluid secretion by slowing osmotic water transport into the acinar lumen, resulting in the secretion of a reduced volume of a hypertonic fluid. (C) Routes of water movement into and out of the brain. Water movement shown through AQP4-expressing glial cells at glia limitans and the blood–brain barrier. (D) Proposed mechanism of AQP-facilitated cell migration, showing water entry into protruding lamellipodia in migrating cells. (E) AQP4-dependent neuroexcitation, showing AQP4-facilitated water transport in glial cells, which communicate with neurons through changes in extracellular space volume and K^+ concentration.

The other anticipated role of AQPs is in active fluid transport across epithelia. AQP5 deletion in mice impairs fluid secretion by salivary (Ma et al., 1999) and airway submucosal (Song and Verkman, 2001) glands, resulting in reduced secretion of a relatively hyperosmolar fluid. Impaired fluid secretion has also been found in AQP1 knockout mice in choroid plexus (Oshio et al., 2005), where cerebrospinal fluid is produced, and in ciliary epithelium (Zhang et al., 2002), where ocular aqueous fluid is produced. AQP1 is involved in the regulation of intracranial and intraocular pressure in these closed fluid compartments. As shown in Fig. 1B, active, near-isosmolar fluid transport involves water movement across a highly water permeable epithelium in response to osmotic gradients produced by salt transport. Reduced epithelial cell water permeability results in the secretion of a relatively low volume of a hyperosmolar fluid. In the various epithelia mentioned above the rates of transepithelial fluid secretion normalized to epithelial surface area are very high, such that the reduced water permeability in AQP deficiency impairs transepithelial osmotic equilibration. In other epithelia having much lower rates of fluid absorption or secretion, including lacrimal gland (Moore et al., 2000), sweat gland (Song et al., 2002), alveolus (Bai et al., 1999; Ma et al., 2000a), and airways (Song et al., 2001), AQP deletion does not impair transepithelial fluid transport. Transepithelial water transport is not rate limiting for fluid secretion when rates of fluid secretion are low.

Unanticipated roles of AQP-facilitated water transport in brain function and cell migration

The main water channel in brain is AQP4, which is expressed in glial cells at fluid–parenchymal interfaces at the blood–brain and ependymal–CSF barriers. In cytotoxic brain edema, water moves into the brain through an intact blood–brain barrier in response to osmotic driving forces. Mice lacking AQP4 showed improved outcome and reduced brain water accumulation compared with wild-type mice in models of cytotoxic brain edema, including water intoxication and ischemic stroke (Manley et al., 2000) and bacterial meningitis (Papadopoulos et al., 2005). Recent results show a remarkably improved outcome in AQP4 null mice following spinal cord injury (Saadoun et al., 2008), which was attributed to reduced spinal cord edema early after injury. AQP deletion also influences tissue water accumulation in stress-induced corneal (Thiagarajah and Verkman, 2002) and retinal (Da and Verkman, 2004) edema, as well as cataract formation (Ruiz-Ederra and Verkman, 2006).

In vasogenic, or 'leaky-vessel' brain edema, excess water moves into the brain by a bulk fluid flow mechanism through a leaky blood–brain barrier, and exits the brain by movement into the CSF through the AQP4-rich glia limitans lining brain ventricles and the brain surface (Fig. 1C). When these water exit routes are blocked in obstructive hydrocephalus, water also moves out of the brain back into microvessels through the blood–brain barrier. Mice lacking AQP4 have a worse clinical outcome and greater brain water accumulation in models of vasogenic brain edema, including cortical-freeze injury and brain tumor (Papadopoulos et al., 2004) and brain abscess (Bloch et al., 2005). AQP4 null mice also manifest an accelerated course of brain swelling in obstructive hydrocephalus (Bloch et al., 2006). We concluded that AQP4 facilitates removal of excess brain water in vasogenic brain edema and hydrocephalus. AQP4 inhibitors are thus predicted to reduce brain swelling in cytotoxic edema, whereas AQP4 enhancers

(activators or upregulators) are predicted to reduce brain swelling in vasogenic edema.

Another unanticipated role of AQPs related to their water-transporting function is in cell migration. Involvement of AQPs in cell migration was discovered following the observation of impaired tumor angiogenesis in AQP1 null mice and subsequent characterization of endothelial cell cultures derived from wild-type and AQP1 null mice (Saadoun et al., 2005a). Based on findings of slowed lamellipodial dynamics in AQP deficiency and AQP polarization to the leading edge of migrating cells, a mechanism of AQP-facilitated cell migration was proposed in which actin cleavage and ion uptake at the tip of a lamellipodium create local osmotic gradients that drive water influx, facilitating lamellipodial extension and cell migration (Fig. 1D) (reviewed by Papadopoulos et al., 2008). AQP-faciliated cell migration has also been found in brain astroglial cells (Saadoun et al., 2005b; Auguste et al., 2007), kidney proximal tubule cells (Hara-Chikuma and Verkman, 2006), corneal epithelial cells (Levin and Verkman, 2006), skin cells (Hara-Chikuma and Verkman, 2008b) and tumor cells (Hu and Verkman, 2006). AQP-facilitated cell migration thus appears to be important in tumor angiogenesis, tumor cell metastasis and spread, and wound healing. These observations offer an explanation for the expression of AQPs in many tumor cell types, and for correlations in some tumors between AQP expression and tumor grade (reviewed by Verkman et al., 2008a).

An additional unexpected role of AQPs is in neural function. AQP4 is expressed in supportive cells adjacent to electrically excitable cells, as in glia *vs* neurons in brain, Müller *vs* bipolar cells in retina, hair *vs* supportive cells in the inner ear, and olfactory receptor neurons *vs* supportive cells in olfactory epithelium. Electrophysiological measurements have demonstrated impaired vision, hearing and olfaction in AQP4 null mice, as demonstrated by increased auditory brainstem response thresholds (Li and Verkman, 2001), reduced electroretinogram potentials (Li et al., 2002), and reduced electro-olfactogram potentials (Lu et al., 2008). In brain, seizure threshold is reduced and seizure duration prolonged in AQP4 deficiency (Binder et al., 2004a). Possible mechanisms for altered neuroexcitation in AQP4 deficiency include impaired K^+ reuptake into glial cells following neuroexcitation, and extracellular space expansion (Fig. 1E). Delayed K^+ uptake from brain extracellular space in AQP4 deficiency has been found (Binder et al., 2006; Padmawar et al., 2005), which may account for their prolonged seizures (Fig. 1D). It has been proposed that AQP4 associates with the inwardly rectifying K^+ channel Kir4.1, such that reduced K^+ channel function in AQP4 deficiency might account for the delay in K^+ clearance. However, patch-clamp studies in Müller cells (Ruiz-Ederra et al., 2007) and brain astroglia (Zhang and Verkman, 2008b) provide evidence against this mechanism. We also found evidence for extracellular space expansion in AQP4 deficiency (Binder et al., 2004b; Zador et al., 2008), in which increased aqueous volume dilutes K^+ exiting from neurons and consequently attenuates changes in extracellular space K^+ concentration. These possibilities for relating AQP4 water transport and altered K^+ dynamics, however, remain speculative.

Roles of AQP-facilitated glycerol transport by aquaglyceroporins

The functional significance of glycerol transport by aquaglyceroporins, such as AQP3 in skin and AQP7 in adipocytes, was for many years unclear. We discovered that AQP3-facilitated glycerol transport in skin is an important determinant of epidermal and stratum corneum hydration (Fig. 2A) (reviewed by Hara-Chikuma and Verkman, 2008c). Mice lacking AQP3, which is normally expressed in the basal layer of proliferating keratinocytes in epidermis, manifest reduced stratum corneum hydration and skin elasticity, and impaired stratum corneum biosynthesis and wound healing (Ma et al., 2002). The reduced skin hydration in AQP3 deficiency is caused by impaired epidermal cell glycerol permeability, resulting in reduced glycerol content in the stratum corneum and epidermis (Hara et al., 2002). Topical or systemic glycerol administration corrected each of these defects (Hara and Verkman, 2003).

A novel role of AQP3 in cell proliferation was found in several AQP3-expressing cell types, including skin, colon and cornea. AQP3 deficient mice manifest impaired cutaneous wound healing (Hara-Chikuma et al., 2008b), colonic epithelial cell regeneration (Thiagarajah et al., 2007) and corneal wound healing (Levin and Verkman, 2006). In each case cell proliferation was found to be impaired. A remarkable tumor phenotype was found in AQP3 null mice, which showed complete resistance to the formation of skin tumors (Hara-Chikuma and Verkman, 2008a). AQP3-dependent epidermal cell proliferation appears to involve reduced cellular glycerol metabolism and biosynthesis, resulting in reduced ATP content and impaired MAP kinase signaling (Fig. 2B). AQP3

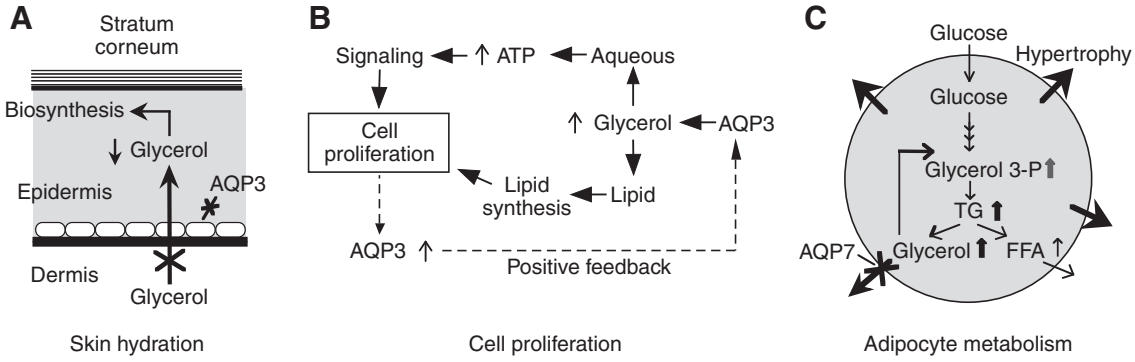

Fig. 2. Roles of AQPs in mammalian physiology based on their glycerol transport function. (A) Reduced glycerol content in epidermis and stratum corneum in skin in AQP3 deficiency, accounting for reduced skin hydration. (B) Proposed mechanism of AQP3-facilitated cell proliferation involving reduced cellular glycerol and consequent reduced ATP energy and biosynthesis. (C) Proposed mechanism for adipocyte hypertrophy in AQP7 deficiency, showing impaired AQP7-dependent glycerol escape from adipocytes resulting in cellular glycerol and triglyceride accumulation. Glycerol 3-P, glycerol 3-phosphate; TG, triacylglycerol; FFA, free fatty acid.

inhibitors may thus have utility in skin tumor prevention and therapy. Recognizing the relationship between AQP3 expression and skin moisturization, several companies have marketed cosmetics containing ingredients claimed to increase AQP3 expression. However, given the relationship between AQP3 expression and skin tumorigenesis, caution seems warranted in the use of AQP3-upregulating cosmetics.

The aquaglyceroporin AQP7 is expressed in the plasma membrane of adipocytes. AQP7 null mice manifest progressive increases in fat mass and adipocyte hypertrophy as they age, with accumulation of glycerol and triglycerides in adipocytes (Hara-Chikuma et al., 2005; Hibuse et al., 2005). Biochemical studies suggested that adipocyte hypertrophy in AQP7 deficiency is the consequence of reduced plasma membrane glycerol permeability, with cellular glycerol accumulation and triglyceride biosynthesis (Fig. 2C). We proposed that increasing adipocyte glycerol permeability, perhaps by enhancers of AQP7 expression, might reverse this process and thus provide a novel therapy for obesity.

AQP roles unrelated to their water and glycerol transport functions

The diverse group of physiological functions described above can be attributed to the plasma membrane water- and/or glycerol-transporting functions of AQPs. Various other roles of AQPs have been proposed. There is controversial evidence that AQPs can transport certain gases, including CO_2, NO and O_2 (reviewed by Wu and Beitz, 2007). Because the permeability of lipid bilayers to these gases is very high, their permeability across cell membranes is predicted to be unstirred layer limited and thus AQP independent, as has been found experimentally for CO_2 (Yang et al., 2000; Fang et al., 2002; Missner et al., 2008). The less membrane-permeable gas NH_3 has been found to pass through AQP8 (Holm et al., 2005), though a study utilizing knockout mice concluded that AQP8-facilitated NH_3 is not of physiological importance (Yang et al., 2006d). There is evidence for transport of small ions, urea and arsenite by some AQPs, though in some cases the findings are controversial and so far no evidence has been reported to support the physiological importance of AQP-facilitated transport of these substances. Evidence for AQP functioning in mitochondria in liver and brain has been proposed (Calamita et al., 2005; Amiry-Moghaddam et al., 2005), though subsequently refuted by direct permeability measurements (Yang et al., 2006b). It is unlikely that AQP-facilitated water or glycerol transport in organellar membranes is of importance to cell functioning because of the high surface-to-volume ratio of organelles and consequent rapid water/solute equilibration even in the absence of AQPs. Finally, as mentioned with regard to neuroexcitation phenomena, various AQP protein–protein interactions have been proposed, such as AQP4–Kir4.1 interaction, though subsequently refuted (Ruiz-Ederra et al., 2007). Stoichiometric interactions between AQPs and ion channels seem unlikely because the membrane density of AQPs is 100- to 1000-fold greater than that of ion channels. Another recently proposed non-transporting role of AQPs is in cell–cell adhesion, including AQP4-facilitated glial cell adhesion (Hiroake et al., 2006). However, direct measurements have refuted the initial findings (Zhang and Verkman, 2008a). Together, the present evidence supports the conclusion that AQPs are involved primarily in plasma membrane water and/or glycerol permeability.

Aquaporin-targeted therapies

Potential clinical indications of AQP modulators

Notwithstanding some differences in human *vs* mouse physiology, the phenotype findings in AQP-deficient mice suggest various clinical indications of AQP modulators. The requirement of AQPs for the formation of a concentrated urine suggests that AQP inhibitors, or 'AQP-aquaretics', would reduce urine concentration, producing a water>salt diuresis. Though inhibitors of AQP2 would have similar aquaretic activity to existing vasopressin receptor-2 antagonists for therapy of hyponaturemia associated with high vasopressin, AQP1 inhibitors are predicted to have utility in diuretic-refractory edematous states, such as severe congestive heart failure, where conventional salt-blocking diuretics are of limited efficacy. Inhibitors of AQP4 are predicted to reduce brain swelling in cytotoxic edema, potentially offering neuroprotection following brain and spinal cord injury, and ischemic stroke, and potentially reducing mortality in infectious meningitis and various encephalitides. Inhibitors of AQPs in tumor cells and microvessels are predicted to reduce tumor spread and angiogenesis, offering adjunctive tumor chemotherapy. Inhibition of AQP4-facilitated glial cell migration is predicted to inhibit glial scar formation following brain and spinal cord injury, promoting axonal regeneration and improving long-term neurological outcome. Topical inhibitors of AQP1 in the eye may reduce intraocular pressure in glaucoma, and inhibitors of AQP3 in the skin may reduce skin cancer. Compounds that increase AQP function, acting by increasing AQP expression, are predicted to have potential efficacy in reducing fat mass in obesity, in accelerating brain water clearance in vasogenic edema, in promoting wound healing and tissue regeneration following injury, and in inhibiting cataractogenesis. Validation of these predictions in humans will require the development of AQP-specific modulators. Challenges will include the identification of potent, AQP subtype-selective inhibitors, and, in the case of AQP4 inhibitors, inhibitors that penetrate the blood–brain barrier. Identification of AQP enhancers presents an even great challenge as AQPs probably already have maximal per-channel function that cannot be further increased, and identification of selective transcriptional upregulators is without precedent in drug discovery.

Aquaporin inhibitors

There are at present no reported AQP inhibitors that are suitable candidates for clinical development. Though mutiple AQPs are inhibited by sulfhydryl-reactive mercurials such as mercury and gold (Niemietz and Tyerman, 2002), these metal ions are non-selective in their action and very toxic. Various candidate blockers of AQP1 have been reported, including tetraethylammonium (Brooks et al., 2000), acetazolamide (Ma et al., 2004) and DMSO (van Hoek et al., 1990); however, a careful evaluation of their inhibition efficacy using sensitive measurement methods indicated little or no AQP1 inhibition by tetraethylammonium or acetazolamide, and apparent inhibition by DMSO resulting from an osmotic clamp effect rather than true inhibition (Yang et al., 2006a). A careful analysis in *Xenopus* oocytes also showed no AQP1 inhibition by acetazolamide or tetraethylammonium (Sogaard and Zeuthen, 2008). Recently, several papers from one group reported AQP4 inhibition by a series of arylsulfonamides, antiepileptic drugs, and related molecules, with strong inhibition at low micromolar concentrations (Huber et al., 2007; Huber et al., 2008a; Huber et al., 2008b); however, these results could not be confirmed, with no inhibition activity found even at high concentrations of any of the putative AQP4 inhibitors (Yang et al., 2008). The identification of *bona fide* AQP inhibitors will likely require high-throughput screening of diverse small-molecule collections, utilizing sensitive assays of water transport function.

Screening methods for identification of AQP modulators

There are a number of possible strategies for identification of AQP modulators by screening of large compound collections. Many methods have been developed to measure water permeability across cell membranes, based largely on changes in cell volume in response to osmotic gradients. Cell volume has been measured in unlabeled cells by light scattering, phase-contrast microscopy and interferometry, and in fluorescently labeled cells by total internal reflection microscopy and confocal microscopy (reviewed by Verkman, 2000). Some of these methods are amenable to platereader or imaging (high-content screening) platforms. Calcein fluorescence quenching provides a simple approach to quantify cell membrane water permeability (Solenov et al., 2004), in which osmotically induced cell shrinking reduces cytoplasmic calcein fluorescence by cytoplasmic protein-mediated quenching. A similar strategy, following the development of second generation green fluorescent protein-based chloride sensors (Galietta et al., 2001), involves measurement of cell membrane water permeability from the time course of fluorescence in labeled cells following osmotic challenge. Cell shrinking produces an instantaneous increase in cytoplasmic chloride concentration and consequent reduction in sensor fluorescence. Challenges in compound screening include the generation of stable cell lines with appropriate AQP expression to allow accurate measurement of water transport rates, and rapid imposition of osmotic gradients to drive cell volume changes.

We recently devised a simple screening method to identify inhibitors of AQP1 water permeability and UT-B urea permeability using human erythrocytes (Levin et al., 2007), which was successful in identifying nanomolar potency UT-B inhibitors of phenylsulfoxyoxozole and benzenesulfonanilide classes. Prior urea analog-based inhibitors have millimolar potency. As shown diagrammatically in Fig. 3, the method involves measurement of erythrocyte lysis after imposing a large, outwardly directed gradient of acetamide, a urea analog that is transported efficiently by UT-B. The acetamide gradient causes cell swelling, which is limited by UT-B-facilitated acetamide efflux. Under appropriate conditions, UT-B inhibition slows acetamide efflux and increases cell lysis, as assayed by near-infrared light scattering. Minor assay modification has allowed identification of AQP1 inhibitors, in which AQP1 inhibition slows water influx and protects against osmotic lysis. Similar lysis-based assays are potentially suitable for studying other AQPs.

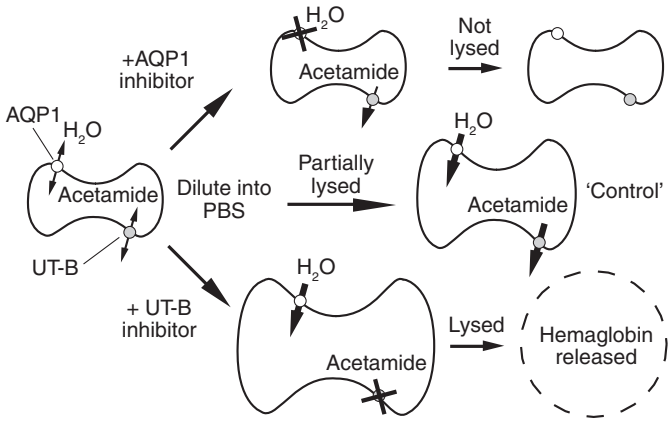

Fig. 3. Erythrocyte osmotic lysis assay for discovery of aquaporins and urea transport inhibitors. Erythrocytes expressing water and urea channels (AQP1 and UT-B) are preloaded with urea or a urea analog, such as acetamide. Following replacement of the external buffer with urea/acetamide-free hypo-osmolar solution, water entry results in cell swelling, which is limited by UT-B-mediated urea/acetamide efflux. Under optimized assay conditions, UT-B-facilitated urea/acetamide produces partial osmotic lysis (middle), whereas AQP1 inhibition slows water influx, preventing lysis (top), and UT-B inhibition impairs urea/acetamide exit resulting in greater lysis (bottom).

Aquaporin-based diagnostics

Antibody-based diagnostics

There is one prominent example of an AQP antibody-based diagnostic test. AQP4 has been implicated as a marker of the central inflammatory demyelinating disease neuromyelitis optica (NMO), or Devic's disease (Wingerchuk et al., 2007). NMO is a unique form of multiple sclerosis (MS) in which inflammatory lesions are restricted to the optic nerve and spinal cord, causing acute ocular pain with loss of vision, and myelitis with symmetric paraplegia, sensory loss and bladder dysfunction. A serum immunoglobulin was discovered in NMO subjects, but not in MS or normal subjects, which was found to target external epitope(s) on AQP4 (Lennon et al., 2005). Seropositivity for NMO-IgG is reasonably sensitive (74%) and specific (>90%) for NMO (Jarius et al., 2008), enabling early diagnostic distinction of NMO from MS. The characteristic vasculocentric deposition of immunoglobulins and complement activation products in NMO has suggested the possibility that the AQP4 autoantibody is involved in NMO disease pathogenesis. However, various observations have challenged the proposed role of AQP4 antibody in disease pathogenesis, such as the lack of correlation of NMO-IgG antibody titer with disease severity, and the restricted sites of NMO lesions compared with the wide distribution of AQP4. Notwithstanding the incomplete understanding of the origin and importance of NMO antibodies in disease pathogenesis, the detection of NMO-IgG has opened a new area in the diagnosis of a neurological disease where alternative tests were not available. There are various other disease states in which AQP antibodies may be of utility for diagnosis and possibly involved in disease pathogenesis. As yet untested possibilities include AQP3 autoantibodies in autoimmune skin diseases and AQP5 autoantibodies in Sjogren's syndrome.

Protein-based diagnostics

Assay of AQP protein content in bodily fluids and tissue specimens may have diagnostic value. The one established example is assay of AQP2 immunoreactive protein in urine for distinguishing among various etiologies of nephrogenic diabetes insipidus (NDI) (Rai et al., 1997; Ishikawa, 2000). The rationale for urinary AQP2 assay is the shedding, by an exosomal mechanism, of a small amount of AQP2 protein when present at the luminal membrane of kidney collecting duct. With suitable caveats, urinary AQP2 protein is a marker of apical membrane AQP2 expression, being absent in NDI caused by AQP2 deficiency or defective cellular processing. However, diagnostic assay of urinary AQP2 has not been widely used because alternative, reliable methods are available to evaluate NDI. The possibility of 'shedding' of other AQPs in urine, or in other bodily fluids, such as aqueous humor or CSF, has not been explored. Another potential role for AQP protein-based diagnostics is in evaluating AQP expression in tissue specimens. Several studies have attempted to correlate AQP expression in tumor cells with tumor grade (reviewed by Verkman et al., 2008a), and AQP expression with human epilepsy (Lee et al., 2004), and ocular (reviewed by Verkman et al., 2008b) and skin (Olsson et al., 2006) diseases. Whether diagnostically useful or unique information can be obtained by such measurements remains to be seen.

Aquaporin genetics and human disease

'Aquaporinopathies'

Though exceedingly rare, there exist loss-of-function mutations in human AQPs. Mutations in AQP2 produce non-X-linked NDI by a recessive mechanism, which involves defective mutant AQP2 protein folding/function, and by a dominant mechanism, which results from endoplasmic reticulum (ER)/Golgi interactions between wild-type and mutant AQP2 that prevent plasma membrane targeting of wild-type AQP2 (reviewed by Bichet, 2006). The incidence of NDI caused by AQP2 mutations is less than one in 20 million births. For other AQPs only a handful of subjects have been identified with loss-of-function mutations. The few subjects that lack functional AQP1, which were identified by blood group screening, are phenotypically normal but manifest defective urinary-concentrating function when deprived of water (King et al., 2001), similar to findings in AQP1 null mice. Because of the rarity of AQP1-deficient individuals, as well as a few subjects that apparently lack functional AQP3 or AQP7 (Roudier et al., 2002; Kondo et al., 2002), and because of wide phenotype variations in humans, little useful information is available about the roles of these AQPs in humans. Mutations in the major intrinsic protein (MIP) of the lens cause congenital cataracts (Berry et al., 2000). MIP (also called AQP0) is homologous to the AQPs, though its function in lens and the relationship between loss-of-function mutations and cataract formation are unclear (reviewed by Verkman et al., 2008b). Disease-causing mutations of other AQPs in humans have not been described.

The rarity of NDI caused by AQP2 mutation precludes clinical development of 'new chemical entities' for therapy because of the large costs involved. NDI patients are treated primarily by water replacement and salt restriction, and in some cases by drugs such as thiazides that impair urinary diluting ability. We have been interested in the possibility of using existing drugs for therapy of recessive NDI caused by defective cellular processing of AQP2 mutants. An emerging paradigm in molecular medicine is the therapy of protein folding diseases by chemical or molecular chaperones, which facilitate folding of the mutant protein by direct binding and/or modulation of components of the molecular quality control machinery. We have focused attention on the T126M mutation in AQP2, one of the mutations causing recessive NDI in humans. Studies in mammalian cell culture models indicated ER retention and protein misfolding (Tamarappoo and Verkman, 1998). We also found that incubation of cell cultures with the 'chemical chaperones' glycerol or trimethylamine-*N*-oxide rescued

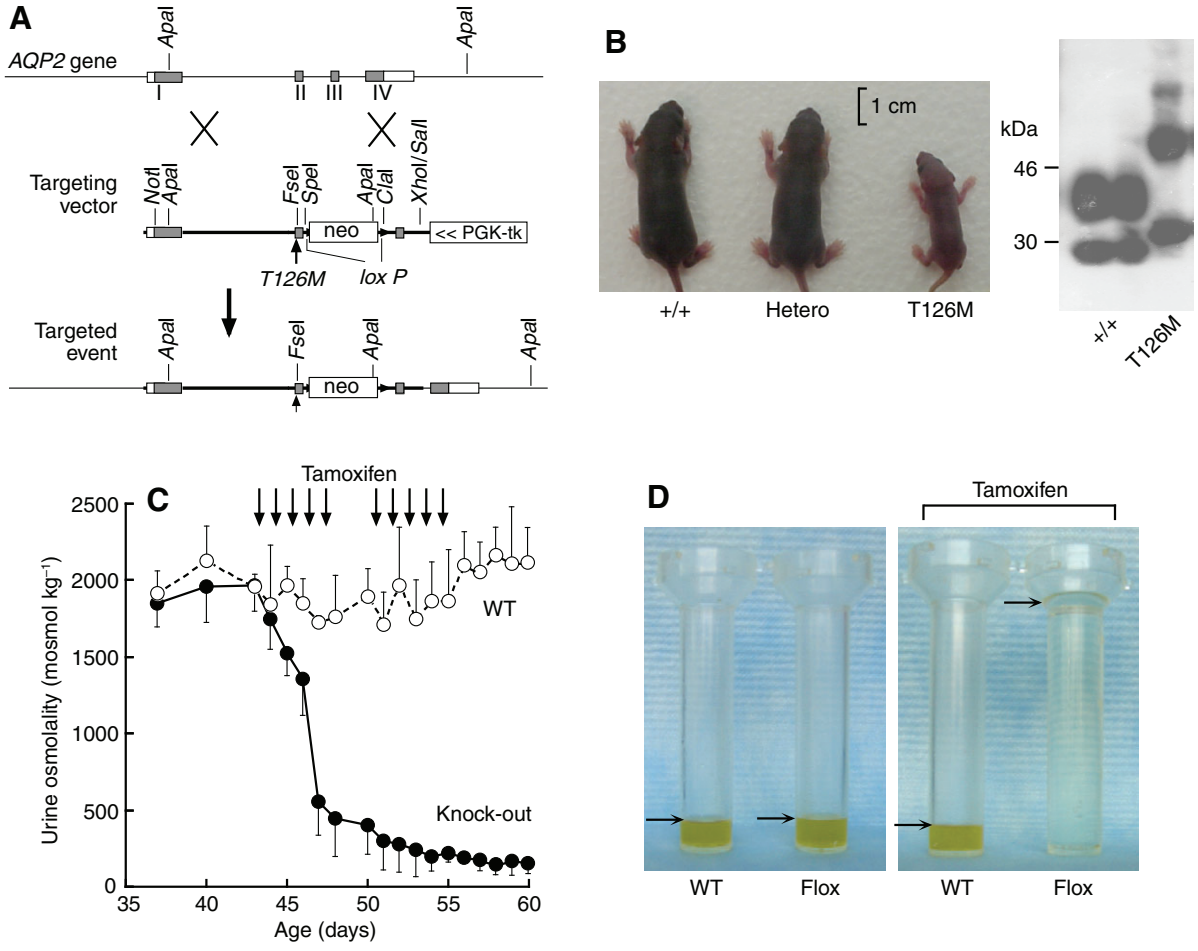

Fig. 4. AQP2 transgenic mouse models of nephrogenic diabetes insipidus. (A) Gene targeting strategy for introducing the T126M mutation into AQP2. Homologous recombination results in replacement of the indicated segment (thick line) of the *AQP2* gene by a 1.8 kb neomycin selection cassette (neo) flanked by loxP sites. PGK-tk, phosphoglycerate promoter–thymidine kinase gene. (B, left) Photograph of mice of indicated genotypes at 5 days after birth. +/+, wild-type; Hetero, heterozygote; T126M, AQP2-T126M mutant. (B, right) Immunoblot of kidney homogenates. (C) Urine osmolality in tamoxifen-treated wild-type mice (WT, open circles) and AQP2 floxed knock-out mice (filled circles) given free access to food and water. Arrows indicate tamoxifen injections. (D) Twenty-four hour urine output in untreated (left) and tamoxifen-treated (right) wild-type and AQP2 knock-out ('flox') mice (arrows indicate urine level).

defective AQP2-T126M cellular processing, resulting in its plasma membrane expression and restoration of cell membrane water permeability (Tamarappoo et al., 1999). However, chemical chaperones are not suitable for use *in vivo* because of the high concentrations required.

For *in vivo* analysis, we created a mouse model of human NDI caused by the T126M AQP2 mutation. Initially, an AQP2-T126M knock-in mouse model was generated by targeted gene replacement using a Cre-loxP strategy in which the targeted gene locus contained an engineered T126M mutation (Fig. 4A) (Yang et al., 2001). Unfortunately, though the homozygous mutant mice appeared normal just after birth, they generally died in the first week of life because of polyuria-induced renal failure (Fig. 4B, left). Immunoblot analysis of kidneys of the mutant mice showed endoglycosidase H-sensitive, core glycosylated AQP2-T126M, indicating ER retention (Fig. 4B, right). As a first step in developing an AQP2-T126M 'conditional knock-in' model of NDI, we generated an inducible mouse model of *AQP2* gene deletion ('conditional knock-out' mouse) manifesting severe polyuria in adult mice (Yang et al., 2006c). LoxP sequences were inserted into introns 1 and 2 in the mouse *AQP2* gene. Mating of germ-line *AQP2*-loxP mice with tamoxifen-inducible Cre-expressing mice produced offspring with inducible homozygous Cre-*AQP2*-loxP. Tamoxifen administration led to Cre recombinase expression and *AQP2* gene excision, resulting in severe polyuria and an inability to concentrate their urine in response to water deprivation (Fig. 4C,D). The adult polyuric mice survived well. To create 'conditional AQP2-T126M knock-in' mouse model, mice heterozygous separately for floxed wild-type AQP2 and AQP2-T126M were bred to produce hemizygous mice containing a floxed wild-type *AQP2* allele and a mutant *AQP2*-T126M allele (Yang et al., 2009). Conditional deletion of the wild-type *AQP2* gene in adult mice by tamoxifen administration produced mice expressing only the mutant AQP2-T126M protein. The conditional knock-in adult mice showed polyuria, urinary hypo-osmolality and ER retention of AQP2-T126M in collecting duct. Screening of candidate protein folding 'correctors' in AQP2-T126M-transfected kidney cells showed increased AQP2-T126M plasma membrane expression with the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), a compound currently in clinical trials for tumor therapy. 17-AAG increased urine osmolality in the AQP2-T126M mice (without effect in AQP2 null mice) and partially rescued defective AQP2-T126M cellular processing. These proof-of-concept findings suggest the possibility of using existing drugs for therapy of some forms of NDI.

Aquaporin polymorphisms as disease markers

The possibility of functionally significant AQP polymorphisms has received little attention, though recent data support further research into this area. Two recent studies have investigated possible AQP4 polymorphisms. Kleffner and colleagues (Kleffner et al., 2008) studied 10 AQP4 polymorphisms in 41 stroke patients with middle cerebral artery occlusion, tentatively identifying one polymorphism associated with increased severity of brain edema. Sorani and colleagues (Sorani et al., 2008) identified 24 AQP4 variants in an ethnically diverse cohort of 188 normal subjects, some of which altered AQP4 water permeability when expressed in cell cultures, though the results were inconclusive because water permeability was not normalized for plasma membrane AQP4 protein expression. In other studies, associations were reported for single nucleotide polymorphisms in AQP1 with priapism in sickle cell disease (Elliott et al., 2007) and with diabetic nephropathy (Ewens et al., 2005). The significance of these observations is unclear. Single nucleotide polymorphisms in AQP7 have also been associated with obesity and type II diabetes (Prudente et al., 2007). Research in disease-related AQP polymorphisms is at a very early stage, with its real impact to be determined. It may be worthwhile, for example, to investigate polymorphisms in AQP4 in brain diseases such as obstructive hydrocephalus, in AQP3 in skin diseases, and in various AQPs in cancer.

Support for AQP research in my lab is acknowledged from the National Institutes of Health, through awards R37 DK35124, R37 EB00415, R01 EY13574, R01 HL59198, R01 HL73856 and P30 DK72517. Deposited in PMC for release after 12 months.

References

Amiry-Moghaddam, M., Lindland, H., Zelenin, S., Roberg, B. A., Gundersen, B. B., Petersen, P., Rinvik, E., Torgner, I. A. and Ottersen, O. P. (2005). Brain mitochondria contain aquaporin water channels: evidence for the expression of a short AQP9 isoform in the inner mitochondrial membrane. *FASEB J.* **19**, 1459-1467.

Auguste, K. I., Jin, S., Uchida, K., Yan, D., Manley, G. T., Papadopoulos, M. C. and Verkman, A. S. (2007). Greatly impaired migration of implanted aquaporin-4-deficient astroglial cells in mouse brain toward a site of injury. *FASEB J.* **21**, 108-116.

Bai, C., Fukuda, N., Song, Y., Ma, T., Matthay, M. A. and Verkman, A. S. (1999). Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J. Clin. Invest.* 103,555-561.

Berry, V., Francis, P., Kaushal, S., Moore, A. and Bhattacharya, S. (2000). Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. *Nat. Genet.* **25**, 15-17.

Bichet, D. G. (2006). Hereditary polyuric disorders: new concepts and differential diagnosis. *Semin. Nephrol.* **26**, 224-233.

Binder, D. K., Oshio, K., Ma, T., Verkman, A. S. and Manley, G. T. (2004a). Increased seizure threshold in mice lacking aquaporin-4 water channels. *NeuroReport* **15**, 259-262.

Binder, D. K., Papadopoulos, M. C., Haggie, P. M. and Verkman, A. S. (2004b). In vivo measurement of brain extracellular space diffusion by cortical surface photobleaching. *J. Neurosci.* **24**, 8049-8056.

Binder, D. K., Yao, X., Sick, T. J., Verkman, A. S. and Manley, G. T. (2006). Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia* **53**, 631-636.

Bloch, O., Papadopoulos, M. C., Manley, G. T. and Verkman, A. S. (2005). Aquaporin-4 gene deletion in mice increases focal edema associated with brain abscess. *J. Neurochem.* **95**, 254-262.

Bloch, O., Manley, G. T. and Verkman, A. S. (2006). Accelerated progression of kaolin-induced hydrocephalus in aquaporin-4 deficient mice. *J. Cereb. Blood Flow. Metab.* **26**, 1527-1537.

Brooks, H. L., Regan, J. W. and Yool, A. J. (2000). Inhibition of aquaporin-1 water permeability by tetraethylammonium: involvement of the loop E pore region. *Mol. Pharmacol.* **57**, 1021-1026.

Calamita, G., Ferri, D., Gena, P., Liquori, G. E., Cavalier, A., Thomas, D. and Svelto, M. (2005). The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. *J. Biol. Chem.* **280**, 17149-17153.

Chou, C. L., Knepper, M. A., Hoek, A. N., Brown, D., Yang, B., Ma, T. and Verkman, A. S. (1999). Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. *J. Clin. Invest.* **103**, 491-496.

Da, T. and Verkman, A. S. (2004). Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. *Invest. Ophthalmol. Vis. Sci.* **45**, 4477-4483.

Deen, P. M., Verdijk, M. A., Knoers, N. V., Wieringa, B., Monnens, L. A., van Os, C. H. and van Oost, B. A. (1994). Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* **264**, 92-95.

Elliott, L., Ashley-Koch, A. E., De Castro, L., Jonassaint, J., Price, J., Ataga, K. I., Levesque, M. C., Brice Weinberg, J., Eckman, J. R., Orringer, E. P. et al. (2007). Genetic polymorphisms associated with priapism in sickle cell disease. *Br. J. Haematol.* **137**, 262-267.

Ewens, K. G., George, R. A., Sharma, K., Ziyadeh, F. N. and Spielman, R. S. (2005). Assessment of 115 candidate genes for diabetic nephropathy by transmission/disequilibrium test. *Diabetes* **54**, 3305-3318.

Fang, X., Yang, B., Matthay, M. A. and Verkman, A. S. (2002). Evidence against aquaporin dependent CO_2 permeability in lung and kidney. *J. Physiol. (Lond.)* **543**, 63-69.

Fujiyoshi, Y., Mitsuoka, K., de Groot, B. L., Phillippsen, A., Grubmuller, H., Agre, P. and Engel, A. (2002). Structure and function of water channels. *Curr. Opin. Struct. Biol.* **12**, 509-515.

Galietta, L. J., Haggie, P. M. and Verkman, A. S. (2001). Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. *FEBS Lett.* **499**, 220-224.

Hara, M. and Verkman, A. S. (2003). Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *Proc. Natl. Acad. Sci. USA* **100**, 7360-7365.

Hara, M., Ma, T. and Verkman, A. S. (2002). Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery. *J. Biol. Chem.* **277**, 46616-46621.

Hara-Chikuma, M. and Verkman, A. S. (2006). Aquaporin-1 facilitates epithelial cell migration in kidney proximal tubule. *J. Am. Soc. Nephrol.* **17**, 39-45.

Hara-Chikuma, M. and Verkman, A. S. (2008a). Prevention of skin tumorigenesis and impairment of epidermal cell proliferation by targeted aquaporin-3 gene disruption. *Mol. Cell. Biol.* **28**, 326-332.
Hara-Chikuma, M. and Verkman, A. S. (2008b). Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *J. Mol. Med.* **96**, 523-529.
Hara-Chikuma, M. and Verkman, A. S. (2008c). Roles of aquaporin-3 in epidermis. *J. Invest. Dermatol.* **128**, 2145-2151.
Hara-Chikuma, M., Sohara, E., Rai, T., Ikawa, M., Okabe, M., Sasaki, S., Uchida, S. and Verkman, A. S. (2005). Progressive adipocyte hypertrophy in aquaporin-7 deficient mice: adipocyte glycerol permeability as a novel regulator of fat accumulation. *J. Biol. Chem.* **280**, 15493-15496.
Hibuse, T., Maeda, N., Funahashi, T., Yamamoto, K., Nagasawa, A., Mizunoya, W., Kishida, K., Inoue, K., Kuriyama, H., Nakamura, T. et al. (2005). Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. *Proc. Natl. Acad. Sci. USA* **102**, 10993-10998.
Hiroaki, Y., Tani, K., Kamegawa, A., Gyobu, N., Nishikawa, K., Suzuki, H., Walz, T., Sasaki, S., Mitsuoka, K., Kimura, K. et al. (2006). Implications of the aquaporin-4 structure on array formation and cell adhesion. *J. Mol. Biol.* **355**, 628-639.
Holm, L. M., Jahn, T. P., Møller, A. L., Schjoerring, J. K., Ferri, D., Klaerke, D. A. and Zeuthen, T. (2005). NH_3 and NH_4^+ permeability in aquaporin-expressing Xenopus oocytes. *Pflugers Arch.* **450**, 415-248.
Hu, J. and Verkman, A. S. (2006). Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. *FASEB J.* **20**, 1892-1894.
Huber, V. J., Tsujita, M., Yamazaki, M., Sakimura, K. and Nakada, T. (2007). Identification of arylsulfonamides as Aquaporin 4 inhibitors. *Bioorg. Med. Chem.* **17**, 1270-1273.
Huber, V. J., Tsujita, M. and Nakada, T. (2008a). Identification of Aquaporin 4 inhibitors using in vitro and in silico methods. *Bioorg. Med. Chem.* **17**, 411-417.
Huber, V. J., Tsujita, M., Kwee, I. L. and Nakada, T. (2008b). Inhibition of Aquaporin 4 by antiepileptic drugs. *Bioorg. Med. Chem* **17**, 418-424.
Ishikawa, S. (2000). Urinary excretion of aquaporin-2 in pathological states of water metabolism. *Ann. Med.* **32**, 90-93.
Jarius, S., Paul, F., Franciotta, D., Waters, P., Zipp, F., Hohlfeld, R., Vincent, A., Wildemann, B. and Vanderbilt. (2008). Aquaporin-4 antibodies in neuromyelitis optica. *Nat. Clin. Pract. Neurol.* **4**, 202-214.
King, L. S., Choi, M., Fernandez, P. C., Cartron, J. P. and Agre, P. (2001). Defective urinary-concentrating ability due to a complete deficiency of aquaporin-1. *N. Engl. J. Med.* **345**, 175-179.
Kleffner, I., Bungeroth, M., Schiffbauer, H., Schäbitz, W. R., Ringelstein, E. B. and Kuhlenbäumer, G. (2008). The role of aquaporin-4 polymorphisms in the development of brain edema after middle cerebral artery occlusion. *Stroke* **9**, 1333-1335.
Kondo, H., Shimomura, I., Kishida, K., Kuriyama, H., Makino, Y., Nishizawa, H., Matsuda, M., Maeda, N., Nagaretani, H., Kihara, S. et al. (2002). Human aquaporin adipose (AQPap) gene. Genomic structure, promoter analysis and functional mutation. *Eur. J. Biochem.* **269**, 1814-1826.
Lee, T. S., Eid, T., Mane, S., Kim, J. H., Spender, D. D., Ottersen, O. P. and Lanerolle, N. C. (2004). Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathol.* **108**, 493-502.
Lennon, V. A., Kryzer, T. J., Pittock, S. J., Verkman, A. S. and Hinson, S. R. (2005). IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J. Exp. Med.* **202**, 473-477.
Levin, M. H. and Verkman, A. S. (2006). Aquaporin-3-dependent cell migration and proliferation during corneal re-epithelialization. *Invest. Ophthalmol. Vis. Sci.* **47**, 4365-4372.
Levin, M. H., de la Fuente, R. and Verkman, A. S. (2007). Urearetics: a small molecule screen yields nanomolar potency inhibitors of urea transporter UT-B. *FASEB J.* **21**, 551-563.
Li, J. and Verkman, A. S. (2001). Impaired hearing in mice lacking aquaporin-4 water channels. *J. Biol. Chem.* **276**, 31233-31237.
Li, J., Patil, R. V. and Verkman, A. S. (2002). Mildly abnormal retinal function in transgenic mice without Muller cell aquaporin-4 water channels. *Invest. Ophthalmol. Vis. Sci.* **43**, 573-579.
Lu, D., Zhang, H., Zador, Z. and Verkman, A. S. (2008). Impaired olfaction in mice lacking aquaporin-4 water channels. *FASEB J.* **22**, 3216-3223.
Ma, B., Xiang, Y., Mu, S. M., Li, T., Yu, H. M. and Li, X. J. (2004). Effects of acetazolamide and anordiol on osmotic water permeability in AQP1-cRNA injected Xenopus oocyte. *Acta Pharmacol. Sin.* **25**, 90-97.
Ma, T., Yang, B., Gillespie, A., Carlson, E. J., Epstein, C. J. and Verkman, A. S. (1997). Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J. Clin. Invest.* **100**, 957-962.
Ma, T., Yang, B., Gillespie, A., Carlson, E. J., Epstein, C. J. and Verkman, A. S. (1998). Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J. Biol. Chem.* **273**, 4296-4299.
Ma, T., Song, Y., Gillespie, A., Carlson, E. J., Epstein, C. J. and Verkman, A. S. (1999). Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J. Biol. Chem.* **274**, 20071-20074.
Ma, T., Fukuda, N., Song, Y., Matthay, M. A. and Verkman, A. S. (2000a). Lung fluid transport in aquaporin-5 knockout mice. *J. Clin. Invest.* **105**, 93-100.
Ma, T., Song, Y., Yang, B., Gillespie, A., Carlson, E. J., Epstein, C. J. and Verkman, A. S. (2000b). Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc. Natl. Acad. Sci. USA* **97**, 4386-4391.
Ma, T., Hara, M., Sougrat, R., Verbavatz, J. M. and Verkman, A. S. (2002). Impaired stratum corneum hydration in mice lacking epidermal water channel aquaporin-3. *J. Biol. Chem.* **277**, 17147-17153.
Manley, G. T., Fujimura, M., Ma, T., Noshita, N., Filiz, F., Bollen, A. W., Chan, P. and Verkman, A. S. (2000). Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat. Med.* **6**, 159-163.
Missner, A., Kügler, P., Saparov, S. M., Sommer, K., Matthai, J. C., Zeidel, M. L. and Pohl, P. (2008). Carbon dioxide transport through membranes. *J. Biol. Chem.* **283**, 25340-25347.
Moore, M., Ma, T., Yang, B. and Verkman, A. S. (2000). Tear secretion by lacrimal glands in transgenic mice lacking water channels AQP1, AQP3, AQP4 and AQP5. *Exp. Eye. Res.* **70**, 557-562.
Niemietz, C. M. and Tyerman, S. D. (2002). New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. *FEBS Lett.* **531**, 443-447.
Olsson, M., Broberg, A., Jernås, M., Carlsson, L., Rudemo, M., Suurküla, M., Svensson, P. A. and Benson, M. (2006). Increased expression of aquaporin 3 in atopic eczema. *Allergy* **61**, 1132-1137.
Oshio, K., Watanabe, H., Song, Y., Verkman, A. S. and Manley, G. T. (2005). Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel aquaporin-1. *FASEB J.* **19**, 76-78.
Padmawar, P., Yao, X., Bloch, O., Manley, G. T. and Verkman, A. S. (2005). K^+ waves in brain cortex visualized using a long-wavelength K^+-sensing fluorescent indicator. *Nat. Methods* **2**, 825-827.
Pallone, T. L., Edwards, A., Ma, T., Silldorff, E. P. and Verkman, A. S. (2000). Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. *J. Clin. Invest.* **105**, 215-222.
Papadopoulos, M. C. and Verkman, A. S. (2005). Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. *J. Biol. Chem.* **280**, 13906-13912.
Papadopoulos, M. C., Manley, G. T., Krishna, S. and Verkman, A. S. (2004). Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J.* **18**, 1291-1293.
Papadopoulos, M. C., Saadoun, S. and Verkman, A. S. (2008). Aquaporins and cell migration. *Pflugers Arch.* **456**, 693-700.
Prudente, S., Flex, E., Morini, E., Turchi, F., Capponi, D., De Cosmo, S., Tassi, V., Guida, V., Avogaro, A., Folli, F. et al. (2007). A functional variant of the adipocyte glycerol channel aquaporin 7 gene is associated with obesity and related metabolic abnormalities. *Diabetes* **56**, 1468-1474.
Rai, T., Sekine, K., Kanno, K., Hata, K., Miura, M., Mizushima, A., Marumo, F. and Sasaki, S. (1997). Urinary excretion of aquaporin-2 water channel protein in human and rat. *J. Am. Soc. Nephrol.* **8**, 1357-1362.
Roudier, N., Ripoche, P., Gane, P., Le Pennec, P. Y., Daniels, G., Cartron, J. P. and Bailly, P. (2002). AQP3 deficiency in humans and the molecular basis of a novel blood group system, GIL. *J. Biol. Chem.* **277**, 45854-45859.
Ruiz-Ederra, J. and Verkman, A. S. (2006). Accelerated cataract formation and reduced lens epithelial water permeability in aquaporin-1deficient mice. *Invest. Opthalmol. Vis. Sci.* **47**, 3960-3967.
Ruiz-Ederra, J., Zhang, H. and Verkman, A. S. (2007). Evidence against functional interaction between aquaporin-4 water channels and Kir4.1 K^+ channels in retinal Müller cells. *J. Biol. Chem.* **282**, 21866-21872.
Saadoun, S., Papadopoulos, M. C., Hara-Chikuma, M. and Verkman, A. S. (2005a). Impairment of angiogenesis and cell migration by targeted of aquaporin-1 gene disruption. *Nature* **434**, 786-792.
Saadoun, S., Papadopoulos, M. C., Watanabe, H., Yan, D., Manley, G. T. and Verkman, A. S. (2005b). Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J. Cell. Sci.* **118**, 5691-5698.
Saadoun, S., Bell, B. A., Verkman, A. S. and Papadopoulos, M. C. (2008). Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice. *Brain* **131**, 1087-1098.
Schnermann, J., Chou, C. L., Ma, T., Traynor, T., Knepper, M. A. and Verkman, A. S. (1998). Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc. Natl. Acad. Sci. USA* **95**, 9660-9664.
Søgaard, R. and Zeuthen, T. (2008). Test of blockers of AQP1 water permeability by a high-resolution method: no effects of tetraethylammonium ions or acetazolamide. *Pflugers Arch.* **456**, 285-292.
Solenov, E., Watanabe, H., Manley, G. T. and Verkman, A. S. (2004). Sevenfold-reduced osmotic water permeability in primary astrocyte cultures from AQP-4-deficient mice, measured by a fluorescence quenching method. *Am. J. Physiol.* 286, C426-C432.
Song, Y. and Verkman, A. S. (2001). Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J. Biol. Chem.* **276**, 41288-41292.
Song, Y., Jayaraman, S., Yang, B., Matthay, M. A. and Verkman, A. S. (2001). Role of aquaporin water channels in airway fluid transport, humidification, and surface liquid hydration. *J. Gen. Physiol.* **117**, 573-582.
Song, Y., Sonawane, N. and Verkman, A. S. (2002). Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice. *J. Physiol.* **541**, 561-568.
Sorani, M. D., Zador, Z., Hurowitz, E., Yan, D., Giacomini, K. M. and Manley, G. T. (2008). Novel variants in human Aquaporin-4 reduce cellular water permeability. *Hum. Mol. Genet.* **17**, 2379-2389.
Tamarappoo, B. K. and Verkman, A. S. (1998). Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. *J. Clin. Invest.* **101**, 2257-2267.
Tamarappoo, B. K., Yang, B. and Verkman, A. S. (1999). Misfolding of mutant aquaporin-2 water channels in nephrogenic diabetes insipidus. *J. Biol. Chem.* **274**, 34825-34831.
Thiagarajah, J. R. and Verkman, A. S. (2002). Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling. *J. Biol. Chem.* **277**, 19139-19144.
Thiagarajah, J. R., Zhao, D. and Verkman, A. S. (2007). Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. *Gut* **56**, 1529-1535.
Van Hoek, A. N., de Jong, M. D. and van Os, C. H. (1990). Effects of dimethylsulfoxide and mercurial sulfhydryl reagents on water and solute permeability of rat kidney brush border membranes. *Biochim. Biophys. Acta* **1030**, 203-210.
Verkman, A. S. (2000). Water permeability measurement in living cells and complex tissues. *J. Membr. Biol.* **173**, 73-87.

Verkman, A. S. (2005). More than just water channels: unexpected cellular roles of aquaporins. *J. Cell Sci.* **118**, 3225-3232.
Verkman, A. S. (2008). Dissecting the role of aquaporins in renal pathophysiology using transgenic mice. *Semin. Nephrol.* **28**, 217-226.
Verkman, A. S., Hara-Chikuma, M. and Papadopoulos, M. C. (2008a). Aquaporins: new players in cancer biology. *J. Mol. Med.* **86**, 523-529.
Verkman, A. S., Ruiz-Ederra, J. and Levin, M. (2008b). Functions of aquaporins in the eye. *Prog. Retin. Eye. Res.* **27**, 420-433.
Wingerchuk, D. M., Lennon, V. A., Lucchinetti, C. F., Pittock, S. J. and Weinshenker, B. G. (2007). The spectrum of neuromyelitis optica. *Lancet Neurol.* **6**, 805-815.
Wu, B. and Beitz, E. (2007). Aquaporins with selectivity for unconventional permeants. *Cell Mol. Life Sci.* **64**, 2413-2421.
Yang, B., Fukuda, N., van Hoek, A. N., Matthay, M. A., Ma, T. and Verkman, A. S. (2000). Carbon dioxide permeability of aquaporin-1 measured in erythrocytes and lung of aquaporin-1 null mice and in reconstituted proteoliposomes. *J. Biol. Chem.* **275**, 2686-2692.
Yang, B., Gillespie, A., Carlson, E. J., Epstein, C. J. and Verkman, A. S. (2001). Neonatal mortality in an aquaporin-2 knock-in mouse model of recessive nephrogenic diabetes insipidus. *J. Biol. Chem.* **276**, 2775-2779.
Yang, B., Kim, J. K. and Verkman, A. S. (2006a). Comparative efficacy of $HgCl_2$ with candidate aquaporin-1 inhibitors DMSO, gold, TEA^+ and acetazolamide. *FEBS Lett.* **580**, 6679-6684.
Yang, B., Zhao, D. and Verkman, A. S. (2006b). Evidence against functionally significant aquaporin expression in mitochondria. *J. Biol. Chem.* **281**, 16202-16206.
Yang, B., Zhao, D., Qian, L. and Verkman, A. S. (2006c). Mouse model of inducible nephrogenic diabetes insipidus produced by floxed aquaporin-2 gene deletion. *Am. J. Physiol.* **291**, F465-F472.
Yang, B., Zhao, D., Solenov, E. and Verkman, A. S. (2006d). Evidence from knockout mice against physiologically significant aquaporin-8 facilitated ammonia transport. *Am. J. Physiol.* 291, C417-C423.
Yang, B., Zhang, H. and Verkman, A. S. (2008). Lack of aquaporin-4 water transport inhibition by antiepileptics and arylsulfonamides. *Bioorg. Med. Chem.* **16**, 7489-7493.
Yang, B., Zhao, D. and Verkman, A. S. (2009). Hsp90 inhibitor partially corrects nephrogenic diabetes insipidus in a conditional knock-in mouse model of human aquaporin-2 mutation. *FASEB J.* **23**, 503-512.
Zador, Z., Magzoub, M., Jin, S., Manley, G. T., Papadopoulos, M. and Verkman, A. S. (2008). Microfiberoptic fluorescence photobleaching reveals size-dependent macromolecule diffusion in extracellular space deep in brain. *FASEB J.* **22**, 326-332.
Zhang, D., Vetrivel, L. and Verkman, A. S. (2002). Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. *J. Gen. Physiol.* **119**, 561-569.
Zhang, H. and Verkman, A. S. (2008a). Evidence against involvement of aquaporin-4 in cell-cell adhesion. *J. Mol. Biol.* **382**, 1136-1143.
Zhang, H. and Verkman, A. S. (2008b). Aquaporin-4 independent Kir4.1 K^+ channel function in brain glial cells. *Mol. Cell Neurosci.* **37**, 1-10.