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REVIEW ARTICLE

Potential role of the glial water channel aquaporin-4 in epilepsy

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Recent studies have implicated glial cells in novel physiological roles in the CNS, such as modulation of synaptic transmission, so it is possible that glial cells might have a functional role in the hyperexcitability that is characteristic of epilepsy. Indeed, alterations in distinct astrocyte membrane channels, receptors and transporters have all been associated with the epileptic state. This paper focuses on the potential roles of the glial water channel aquaporin-4 (AQP4) in modulating brain excitability and in epilepsy. We review studies of seizure phenotypes, K^+ homeostasis and extracellular space physiology of mice that lack AQP4 ($AQP4^{-/-}$ mice) and discuss the human studies demonstrating alterations of AQP4 in specimens of human epilepsy tissue. We conclude with new studies of AQP4 regulation by seizures and discuss its potential role in the development of epilepsy (epileptogenesis). Although many questions remain unanswered, the available data indicate that AQP4 and its molecular partners might represent important new therapeutic targets.

Keywords: Epilepsy, potassium, seizure, transport, water

INTRODUCTION

Glial cells and epilepsy

Epilepsy, which affects ~1% of the population, comprises a group of disorders of the brain that are characterized by the periodic and unpredictable occurrence of seizures. It is clear that epilepsy is a major public health problem in that those affected experience the periodic and unpredictable occurrence of seizures leading to impairment of consciousness. This handicap severely impairs the performance of many tasks and, secondarily, the procurement and maintenance of steady employment. Elucidating the cellular and molecular mechanisms of seizure generation might lead to novel antiepileptic drug (AED) therapies.

Most current existing AEDs act on widely expressed ion channels that directly control neuronal excitability (Rogawski and Loscher, 2004). For example, Na^+ channel blockers (e.g. phenytoin) reduce the rate and/or rise of neuronal action potentials and, thus, inhibit high-frequency neuronal firing. GABA receptor agonists (e.g. phenobarbital) increase the efficacy of GABAergic synapses, thus, increasing inhibitory synaptic transmission. These existing medications have at least two major drawbacks. First, even with optimal current AED therapy, ~30% of patients have poor seizure control and become medically refractory. Secondly, because these medications act as general CNS depressants and must be taken chronically for seizure suppression, they also have marked inhibitory effects on cognition and cognitive development.

Glial cells are involved in many important physiological functions, such as sequestration and/or redistribution of K^+ during neural activity, neurotransmitter cycling and provision of energy substrates to neurons (Ransom *et al.*, 2003). Several recent lines of evidence strongly indicate that glial cells are potential novel targets for epilepsy (Binder and Steinhäuser, 2006). First, many studies now link glial cells to modulation of synaptic transmission (Volterra and Steinhäuser, 2004; Volterra and Meldolesi, 2005). Secondly, functional alterations of specific glial membrane channels and receptors have been discovered in epileptic tissue (Heinemann *et al.*, 2000; Hinterkeuser *et al.*, 2000; Kivi *et al.*, 2000; Steinhäuser and Seifert, 2002; de Lanerolle and Lee, 2005; Seifert *et al.*, 2006). Thirdly, direct stimulation of astrocytes is sufficient for neuronal synchronization in epilepsy models (Tian *et al.*, 2005), although see also Fiacco *et al.* (2007). Thus, if the cellular and molecular mechanisms by which glial cells (especially astrocytes) modulate excitability are better understood, specific antiepileptic therapies based selectively on modulation of glial receptors and channels that are likely to have fewer deleterious side effects can be contemplated.

Aquaporins

The aquaporins (AQPs) are a family of membrane proteins that function as 'water channels' in many cell types and tissues in which fluid transport is crucial (Amiry-Moghaddam and Ottersen, 2003; Verkman, 2005). The AQPs are small hydrophobic integral membrane proteins

(~30 kDa monomer) that facilitate bidirectional water transport in response to osmotic gradients (Verkman, 2002). Multiple mammalian AQPs have been identified including AQP0, AQP1, AQP2, AQP4 and AQP5, which transport water only (AQPs), and AQP3, AQP7 and AQP9, which also transport glycerol (aquaglyceroporins) (Agre *et al.*, 2002; Verkman, 2002).

AQP4 (Hasegawa *et al.*, 1994; Jung *et al.*, 1994; Yang *et al.*, 1995) is of particular interest in neuroscience because it is expressed in brain and spinal cord by glial cells, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels and astrocyte membranes that ensheathe glutamatergic synapses (Nielsen *et al.*, 1997; Rash *et al.*, 1998; Nagelhus *et al.*, 2004; Oshio *et al.*, 2004). Activity-induced radial water fluxes in the neocortex have been demonstrated that might represent water movement via AQP channels in response to physiological activity (Holthoff and Witte, 2000; Niermann *et al.*, 2001). Interestingly, AQP4 is a structural component of membrane orthogonal arrays of particles (OAPs) seen in freeze-fracture electron micrographs (Frigeri *et al.*, 1995; Yang *et al.*, 1996; Verbavatz *et al.*, 1997).

AQP4$^{-/-}$ mice

AQP4$^{-/-}$ mice were generated by targeted gene disruption in 1997 (Ma *et al.*, 1997). These mice are grossly normal phenotypically, do not manifest overt neurological abnormalities, altered blood–brain barrier properties, abnormal baseline intracranial pressure, impaired osmoregulation or obvious brain dysmorphology (Fig. 1). They have normal blood chemistries and hematologies, normal expression of other brain AQPs (AQP1 and AQP9) and their only non-neural phenotype is a mild impairment in maximal urine-concentrating ability (Ma *et al.*, 1997).

Interestingly, more detailed studies of brain tissue from AQP4$^{-/-}$ mice demonstrate reduced osmotic water permeability as measured in isolated membrane vesicles (Ma *et al.*, 1997), brain slices (Solenov *et al.*, 2002) and intact brain (Thiagarajah *et al.*, 2005). Also, water permeability is reduced seven-fold in primary astrocyte cultures from AQP4$^{-/-}$ mice as measured by a calcein fluorescence-quenching method (Solenov *et al.*, 2004), and similar results have been obtained with AQP4 RNAi knockdown experiments in wild-type astrocytes (Nicchia *et al.*, 2003). These data demonstrate directly that AQP4 provides the predominant pathway for transmembrane water movement in astrocytes.

In vivo studies of these mice demonstrated a functional role for AQP4 in brain water transport. AQP4$^{-/-}$ mice have markedly decreased accumulation of brain water (cerebral edema) following water intoxication and focal cerebral ischemia (Manley *et al.*, 2000) and impaired clearance of brain water in models of vasogenic edema (Papadopoulos *et al.*, 2004). This corresponds to impaired water flux into (in the case of cytotoxic edema) and out of (in the case of vasogenic edema) the brain, which makes sense based on the bidirectional nature of water flux across the AQP4 membrane channel at the blood–brain barrier (Manley *et al.*, 2004). Similarly, mice deficient in dystrophin or α-syntrophin, in which there is mislocalization of the AQP4 protein (Frigeri *et al.*, 2001; Neely *et al.*, 2001; Vajda *et al.*, 2002), also demonstrate attenuated cerebral edema (Vajda *et al.*, 2002; Amiry-Moghaddam *et al.*, 2003a).

Altered extracellular space in AQP4$^{-/-}$ mice

We also hypothesized that the physiology of the extracellular space (ECS) in the brain might be altered in AQP4$^{-/-}$ mice because of impaired glial water uptake. The ECS in the brain comprises ~20% of brain tissue volume, consisting of a jelly-like matrix in which neurons, glia and blood vessels are embedded (Sykova, 1997). The ECS contains ions, neurotransmitters, metabolites, peptides and extracellular matrix molecules, forming the microenvironment for all cells in the brain and mediating glia–neuron communication via diffusible messengers, metabolites and ions (Fields and Stevens-Graham, 2002).

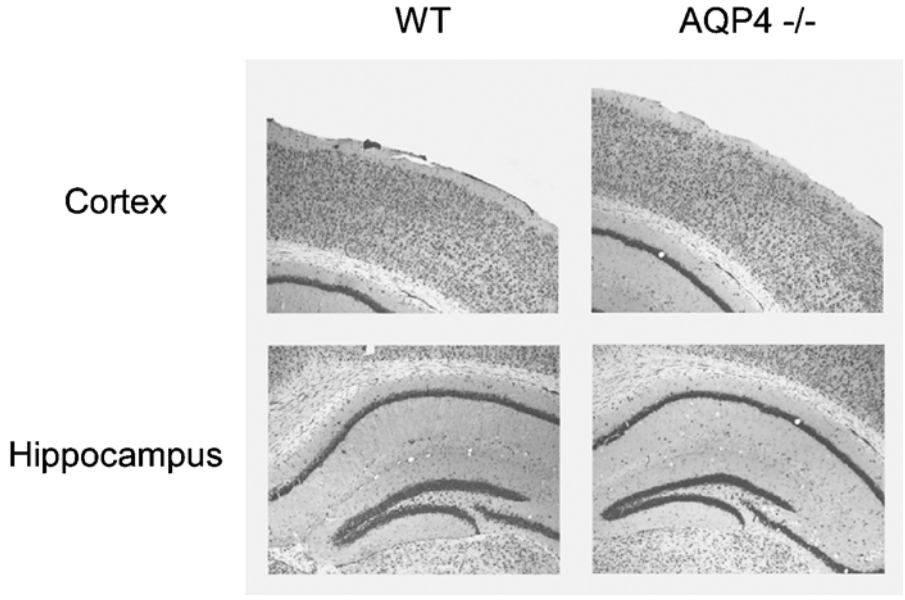

Fig. 1. Nissl stain of adult wild-type and AQP4$^{-/-}$ mice. No obvious developmental abnormalities in the cortex and hippocampus at the light microscopic level are observed. Modified from Binder *et al.* (2004a).

To examine the physiology of the ECS *in vivo*, we have developed and validated a novel cortical fluorescence recovery after photobleaching (cFRAP) method to measure the diffusion of fluorescently-labeled macromolecules in the cortex (Binder *et al.*, 2004b). ECS in mouse brain was labeled by exposure of the intact dura to fluorescein-dextrans (M_r 4 kD, 70 kD and 500 kD) after craniectomy (Fig. 2A). Fluorescein-dextran diffusion was detected by fluorescence recovery after laser-induced cortical photobleaching using confocal optics (Fig. 2B). FITC-dextran diffusion was slowed ~three-fold in brain ECS relative to solution (Fig. 2C). The cortical photobleaching approach was applied to brain edema, seizure initiation and AQP4 deficiency. Cytotoxic brain edema (produced by water intoxication) or seizure activity (produced by convulsants) slowed diffusion by >10-fold and created dead-space microdomains in which free diffusion was prevented (not shown). The hindrance to diffusion was greater for the larger fluorescein dextrans. Interestingly, slowed ECS diffusion preceded electroencephalographic seizure activity (Fig. 3) (Binder *et al.*, 2004b). In contrast to the slowed diffusion produced by brain edema and seizure activity, ECS diffusion was faster in $AQP4^{-/-}$ mice (Fig. 2D), indicating ECS expansion in AQP4 deficiency. In follow-up studies, cortical surface photobleaching has been used to demonstrate accelerated macromolecule diffusion in the expanded ECS in vasogenic edema (Papadopoulos *et al.*, 2005).

Seizure phenotype of $AQP4^{-/-}$ mice

Chemoconvulsant studies

In view of the role of AQP4 in cerebral water balance and ECS physiology, we aimed to test seizure phenotypes in $AQP4^{-/-}$ mice. Seizure susceptibility has been examined using the convulsant (GABA antagonist) pentylenetetrazol (PTZ) (Binder *et al.*, 2004a). At 40 mg kg^{-1} PTZ (i.p.), all wild-type mice exhibited seizure activity, whereas 6 out of 7 $AQP4^{-/-}$ mice did not exhibit seizure activity. At 50 mg kg^{-1} PTZ, both groups exhibited seizure activity; however, the latency to generalized (tonic–clonic) seizures was longer in $AQP4^{-/-}$ mice (Binder *et al.*, 2004a). Seizure propensity is exquisitely sensitive to ECS volume (Schwartzkroin *et al.*, 1998), so we hypothesized that the expanded ECS in AQP4 deficient mice is responsible for the increased seizure

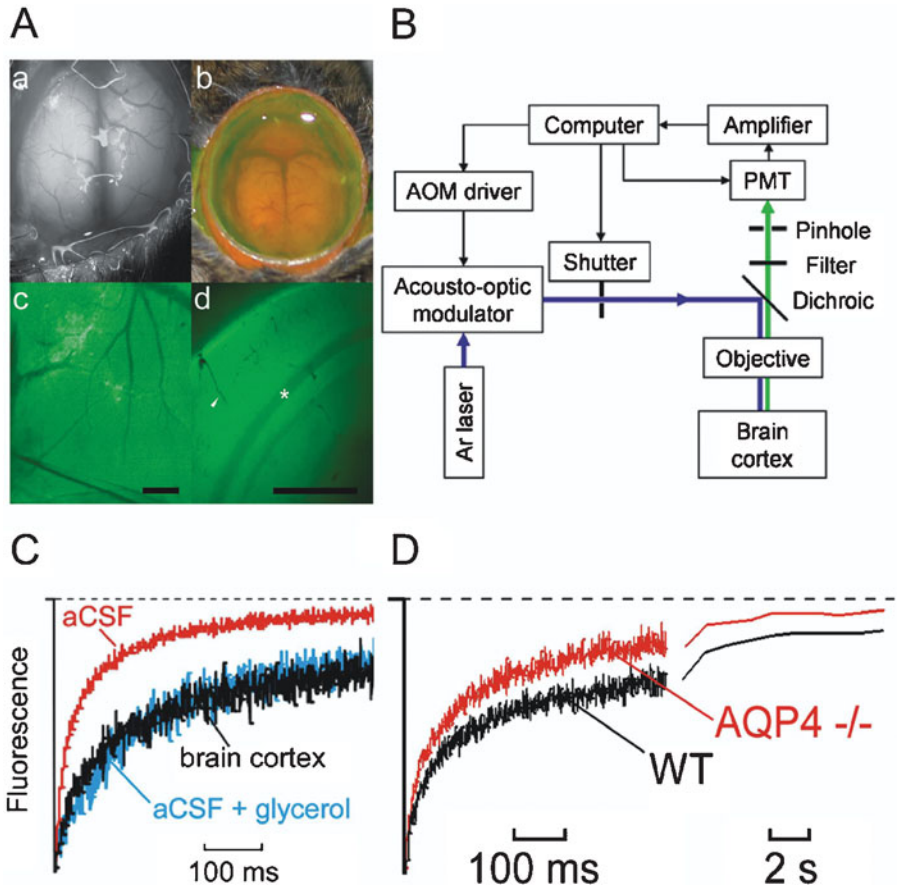

Fig. 2. *In vivo* cortical surface photobleaching. (A) *In vivo* loading of brain ECS by fluorescein-dextrans. a: Brain surface exposure following craniectomy showing cortical blood vessels and intact dura. b: Transdural loading of brain ECS showing a cylindrical dam that contains artificial CSF (aCSF) solution of fluorescein-dextran. c: Fluorescence image of cortical surface after dye loading (scale bar, 1 mm). d: Coronal, 300-μm brain slice obtained *ex vivo* following loading demonstrates fluorescence loading of cortex (scale bar, 1 mm). A gradient of fluorescence signal is observed from cortical surface (top left) to dorsal hippocampus (bottom right). Arrowhead, cortical blood vessel; asterisk, white matter. (B) Photobleaching apparatus. A laser beam is modulated by an acousto-optic modulator and directed onto the surface of the cortex using a dichroic mirror and objective lens. Emitted fluorescence is focused through a pinhole and detected by a gated photomultiplier (PMT). (C) *In vivo* fluorescence recovery for 4 kD FITC-dextran in cortex of wild-type mouse (black) shown in comparison to aCSF (red) and 30% glycerol in aCSF (blue, relative viscosity 2.7). (D) Enhanced ECS diffusion in mice lacking the glial water channel AQP4. Fluorescence recovery curves for 4 kD FITC-dextran in the cortex of wild-type (black) and $AQP4^{-/-}$ (red) mice. Modified from Binder *et al.* (2004b).

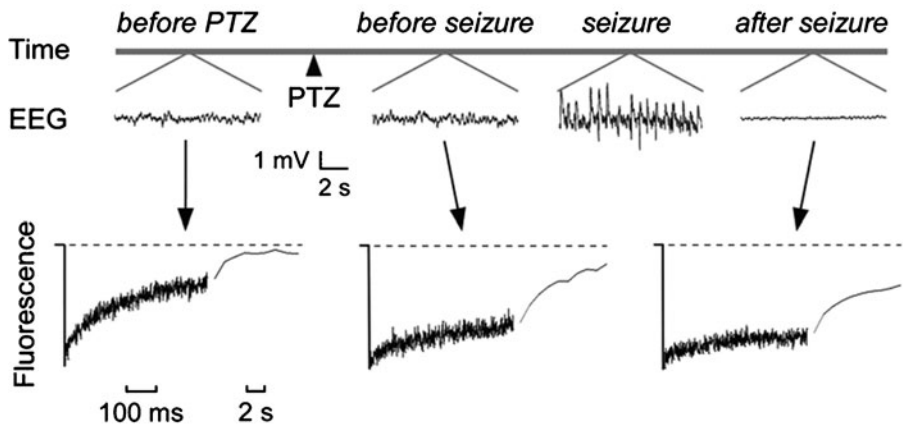

Fig. 3. Reduced macromolecular diffusion in brain ECS associated with seizures. *Top*: Electroencephalographic recordings before and after intraperitoneal injection of PTZ (100 mg kg^{-1}). *Middle*: EEG seizure recording. *Bottom*: Fluorescence recovery curves for 70 kD fluorescein-dextran before PTZ administration, after PTZ but before EEG seizure activity, and following seizure activity. Note the change in signal before EEG seizure onset, which indicates the possibility of seizure detection. From Binder *et al.* (2004b).

threshold. Thus, more intense stimuli (e.g. either higher PTZ doses or a longer time after PTZ) might be required to overcome the expanded ECS of $AQP4^{-/-}$ mice in order to initiate a seizure.

Electrical stimulation in vivo

In order to analyze the seizure phenotype of $AQP4^{-/-}$ mice in greater detail, we employed *in vivo* electroencephalographic (EEG) characterization with stimulation and recording (Binder *et al.*, 2006). $AQP4^{-/-}$ mice and wild-type controls were implanted in the right dorsal hippocampus with bipolar electrodes. Following postoperative recovery, electrical stimulations were given to assess electrographic seizure threshold and duration. Mice were awake and freely moving at the onset of stimulation (Fig. 4A). Baseline EEG in wild-type and $AQP4^{-/-}$ mice was indistinguishable, and there was no difference in baseline EEG power spectra (data not shown). Representative examples of hippocampal stimulation-evoked seizures for wild-type versus $AQP4^{-/-}$ mice are shown in Fig. 4B. $AQP4^{-/-}$ mice have a higher mean electrographic seizure threshold (167 ± 17 μA) than wild-type controls (114 ± 10 μA) ($P<0.01$) (Fig. 4C), consistent with the earlier PTZ studies (Binder *et al.*, 2004a). In addition, $AQP4^{-/-}$ mice have more prolonged stimulation-evoked seizures (33 ± 2 sec) compared to wild-type controls (13 ± 2 sec) (Fig. 4D). The only change in behavior during seizures (in both genotypes) is postural immobility (Grade 1 in the Racine classification) (Racine, 1972).

Altered K^+ homeostasis in $AQP4^{-/-}$ mice

More impressive than the effect on seizure threshold is the nearly three-fold increase in seizure duration in $AQP4^{-/-}$ mice. We hypothesized that the absence of AQP4 was associated with impaired K^+ reuptake. Impaired K^+ clearance from the ECS following the intense neuronal activity accompanying the seizure would lead to prolonged depolarization of neurons and inhibit seizure termination (Rutecki *et al.*, 1985; Yaari *et al.*, 1986; Traynelis and Dingledine, 1988; Steinhäuser and Seifert, 2002). Indeed, in addition to modulating water transport in the brain, AQP4 and its known molecular partners have been hypothesized to modulate ion homeostasis (Manley *et al.*, 2004; Simard and Nedergaard, 2004). During rapid neuronal firing, extracellular $[K^+]$ increases from ~3 mM to a maximum of 10–12 mM; and K^+ released by active neurons is thought to be primarily taken up by glial cells (Heinemann and Lux, 1977; Sykova, 1997; Xiong and Stringer, 1999; Somjen, 2002). Such K^+ reuptake into glial cells might be AQP4-dependent because water influx coupled to K^+ influx is thought to underlie activity-induced glial cell swelling (Walz, 1987; Walz, 1992). In support of this possibility is the subcellular colocalization of AQP4 with the inwardly rectifying K^+ channel $K_{ir}4.1$ in the retina both by electron micrographic and co-immunoprecipitation analyses (Connors *et al.*, 2004; Nagelhus *et al.*, 2004). $K_{ir}4.1^{-/-}$ mice, like $AQP4^{-/-}$ mice (Li and Verkman, 2001; Li *et al.*, 2002), demonstrate abnormal retinal and cochlear physiology presumably due to altered K^+ homeostasis (Kofuji *et al.*, 2000; Neusch *et al.*, 2001; Marcus *et al.*, 2002; Rozengurt *et al.*, 2003). $K_{ir}4.1$ is thought to contribute to K^+ reuptake and spatial K^+ buffering by glial cells (Newman *et al.*, 1984; Newman, 1986; Newman and Karwoski, 1989; Newman, 1993), and pharmacological or genetic inactivation of $K_{ir}4.1$ leads to impairment of extracellular K^+ regulation (Ballanyi *et al.*, 1987; Kofuji *et al.*, 2000; Kofuji and Newman, 2004; Neusch *et al.*, 2006; Djukic *et al.*, 2007).

To address the possibility that AQP4 deficiency was associated with a deficit in K^+ homeostasis, we examined K^+ dynamics *in vivo* in $AQP4^{-/-}$ mice (Binder *et al.*, 2006). K^+ measurements were made from intact cortex with double-barreled K^+-sensitive microelectrodes (Fig. 5A). Before and after each *in vivo* measurement, K^+ calibration curves were measured (Fig. 5B). Stimulation produced an amplitude-dependent rise in $[K^+]_o$ that rapidly returned to baseline over a few seconds (Fig. 5C). The kinetics of K^+ rise and decay were quite different in $AQP4^{-/-}$ mice; in particular, there was a markedly slower rise and decay time for post-stimulus changes in $[K^+]_o$ in the $AQP4^{-/-}$ mice (Fig. 5D).

These data indicate a deficit in extracellular K^+ clearance in $AQP4^{-/-}$ mice. Neither baseline $[K^+]_o$ nor the 'Lux-Heinemann ceiling' level of activity-induced physiological $[K^+]_o$ elevation (~12 mM) (Heinemann and Lux, 1977; Somjen, 2002) were altered in AQP4 deficiency, indicating

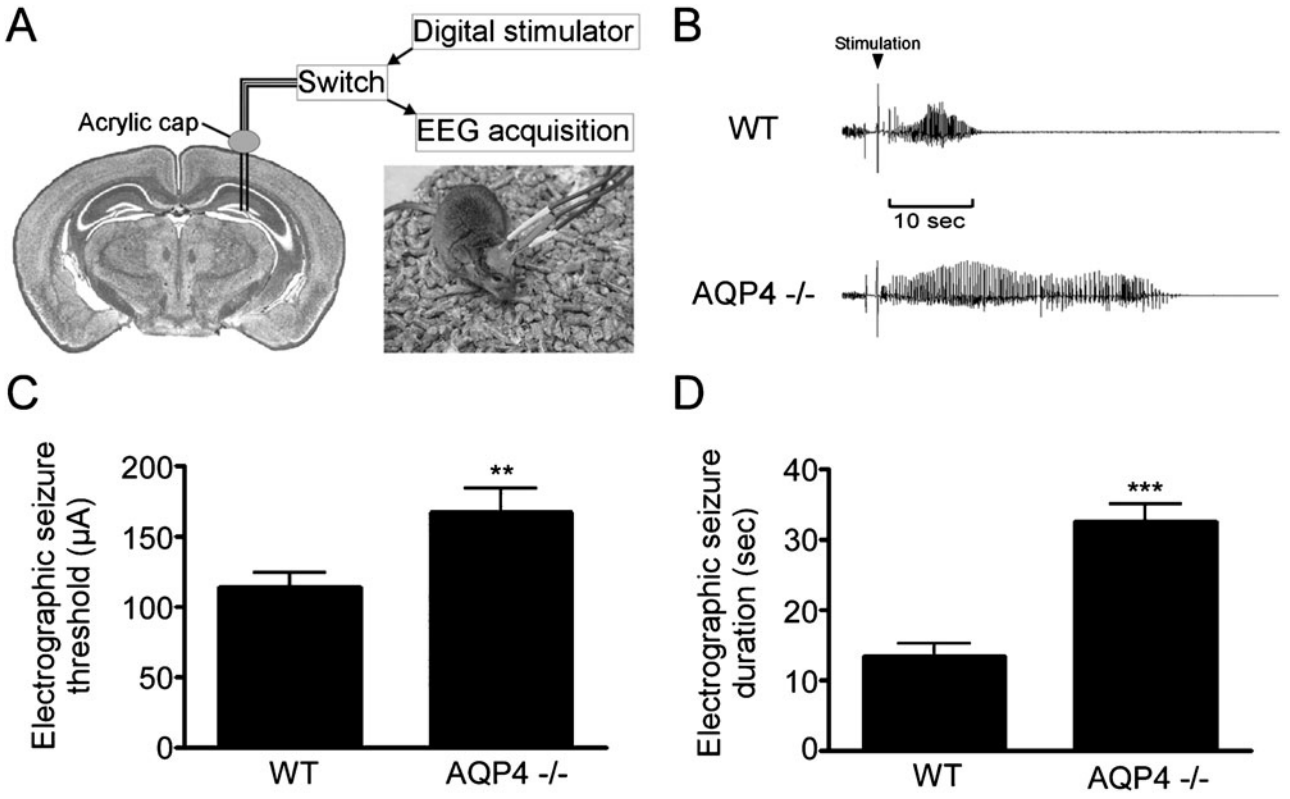

Fig. 4. Electrographic seizure threshold and duration in wild-type and AQP4$^{-/-}$ mice. (A) Bipolar electrodes implanted in the right hippocampus were connected to a stimulator and digital EEG acquisition system. Mice were awake and behaving normally at the onset of stimulation (inset). (B) Representative electroencephalograms from wild-type and AQP4$^{-/-}$ mice. The baseline EEG before stimulation is similar (left). Hippocampal stimulation-induced electrographic seizures are shown for a wild-type mouse (top) and an AQP4$^{-/-}$ mouse (bottom). The wild-type mouse had an 11-sec seizure, whereas the AQP4$^{-/-}$ mouse had a much longer seizure (37 secs). Behavioral arrest was observed in both animals during the seizure. Post-ictal depression is evident on the EEG in both cases. (C) Electrographic seizure threshold (μA) (mean ± SEM) in WT *vs.* AQP4$^{-/-}$ mice. AQP4$^{-/-}$ mice had a higher electrographic seizure threshold than wild-type controls. (D) Electrographic seizure duration (sec) (mean ± SEM) following hippocampal stimulation in wild-type and AQP4$^{-/-}$ mice. AQP4$^{-/-}$ mice had longer stimulation-evoked seizures compared to wild-type controls. From Binder *et al.* (2006).

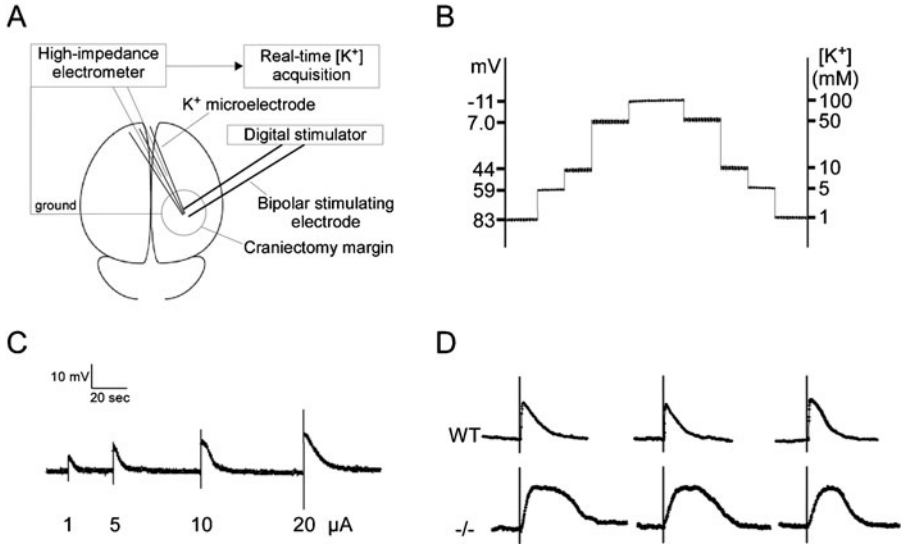

Fig. 5. *In vivo* K$^+$ measurements in wild-type and AQP4$^{-/-}$ mice. (A) Double-barreled, K$^+$-selective microelectrodes were introduced through a small craniectomy and connected to a high-impedance electrometer for real-time [K$^+$] acquisition. A bipolar electrode connected to a digital stimulator was positioned to provide direct current stimulation of the cortex at varying intensities. (B) Sample calibration curve for a K$^+$-sensitive microelectrode placed in serial K$^+$ standard solutions. (C) Representative family of K$^+$ curves in a wild-type mouse following stimulation intensities of 1, 5, 10 and 20 μA. (D) Representative 20-μA stimulation curves from three wild-type mice (top) and three AQP4$^{-/-}$ mice (bottom). Calibration in C applies to D. From Binder *et al.* (2006).

that basic K^+ homeostasis was intact. However, there was a distinction in kinetics, with marked delay in rise and decay time. A similar delay in K^+ kinetics was observed following cortical spreading depression in $AQP4^{-/-}$ mice using a fluorescent K^+ sensor (Padmawar *et al.*, 2005). Slowed $[K^+]_o$ rise time is consistent with increased ECS volume fraction in $AQP4^{-/-}$ mice (Binder *et al.*, 2004b). Slowed $[K^+]_o$ decay is possibly caused by impaired K^+ reuptake into $AQP4^{-/-}$ astrocytes. We found no alteration in expression of $K_{ir}4.1$ protein in $AQP4^{-/-}$ mice (Binder *et al.*, 2006), but their colocalization and subcellular distribution in the hippocampus remain to be characterized. Interestingly, recent investigation of this possibility *in vitro* demonstrated no alterations in membrane potential, barium-sensitive $K_{ir}4.1$ K^+ current or current-voltage curves in $AQP4^{-/-}$ retinal Müller cells (Ruiz-Ederra *et al.*, 2007) or brain astrocytes (Zhang and Verkman, 2008). Lack of alteration of K^+ channels in $AQP4^{-/-}$ mice indicates that the slowed $[K^+]_o$ decay might be a secondary effect of slowed water extrusion ('deswelling') following stimulation, but this has not been demonstrated directly.

Mice deficient in α-syntrophin, an adapter protein in the dystrophin-containing protein complex (Neely *et al.*, 2001), manifest altered subcellular distribution of AQP4, and afferent stimulation of hippocampal slices from α-syntrophin-deficient mice also demonstrated a deficit in extracellular K^+ clearance following evoked neuronal activity (Amiry-Moghaddam *et al.*, 2003b). Together with our findings, these data indicate that AQP4 and its molecular partners together comprise a multifunctional 'unit' responsible for clearance of K^+ and/or H_2O following neural activity.

Water and K^+ dysregulation in seizure susceptibility and in human epilepsy

Modulation of water and K^+ homeostasis by AQP4 might dramatically affect seizure susceptibility. First, brain tissue excitability is exquisitely sensitive to osmolarity and the size of the ECS (Schwartzkroin *et al.*, 1998). Decreasing ECS volume produces hyperexcitability and enhanced epileptiform activity (Dudek *et al.*, 1990; Roper *et al.*, 1992; Chebabo *et al.*, 1995; Pan and Stringer, 1996); conversely, increasing ECS volume with hyperosmolar medium attenuates epileptiform activity (Traynelis and Dingledine, 1989; Dudek *et al.*, 1990; Pan and Stringer, 1996; Haglund and Hochman, 2005). These experimental data parallel extensive clinical experience indicating that hypo-osmolar states such as hyponatremia lower seizure threshold whereas hyperosmolar states elevate seizure threshold (Andrew *et al.*, 1989). Second, millimolar and even submillimolar increases in extracellular K^+ concentration powerfully enhance epileptiform activity in the hippocampus (Rutecki *et al.*, 1985; Yaari *et al.*, 1986; Traynelis and Dingledine, 1988; Feng and Durand, 2006). High-K^+ reliably induces epileptiform activity in hippocampal slices from humans with intractable temporal lobe epilepsy (Gabriel *et al.*, 2004).

The most common pathology in patients with medically-intractable temporal lobe epilepsy is mesial temporal sclerosis (MTS), which is characterized by marked neuronal cell loss in specific hippocampal areas, gliosis, and microvascular proliferation (Blümcke *et al.*, 1999). Emerging work also demonstrates dysregulation of water and K^+ homeostasis in patients with MTS. First, imaging studies demonstrate abnormal T2 prolongation by MRI in the epileptic hippocampus, thought to be due to increased water content (Mitchell *et al.*, 1999). This is accompanied by alterations in apparent diffusion coefficient (ADC) with diffusion-weighted MRI imaging (Hugg *et al.*, 1999). Second, the expression and subcellular localization of AQP4 have been shown recently to be altered in sclerotic hippocampi obtained from patients with MTS. Using immunohistochemistry, rt-PCR and gene chip analysis, Lee *et al.* demonstrated an overall increase in AQP4 expression in sclerotic epilepsy tissue (Lee *et al.*, 2004). However, using quantitative immunogold electron microscopy, the same group found that there is mislocalization of AQP4 in the human epileptic hippocampus, with reduction in perivascular membrane expression (Eid *et al.*, 2005). Thus, although there is an overall increase in AQP4 content by Western blot, rt-PCR and gene chip analysis, the subcellular distribution of AQP4 in MTS tissue is changed. Their hypothesis is that reduction in perivascular AQP4 expression might lead to water and K^+ dysregulation in the epileptic hippocampus, potentially contributing to hyperexcitability. This provocative finding needs to be replicated in further human epilepsy tissue samples.

Dysfunction of astroglial K_{ir} channels has also been found in specimens from patients with temporal lobe epilepsy (Heinemann *et al.*, 2000; Steinhäuser and Seifert, 2002). First, using ion-sensitive microelectrodes, Kivi *et al.* demonstrated an impairment of glial barium-sensitive K^+ uptake in the CA1 region of MTS specimens (Kivi *et al.*, 2000). Secondly, using patch-clamp analysis, Hinterkeuser *et al.* demonstrated a dramatic reduction in astroglial K_{ir} currents in MTS tissue (Hinterkeuser *et al.*, 2000). Reduction in K_{ir} currents is expected to contribute to hyperexcitability; excellent recent evidence for this comes from the finding of stress-induced seizures in conditional $K_{ir}4.1^{-/-}$ mice from Ken McCarthy's laboratory (Djukic *et al.*, 2007).

Disruption of the dystrophin-associated protein complex

A key potential mechanism for altered subcellular distribution of AQP4 during epileptogenesis is disruption of the dystrophin-associated protein complex (DAPC), which has an essential role in anchoring of AQP4 at the perivascular astrocyte membrane. First, in dystrophin-null transgenic (*mdx*) mice, immunofluorescence and immunoelectron microscopic analyses revealed a dramatic reduction of AQP4 in astroglial endfeet surrounding capillaries and at the glia limitans (cerebrospinal fluid-brain interface) despite no alteration in total AQP4 protein (Vajda *et al.*, 2002). Secondly, mice deficient in α-syntrophin, an adapter protein associated with dystrophin (Neely *et al.*, 2001), show marked loss of AQP4 from perivascular and subpial membranes as judged by quantitative immunogold electron microscopy (Amiry-Moghaddam *et al.*, 2003a). These studies indicate that alterations in components of the DAPC might secondarily affect the subcellular targeting and function of AQP4. In this regard, α-syntrophin-deficient mice also demonstrated a deficit in extracellular K^+ clearance following evoked neuronal activity (Amiry-Moghaddam *et al.*, 2003b). A recent study also demonstrates that dystrophin localization

at the astrocytic endfoot is dependent on syntrophin (Bragg *et al.*, 2006).

What are the functional consequences of loss of dystrophin or syntrophin on seizure susceptibility? Dystrophin-null (*mdx*) mice have altered seizure susceptibility in response to various chemical convulsants; in particular, *mdx* mice show enhanced seizure severity and a shorter latency in the development of chemical kindling produced by administration of PTZ (De Sarro *et al.*, 2004). Mice deficient in α-syntrophin were found to have an increased intensity of hyperthermia-evoked seizures (Amiry-Moghaddam *et al.*, 2003b). It is interesting to note that there is an increased incidence of epilepsy in forms of human muscular dystrophy in which this complex is affected (Tsao and Mendell, 2006).

Disruption of the DAPC has also been demonstrated in human epileptic tissue. In the study in which reduced perivascular membrane expression of AQP4 was noted (Eid *et al.*, 2005), the authors also studied perivascular dystrophin expression. Like AQP4 expression, perivascular dystrophin expression is reduced in tissue from the sclerotic epileptic hippocampus (Eid *et al.*, 2005). Thus, subcellular alteration in AQP4 expression might result from alterations in dystrophin and/or other members of the DAPC.

Methodological issues

When assessing the contribution of AQP4 and its molecular partners to epileptogenesis it is necessary to examine the methodology in the reported studies. First, studies of human tissue represent the 'endpoint' of an already long-standing epileptogenic process. A sclerotic hippocampus with significant neuronal cell loss and gliosis might show many molecular changes in glial cells including the reported altered distribution of AQP4 (Lee *et al.*, 2004; Eid *et al.*, 2005), but what is unclear from these studies is whether dysregulation of AQP4 represents a cause or consequence of epileptogenesis. Appropriate animal models of epilepsy permit dissection of the process of epileptogenesis in greater detail. In particular, in such models it is possible to examine the transition from a normal to an epileptic brain as assessed by *in vivo* electrophysiological recordings of spontaneous seizures following an initial epileptogenic insult. Description of the molecular changes in glial cells during epileptogenesis is still in its infancy (Binder and Steinhäuser, 2006), but will be facilitated by studies in such *in vivo* models. Unlike *in vivo* models, studies with *ex vivo* slices enable the delineation of tissue and synaptic physiology in greater detail (Amiry-Moghaddam *et al.*, 2003b) but at the expense of isolating only a part of the *in vivo* network. Thus, a fruitful combination of methodologies for future study will include *in vivo* electrophysiological recording for validation of epileptogenesis, *ex vivo* slice physiology, and examination of the cellular and molecular changes in glial cells leading up to the development of spontaneous seizures.

Unanswered questions

The studies discussed above indicate novel roles for AQP4 in the control of seizure susceptibility (Binder *et al.*, 2004a; Binder *et al.*, 2006), K^+ homeostasis (Padmawar *et al.*, 2005; Binder *et al.*, 2006) and ECS physiology (Binder *et al.*, 2004b). These findings, together with the changes in human epileptic tissue (Lee *et al.*, 2004; Eid *et al.*, 2005), lead to the unifying hypothesis that AQP4 and its molecular partners might have a functional role in epilepsy (Dudek and Rogawski, 2005). In this context, and based on the recent burgeoning literature on glial–neuronal interactions and gliovascular interactions, several fascinating unanswered questions arise including:

- What is the expression pattern of AQP4 within the hippocampus, a structure that is crucial to epileptogenesis? Early studies have demonstrated the perivascular localization of AQP4 (Nielsen *et al.*, 1997; Badaut *et al.*, 2000; Badaut *et al.*, 2002; Nagelhus *et al.*, 2004) but did not examine the region-specific expression of AQP4 in the brain in detail.
- What are the cellular/molecular events that cause AQP4 upregulation during epileptogenesis? Seizure activity is accompanied by a variety of changes in gene expression, and either repeated or prolonged seizure activity leads to glial changes, ultimately including the proliferation of 'reactive' astrocytes (gliosis). Previous studies indicate upregulation and altered distribution of AQP4 protein in sclerotic tissue resected from patients with MTS (Lee *et al.*, 2004; Eid *et al.*, 2005). However, it is unclear whether AQP4 is upregulated by seizure activity *per se* and/or by other processes occurring during epileptogenesis (development of epilepsy), such as cell death, inflammation and gliosis (Vezzani and Granata, 2005). AQP4 is upregulated dramatically in reactive astrocytes following injury (Vizuete *et al.*, 1999; Saadoun *et al.*, 2002; Badaut *et al.*, 2003) but might also be regulated by physiological stimuli (Saito *et al.*, 2005). To date, no studies of seizure regulation of AQP4 have been reported in animal models. Distinct epilepsy models in which epileptogenesis occurs in the absence of detectable cell death would be useful in determining the threshold for AQP4 regulation (Bender *et al.*, 2004; Dubé *et al.*, 2007).
- What is the time course of AQP4 regulation? Whether AQP4 regulation either precedes or follows epileptogenesis impacts its functional role as cause or consequence. To address this question requires concurrent video–EEG monitoring during epileptogenesis to determine the exact time of onset of spontaneous seizures.
- What is the cell type-specificity of AQP4 expression and regulation? Colocalization with glial markers will be crucial to determine cell type-specificity. The description of novel classes of hippocampal glial cells (GluT and GluR cells) with different morphology and functional properties (Matthias *et al.*, 2003; Wallraff *et al.*, 2004; Jabs *et al.*, 2005; Nishiyama *et al.*, 2005) makes it particularly important to clearly identify which 'glial cell' expresses AQP4. Whereas AQP4 is thought to be expressed predominantly by 'classical' astrocytes (GluT cells), some have reported expression in other cell types, such as microglia (Tomas-Camardiel *et al.*, 2004).
- How does the regulation of AQP4 relate to angiogenesis during epileptogenesis? It is known that epileptic tissue contains many more microvessels, probably caused partly by upregulation of vascular endothelial growth factor (VEGF) following seizures (Croll *et al.*, 2004; Nicoletti *et al.*, 2008; Rigau *et al.*, 2007). Recently, administration of VEGF itself has been shown to upregulate AQP4 (Rite

et al., 2007), and it will be interesting to study whether AQP4 distribution and/or function may be unique in 'epileptic vasculature'. In this regard, recent studies convincingly demonstrate astrocyte control of cerebrovascular tone (Gordon *et al.*, 2007).

- In a related question, what is the subcellular distribution of AQP4 at the astroglial endfeet following epileptogenesis and how might subcellular redistribution of AQP4 affect water and K^+ homeostasis in the epileptic brain? Might either improved targeting or localization of AQP4 be an effective therapy in an epileptic brain? A recent study shows that the extracellular matrix protein agrin might be a crucial regulator of AQP4 membrane trafficking and polarity (Noell *et al.*, 2007).
- Seizure regulation of AQP4 expression does not prove pathophysiological significance. Comparing the rate of epileptogenesis in wild-type and $AQP4^{-/-}$ mice with quantitative, *in vivo,* real-time video-EEG recording would help to provide functional evidence for a role of AQP4 as either pro- or anti-epileptogenic. If fruitful, these experiments might lead to the concept of AQP modulation as a novel antiepileptic strategy. As pharmacological activators/inhibitors of AQP4 become available they can be tested in relevant animal models.

CONCLUSIONS

Compelling evidence indicates that the glial water channel AQP4 has a fundamental role in water transport in the brain. AQP4 is expressed in astrocytes and, along with the inwardly-rectifying K^+ channel $K_{ir}4.1$, is thought to underlie the reuptake of H_2O and K^+ into glial cells during neural activity. Because osmolarity and K^+ have powerful effects on seizure susceptibility, AQP4 and its molecular partners might represent novel therapeutic targets for the control of seizures. Transgenic mice that lack AQP4 have significantly prolonged seizure duration, which is associated with a deficit in extracellular K^+ clearance. The available human studies indicate dysfunctional K^+ homeostasis in human epileptic tissue, and upregulation and altered subcellular distribution of AQP4. The relevance of these findings to hippocampal epileptogenesis and to human epilepsy requires further study. Restoration of water and K^+ homeostasis in epileptic tissue constitutes a novel therapeutic concept. AQP4 occupies a pivotal position: concentrated in both astrocytic endfeet and in astroglial processes investing synapses, it might be involved in glial–neuronal interaction of water and ion fluxes and in glial–vascular interactions across the blood–brain barrier. Thus, together with its molecular partners, it might havea crucial role both during active tissue metabolism and in pathophysiological states.

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STATEMENT OF INTEREST

None.

REFERENCES

Agre P., King L.S., Yasui M., Guggino W.B., Ottersen O.P., Fujiyoshi Y. *et al.* (2002) Aquaporin water channels – from atomic structure to clinical medicine. *Journal of Physiology* 542, 3–16.

Amiry-Moghaddam M., Otsuka T., Hurn P.D., Traystman R.J., Haug F.M., Froehner S.C. *et al.* (2003a) An alpha-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proceedings of the National Academy of Sciences of the U.S.A.* 100, 2106–2111.

Amiry-Moghaddam M. and Ottersen O.P. (2003) The molecular basis of water transport in the brain. *Nature Reviews Neuroscience* 4, 991–1001.

Amiry-Moghaddam M., Williamson A., Palomba M., Eid T., de Lanerolle N.C., Nagelhus E.A. *et al.* (2003b) Delayed K^+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. *Proceedings of the National Academy of Sciences of the U.S.A.* 100, 13615–13620.

Andrew R.D., Fagan M., Ballyk B.A. and Rosen A.S. (1989) Seizure susceptibility and the osmotic state. *Brain Research* 498, 175–180.

Badaut J., Brunet J.F., Grollimund L., Hamou M.F., Magistretti P.J., Villemure J.G. *et al.* (2003) Aquaporin-1 and aquaporin-4 expression in human brain after subarachnoid hemorrhage and in peritumoral tissue. *Acta Neurochirurgica. Supplement* 86, 495–498.

Badaut J., Lasbennes F., Magistretti P.J. and Regli L. (2002) Aquaporins in brain: distribution, physiology, and pathophysiology. *Journal of Cerebral Blood Flow and Metabolism* 22, 367–378.

Badaut J., Verbavatz J.M., Freund-Mercier M.J. and Lasbennes F. (2000) Presence of aquaporin-4 and muscarinic receptors in astrocytes and ependymal cells in rat brain: a clue to a common function? *Neuroscience Letters* 292, 75–78.

Ballanyi K., Grafe P. and ten Bruggencate G. (1987) Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. *Journal of Physiology* 382, 159–174.

Bender R.A., Dubé C. and Baram T.Z. (2004) Febrile seizures and mechanisms of epileptogenesis: insights from an animal model. *Advances in Experimental Medicine and Biology* 548, 213–225.

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. *Journal of Neuroscience* 24, 8049–8056.

Binder D.K. and Steinhäuser C. (2006) Functional changes in astroglial cells in epilepsy. *Glia* 54, 358–368.

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.

Blümcke I., Beck H., Lie A.A. and Wiestler O.D. (1999) Molecular neuropathology of human mesial temporal lobe epilepsy. *Epilepsy Research* 36, 205–223.

Bragg A.D., Amiry-Moghaddam M., Ottersen O.P., Adams M.E. and Froehner S.C. (2006) Assembly of a perivascular astrocyte protein scaffold at the mammalian blood-brain barrier is dependent on alpha-syntrophin. *Glia* 53, 879–890.

Chebabo S.R., Hester M.A., Aitken P.G. and Somjen G.G. (1995) Hypotonic exposure enhances synaptic transmission and triggers spreading depression in rat hippocampal tissue slices. *Brain Research* 695, 203–216.

Connors N.C., Adams M.E., Froehner S.C. and Kofuji P. (2004) The potassium channel Kir4.1 associates with the dystrophin-glycoprotein complex via alpha-syntrophin in glia. *Journal of Biological Chemistry* 279, 28387–28392.

Croll S.D., Goodman J.H. and Scharfman H.E. (2004) Vascular endothelial growth factor (VEGF) in seizures: a double-edged sword. *Advances in Experimental Medicine and Biology* 548, 57–68.

de Lanerolle N.C. and Lee T.S. (2005) New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. *Epilepsy and Behaviour* 7, 190–203.

De Sarro G., Ibbadu G.F., Marra R., Rotiroti D., Loiacono A., Donato Di Paola E. *et al.* (2004) Seizure susceptibility to various convulsant stimuli in dystrophin-deficient *mdx* mice. *Neuroscience Research* 50, 37–44.

Djukic B., Casper K.B., Philpot B.D., Chin L.S. and McCarthy K.D. (2007) Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. *Journal of Neuroscience* 27, 11354–11365.

Dubé C.M., Brewster A.L., Richichi C., Zha Q. and Baram T.Z. (2007) Fever, febrile seizures and epilepsy. *Trends in Neurosciences* 30, 490–496.

Dudek F.E., Obenhaus A. and Tasker J.G. (1990) Osmolality-induced changes in extracellular volume alter epileptiform bursts independent of chemical synapses in the rat: importance of non-synaptic mechanisms in hippocampal epileptogenesis. *Neuroscience Letters* 120, 267–270.

Dudek F.E. and Rogawski M.A. (2005) Regulation of brain water: is there a role for aquaporins in epilepsy? *Epilepsy Currents* 5, 104–106.

Eid T., Lee T.S., Thomas M.J., Amiry-Moghaddam M., Bjornsen L.P., Spencer D.D. *et al.* (2005) Loss of perivascular aquaporin-4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proceedings of the National Academy of Sciences of the U.S.A.* 102, 1193–1198.

Feng Z. and Durand D.M. (2006) Effects of potassium concentration on firing patterns of low-calcium epileptiform activity in anesthetized rat hippocampus: inducing of persistent spike activity. *Epilepsia* 47, 727–736.

Fiacco T.A., Agulhon C., Taves S.R., Petravicz J., Casper K.B., Dong X. *et al.* (2007) Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. *Neuron* 54, 611–626.

Fields R.D. and Stevens-Graham B. (2002) New insights into neuron-glia communication. *Science* 298, 556–562.

Frigeri A., Gropper M.A., Umenishi F., Kawashima M., Brown D. and Verkman A.S. (1995) Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. *Journal of Cell Science* 108, 2993–3002.

Frigeri A., Nicchia G.P., Nico B., Quondamatteo F., Herken R., Roncali L. *et al.* (2001) Aquaporin-4 deficiency in skeletal muscle and brain of dystrophic *mdx* mice. *FASEB Journal* 15, 90–98.

Gabriel S., Njunting M., Pomper J.K., Merschhemke M., Sanabria E.R., Eilers A. *et al.* (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. *Journal of Neuroscience* 24, 10416–10430.

Gordon G.R., Mulligan S.J. and MacVicar B.A. (2007) Astrocyte control of the cerebrovasculature. *Glia* 55, 1214–1221.

Haglund M.M. and Hochman D.W. (2005) Furosemide and mannitol suppression of epileptic activity in the human brain. *Journal of Neurophysiology* 94, 907–918.

Hasegawa H., Ma T., Skach W., Matthay M.A. and Verkman A.S. (1994) Molecular cloning of a mercurial-insensitive water channel expressed in selected water-transporting tissues. *Journal of Biological Chemistry* 269, 5497–5500.

Heinemann U., Gabriel S., Jauch R., Schulze K., Kivi A., Eilers A. *et al.* (2000) Alterations of glial cell function in temporal lobe epilepsy. *Epilepsia* 41, S185–189.

Heinemann U. and Lux H.D. (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. *Brain Research* 120, 231–249.

Hinterkeuser S., Schroder W., Hager G., Seifert G., Blümcke I., Elger C.E. *et al.* (2000) Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *European Journal of Neuroscience* 12, 2087–2096.

Holthoff K. and Witte O.W. (2000) Directed spatial potassium redistribution in rat neocortex. *Glia* 29, 288–292.

Hugg J.W., Butterworth E.J. and Kuzniecky R.I. (1999) Diffusion mapping applied to mesial temporal lobe epilepsy: preliminary observations. *Neurology* 53, 173–176.

Jabs R., Pivneva T., Huttmann K., Wyczynski A., Nolte C., Kettenmann H. *et al.* (2005) Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. *Journal of Cell Science* 118, 3791–3803.

Jung J.S., Bhat R.V., Preston G.M., Guggino W.B., Baraban J.M. and Agre P. (1994) Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proceedings of the National Academy of Sciences of the U.S.A.* 91, 13052–13056.

Kivi A., Lehmann T.N., Kovacs R., Eilers A., Jauch R., Meencke H.J. *et al.* (2000) Effects of barium on stimulus-induced rises of [K+]o in human epileptic non-sclerotic and sclerotic hippocampal area CA1. *European Journal of Neuroscience* 12, 2039–2048.

Kofuji P., Ceelen P., Zahs K.R., Surbeck L.W., Lester H.A. and Newman E.A. (2000) Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. *Journal of Neuroscience* 20, 5733–5740.

Kofuji P. and Newman E.A. (2004) Potassium buffering in the central nervous system. *Neuroscience* 129, 1045–1056.

Lee T.S., Eid T., Mane S., Kim J.H., Spencer D.D., Ottersen O.P. *et al.* (2004) Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathology (Berlin)* 108, 493–502.

Li J., Patil R.V. and Verkman A.S. (2002) Mildly abnormal retinal function in transgenic mice without Muller cell aquaporin-4 water channels. *Investigative Ophthalmology and Visual Science* 43, 573–579.

Li J. and Verkman A.S. (2001) Impaired hearing in mice lacking aquaporin-4 water channels. *Journal of Biological Chemistry* 276, 31233–31237.

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. *Journal of Clinical Investigation* 100, 957–962.

Manley G.T., Binder D.K., Papadopoulos M.C. and Verkman A.S. (2004) New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. *Neuroscience* 129, 983–991.

Manley G.T., Fujimura M., Ma T., Noshita N., Filiz F., Bollen A.W. *et al.* (2000) Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nature Medicine* 6, 159–163.

Marcus D.C., Wu T., Wangemann P. and Kofuji P. (2002) KCNJ10 (Kir4.1) potassium channel knockout abolishes endocochlear potential. *American Journal of Physiology. Cell Physiology* 282, C403–C407.

Matthias K., Kirchhoff F., Seifert G., Huttmann K., Matyash M., Kettenmann H. *et al.* (2003) Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *Journal of Neuroscience* 23, 1750–1758.

Mitchell L.A., Jackson G.D., Kalnins R.M., Saling M.M., Fitt G.J., Ashpole R.D. *et al.* (1999) Anterior temporal abnormality in temporal lobe epilepsy: a quantitative MRI and histopathologic study. *Neurology* 52, 327–336.

Nagelhus E.A., Mathiisen T.M. and Ottersen O.P. (2004) Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with Kir4.1. *Neuroscience* 129, 905–913.

Neely J.D., Amiry-Moghaddam M., Ottersen O.P., Froehner S.C., Agre P. and Adams M.E. (2001) Syntrophin-dependent expression and localization of aquaporin-4 water channel protein. *Proceedings of the National Academy of Sciences of the U.S.A.* 98, 14108–14113.

Neusch C., Papadopoulos N., Muller M., Maletzki I., Winter S.M., Hirrlinger J. *et al.* (2006) Lack of the Kir4.1 channel subunit abolishes K^+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K^+ regulation. *Journal of Neurophysiology* 95, 1843–1852.

Neusch C., Rozengurt N., Jacobs R.E., Lester H.A. and Kofuji P. (2001) Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. *Journal of Neuroscience* 21, 5429–5438.

Newman E.A. (1986) High potassium conductance in astrocyte endfeet. *Science* 233, 453–454.

Newman E.A. (1993) Inward-rectifying potassium channels in retinal glial (Muller) cells. *Journal of Neuroscience* 13, 3333–3345.

Newman E.A., Frambach D.A. and Odette L.L. (1984) Control of extracellular potassium levels by retinal glial cell K^+ siphoning. *Science* 225, 1174–1175.

Newman E.A. and Karwoski C.J. (1989) Spatial buffering of light-evoked potassium increases by retinal glial (Muller) cells. *Acta Physiologica Scandinavica. Supplementum* 582, 51.

Nicchia G.P., Frigeri A., Liuzzi G.M. and Svelto M. (2003) Inhibition of aquaporin-4 expression in astrocytes by RNAi determines alteration in cell morphology, growth, and water transport and induces changes in ischemia-related genes. *FASEB Journal* 17, 1508–1510.

Nicoletti J.N., Shah S.K., McCloskey D.P., Goodman J.H., Elkady A., Atassi H. *et al.* (2008) Vascular endothelial growth factor is up-regulated after status epilepticus and protects against seizure-induced neuronal loss in hippocampus. *Neuroscience* 151, 232–241.

Nielsen S., Nagelhus E.A., Amiry-Moghaddam M., Bourque C., Agre P. and Ottersen O.P. (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *Journal of Neuroscience* 17, 171–180.

Niermann H., Amiry-Moghaddam M., Holthoff K., Witte O.W. and Ottersen O.P. (2001) A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. *Journal of Neuroscience* 21, 3045–3051.

Nishiyama A., Yang Z. and Butt A. (2005) Astrocytes and NG2-glia: what's in a name? *Journal of Anatomy* 207, 687–693.

Noell S., Fallier-Becker P., Beyer C., Kroger S., Mack A.F. and Wolburg H. (2007) Effects of agrin on the expression and distribution of the water channel protein aquaporin-4 and volume regulation in cultured astrocytes. *European Journal of Neuroscience* 26, 2109–2118.

Oshio K., Binder D.K., Yang B., Schecter S., Verkman A.S. and Manley G.T. (2004) Expression of aquaporin water channels in mouse spinal cord. *Neuroscience* 127, 685–693.

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. *Nature Methods* 2, 825–827.

Pan E. and Stringer J.L. (1996) Influence of osmolality on seizure amplitude and propagation in the rat dentate gyrus. *Neuroscience Letters* 207, 9–12.

Papadopoulos M.C., Binder D.K. and Verkman A.S. (2005) Enhanced macromolecular diffusion in brain extracellular space in mouse models of vasogenic edema measured by cortical surface photobleaching. *FASEB Journal* 19, 425–427.

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 Journal* 18, 1291–1293.

Racine R.J. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalography and Clinical Neurophysiology* 32, 281–294.

Ransom B., Behar T. and Nedergaard M. (2003) New roles for astrocytes (stars at last). *Trends in Neurosciences* 26, 520–522.

Rash J.E., Yasumura T., Hudson C.S., Agre P. and Nielsen S. (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proceedings of the National Academy of Sciences of the U.S.A.* 95, 11981–11986.

Rigau V., Morin M., Rousset M.C., de Bock F., Lebrun A., Coubes P. *et al.* (2007) Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain* 130, 1942–1956.

Rite I., Machado A., Cano J. and Venero J.L. (2007) Intracerebral VEGF injection highly upregulates AQP4 mRNA and protein in the perivascular space and glia limitans externa. *Neurochemistry International* 52, 897–903.

Rogawski M.A. and Loscher W. (2004) The neurobiology of antiepileptic drugs. *Nature Reviews Neuroscience* 5, 553–564.

Roper S.N., Obenhaus A. and Dudek F.E. (1992) Osmolality and nonsynaptic epileptiform bursts in rat CA1 and dentate gyrus. *Annals of Neurology* 31, 81–85.

Rozengurt N., Lopez I., Chiu C.S., Kofuji P., Lester H.A. and Neusch C. (2003) Time course of inner ear degeneration and deafness in mice lacking the Kir4.1 potassium channel subunit. *Hearing Research* 177, 71–80.

Ruiz-Ederra J., Zhang H. and Verkman A.S. (2007) Evidence against functional interaction between aquaporin-4 water channels and Kir4.1 potassium channels in retinal Muller cells. *Journal of Biological Chemistry* 282, 21866–21872.

Rutecki P.A., Lebeda F.J. and Johnston D. (1985) Epileptiform activity induced by changes in extracellular potassium in hippocampus. *Journal of Neurophysiology* 54, 1363–1374.

Saadoun S., Papadopoulos M.C., Davies D.C., Krishna S. and Bell B.A. (2002) Aquaporin-4 expression is increased in oedematous human brain tumours. *Journal of Neurology, Neurosurgery and Psychiatry* 72, 262–265.

Saito N., Ikegami H. and Shimada K. (2005) Effect of water deprivation on aquaporin-4 (AQP4) mRNA expression in chickens (Gallus domesticus). *Brain Research Molecular Brain Research* 141, 193–197.

Schwartzkroin P.A., Baraban S.C. and Hochman D.W. (1998) Osmolarity, ionic flux, and changes in brain excitability. *Epilepsy Research* 32, 275–285.

Seifert G., Schilling K. and Steinhauser C. (2006) Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nature Reviews Neuroscience* 7, 194–206.

Simard M. and Nedergaard M. (2004) The neurobiology of glia in the context of water and ion homeostasis. *Neuroscience* 129, 877–896.

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. *American Journal of Physiology. Cell Physiology* 286, C426–C432.

Solenov E.I., Vetrivel L., Oshio K., Manley G.T. and Verkman A.S. (2002) Optical measurement of swelling and water transport in spinal cord slices from aquaporin null mice. *Journal of Neuroscience Methods* 113, 85–90.

Somjen G.G. (2002) Ion regulation in the brain: implications for pathophysiology. *Neuroscientist* 8, 254–267.

Steinhäuser C. and Seifert G. (2002) Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *European Journal of Pharmacology* 447, 227–237.

Sykova E. (1997) The extracellular space in the CNS: its regulation, volume and geometry in normal and pathological neuronal function. *Neuroscientist* 3, 28–41.

Thiagarajah J.R., Papadopoulos M.C. and Verkman A.S. (2005) Noninvasive early detection of brain edema in mice by near-infrared light scattering. *Journal of Neuroscience Research* 80, 293–299.

Tian G.F., Azmi H., Takano T., Xu Q., Peng W., Lin J. *et al.* (2005) An astrocytic basis of epilepsy. *Nature Medicine* 11, 973–981.

Tomas-Camardiel M., Venero J.L., de Pablos R.M., Rite I., Machado A. and Cano J. (2004) In vivo expression of aquaporin-4 by reactive microglia. *Journal of Neurochemistry* 91, 891–899.

Traynelis S.F. and Dingledine R. (1988) Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *Journal of Neurophysiology* 59, 259–276.

Traynelis S.F. and Dingledine R. (1989) Role of extracellular space in hyperosmotic suppression of potassium-induced electrographic seizures. *Journal of Neurophysiology* 61, 927–938.

Tsao C.Y. and Mendell J.R. (2006) Coexisting muscular dystrophies and epilepsy in children. *Journal of Child Neurology* 21, 148–150.

Vajda Z., Pedersen M., Fuchtbauer E.M., Wertz K., Stodkilde-Jorgensen H., Sulyok E. *et al.* (2002) Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. *Proceedings of the National Academy of Sciences of the U.S.A.* 99, 13131–13136.

Verbavatz J.M., Ma T., Gobin R. and Verkman A.S. (1997) Absence of orthogonal arrays in kidney, brain and muscle from transgenic knockout mice lacking water channel aquaporin-4. *Journal of Cell Science* 110, 2855–2860.

Verkman A.S. (2002) Physiological importance of aquaporin water channels. *Annals of Medicine* 34, 192–200.

Verkman A.S. (2005) More than just water channels: unexpected cellular roles of aquaporins. *Journal of Cell Science* 118, 3225–3232.

Vezzani A. and Granata T. (2005) Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia* 46, 1724–1743.

Vizuete M.L., Venero J.L., Vargas C., Ilundain A.A., Echevarria M., Machado A. *et al.* (1999) Differential upregulation of aquaporin-4 mRNA expression in reactive astrocytes after brain injury: potential role in brain edema. *Neurobiology of Disease* 6, 245–258.

Volterra A. and Meldolesi J. (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nature Reviews Neuroscience* 6, 626–640.

Volterra A. and Steinhäuser C. (2004) Glial modulation of synaptic transmission in the hippocampus. *Glia* 47, 249–257.

Wallraff A., Odermatt B., Willecke K. and Steinhäuser C. (2004) Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia* 48, 36–43.

Walz W. (1987) Swelling and potassium uptake in cultured astrocytes. *Canadian Journal of Physiology and Pharmacology* 65, 1051–1057.

Walz W. (1992) Mechanism of rapid K^+-induced swelling of mouse astrocytes. *Neuroscience Letters* 135, 243–246.

Xiong Z.Q. and Stringer J.L. (1999) Astrocytic regulation of the recovery of extracellular potassium after seizures in vivo. *European Journal of Neuroscience* 11, 1677–1684.

Yaari Y., Konnerth A. and Heinemann U. (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. II. Role of extracellular potassium. *Journal of Neurophysiology* 56, 424–438.

Yang B., Brown D. and Verkman A.S. (1996) The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. *Journal of Biological Chemistry* 271, 4577–4580.

Yang B., Ma T. and Verkman A.S. (1995) cDNA cloning, gene organization, and chromosomal localization of a human mercurial insensitive water channel. Evidence for distinct transcriptional units. *Journal of Biological Chemistry* 270, 22907–22913.

Zhang H. and Verkman A.S. (2008) Aquaporin-4 independent Kir4.1 K^+ channel function in brain glial cells. *Molecular and Cellular Neuroscience* 37, 1–10.

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