Increased seizure threshold in mice lacking aquaporin-4 water channels

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Mice deficient in the glial water channel aquaporin-4 (AQP4) show decreased cerebral edema and improved neurological outcome following water intoxication or ischemic challenge. In this report, we tested seizure susceptibility in AQP4$^{-/-}$ mice. AQP4$^{-/-}$ mice and wild-type controls were given the chemoconvulsant pentylenetetrazol (PTZ) and monitored for seizure activity. At 40 mg/kg PTZ, all wild-type mice exhibited seizure activity, whereas six of seven AQP4$^{-/-}$ mice did not exhibit seizure activity. At 50 mg/kg PTZ, both groups exhibited seizure activity; however, the latency to generalized (tonic-clonic) seizures was significantly lower in wild-type than AQP4$^{-/-}$ mice. These results suggest that glial water channels may modulate brain excitability and the initiation and generalization of seizure activity. *NeuroReport* 15:259–262 © 2004 Lippincott Williams & Wilkins.

Key words: Aquaporin-4; Brain edema; Epilepsy; Glial cells; Water transport

INTRODUCTION

Seizure activity leads to cerebral edema *in vivo*, and regulation of cell volume and swelling have been implicated in seizure propagation *in vitro*. Decreasing extracellular space (ECS) volume by exposure of tissue slices to hypotonic bathing solutions produces hyperexcitability and enhanced epileptiform activity [1–5]. Conversely, hyperosmolar medium attenuates epileptiform activity in hippocampal slices [3,5,6]. Furosemide, a chloride cotransport inhibitor that blocks seizure-induced cell swelling, inhibits epileptiform activity *in vitro* and *in vivo* [7,8]. These experimental data parallel extensive clinical experience indicating that hypo-osmolar states such as hyponatremia lower seizure threshold while hyperosmolar states elevate seizure threshold [9]. Thus, brain tissue excitability appears to be exquisitely sensitive to osmolarity and the size of the extracellular space.

Because of the importance of cell swelling and water transport in determining ECS volume, we hypothesized that water transport facilitated by glial aquaporin channels may modulate intrinsic brain excitability. 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, such as the kidney, secretory glands, gastrointestinal tract, lung, and brain [10,11]. Aquaporin-4 (AQP4) is expressed widely throughout the brain, particularly at brain-blood and brain-cerebrospinal fluid (CSF) interfaces where it is thought to play a role in edema formation and CSF absorption. AQP4 is abundantly expressed by glial cells lining the ependymal and pial surfaces that are in contact with CSF in the ventricular system and subarachnoid space [12]. Highly polarized AQP4 expression is also found in astrocytic foot processes near or in direct contact with blood vessels [12]. Recently, we found that mice deficient in AQP4 (AQP4$^{-/-}$) had decreased cerebral edema and improved neurological outcome following water intoxication and focal cerebral ischemia [13]. In view of the potential role of AQP4 in mediating water fluxes in response to neuronal activity and perhaps in seizure-induced edema, we examined seizure susceptibility in AQP4$^{-/-}$ mice.

MATERIALS AND METHODS

All animal procedures were performed with an approved protocol from the UCSF Committee on Animal Research (CAR).

AQP4$^{-/-}$ mice: AQP4$^{-/-}$ mice in a CD1 genetic background were generated as described previously [14]. These mice lack detectable AQP4 protein by immunoblot and immunocytochemical analysis, and phenotypically have normal growth, development, survival, and neuromuscular function except for a mild defect in maximal urinary concentrating ability produced by decreased water permeability in the inner medullary collecting duct.

Immunohistochemistry: An affinity-purified polyclonal rabbit anti-rat AQP4 antibody (Chemicon, Temecula, CA) was used at 1:500. Briefly, mice were anesthetized with 2,2,2-tribromoethanol (0.5 mg/g, i.p.), perfused with 2% paraformaldehyde (pH 7.4) in phosphate-buffered saline (PBS) and sacrificed. Brains were dissected, fixed in perfusion buffer for 6 h, cryoprotected in 20% sucrose/PBS, frozen and

14 μm coronal sections were cut on a cryostat and slide-mounted. Endogenous peroxidase activity was quenched with 3% H_2O_2/methanol for 10 min, and slides were blocked in 5% normal goat serum (Vector, Burlingame, CA) and incubated in primary antibody (1:500) overnight at 4°C. After extensive washing, slides were incubated in 1:500 biotinylated goat anti-rabbit IgG (Vector, Burlingame, CA) for 1 h followed by 1:100 ABC reagent (Vector, Burlingame, CA), developed with diaminobenzidine (Sigma, Saint Louis, MO), counter-stained with hematoxylin (Biomeda, Foster City, CA), dehydrated and cover-slipped.

PTZ injections and seizure observations: AQP4$^{-/-}$ mice or wild-type controls (+/+) were given the chemoconvulsant pentylenetetrazol (PTZ, Sigma, USA) at one of two doses (40 mg/kg or 50 mg/kg, i.p.) and monitored for seizure activity by an observer blinded to genotype. After an initial period of immobility or normal activity post-injection, generalized seizures manifested as the development of forelimb and hindlimb clonus occasionally accompanied by rearing and falling. Latency of generalized seizures were recorded. The significance of differences in means between wild-type controls and AQP4$^{-/-}$ mice was assessed by two-tailed *t*-test.

Electrode implantation and EEG recording: AQP4$^{-/-}$ mice ($n = 2$) and wild-type littermates ($n = 2$) were anesthetized with 2,2,2,-tribromoethanol (125 mg/kg, i.p.) and placed in a standard mouse stereotaxic frame. Bipolar electrodes made from Teflon-coated stainless steel wire were implanted in the right somatosensory cortex (bregma as reference: 0.5 mm posterior; 2.7 mm lateral; 1.0 mm below dura) by standard techniques [15]. Mice were then allowed to recover for 2 days prior to PTZ administration. Electroencephalographic (EEG) activity was monitored continuously before and after PTZ administration via a digital signal acquisition system (Biopac Inc.).

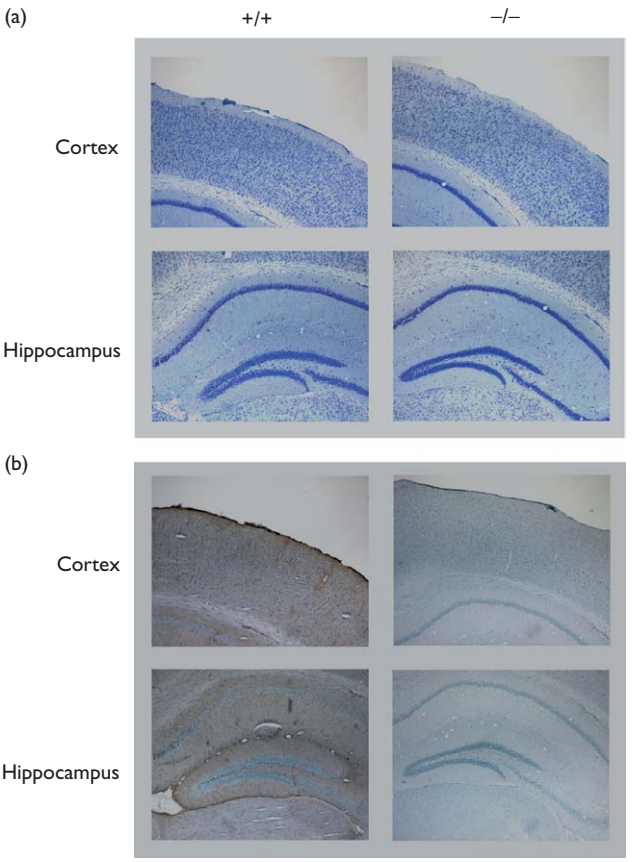

Fig. 1. Histological and immunohistochemical analysis of wild-type (+/+) and AQP4 null (AQP4$^{-/-}$) mice. (**a**) Nissl stain of coronal section of cortex and dorsal hippocampus. (**b**) AQP4 immunohistochemistry. Immunoreactivity was seen in +/+ mice around cortical vessels, ependymal cells, and the glia limitans at the pial border. Sections are counterstained with hematoxylin.

RESULTS

No gross or cellular morphological differences between AQP4$^{-/-}$ and wild-type littermates were seen in hippocampus or cortex by Nissl stain (Fig. 1a). Immunohistochemical analysis in wild-type mice using an affinity-purified polyclonal antibody demonstrated intense AQP4 immunoreactivity around parenchymal vessels, ventricular ependyma, glia limitans, and diffuse immunoreactivity in neuropil (Fig. 1b), similar to that reported in rats [12]. AQP4 immunoreactivity was absent in the AQP4$^{-/-}$ mice (Fig. 1b).

To examine seizure susceptibility, the chemoconvulsant pentylenetetrazol (PTZ, 40 or 50 mg/kg, i.p.) was administered to AQP4$^{-/-}$ and wild-type mice, and the latencies to generalized seizure activity were recorded. At 40 mg/kg PTZ, all of six wild-type mice exhibited seizure activity, whereas six of seven mice deficient in AQP4 did not exhibit any seizure activity (Fig. 2a). At 50 mg/kg PTZ, both groups exhibited seizure activity; however, the latency to generalized tonic-clonic seizures was significantly lower in wild-type mice (190 ± 24 s, $n = 9$) than AQP4$^{-/-}$ mice (298 ± 21 s, $n = 9$, $p < 0.02$; Fig. 2a). To confirm that the behavioral seizures were associated with electroencephalographic seizure activity, a subset of mice were prepared with indwelling cortical electrodes for seizure recording. Baseline EEG in AQP4$^{-/-}$ mice was indistinguishable from that of PTZ wild-type littermates (data not shown). Following PTZ administration, in all cases behavioral manifestations of generalized seizures were accompanied by electroencephalographic seizure activity (Fig. 2b). Aside from the differences in seizure threshold and latency as described above, the electrographic seizures were similar in wild-type and AQP4$^{-/-}$ mice. Seizure termination was accompanied by post-ictal depression on EEG in both wild-type and AQP4$^{-/-}$ mice (Fig. 2b).

DISCUSSION

These results demonstrate that absence of AQP4 increases seizure threshold and latency to generalized seizure. This is the first evidence for the involvement of glial aquaporin water channels and water transport in modulation of brain excitability.

There is increasing evidence that water movement in the brain involves aquaporin channels. AQP4 is expressed ubiquitously by glial cells throughout the brain, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels [12]. Activity-induced radial water fluxes in neocortex have been demonstrated

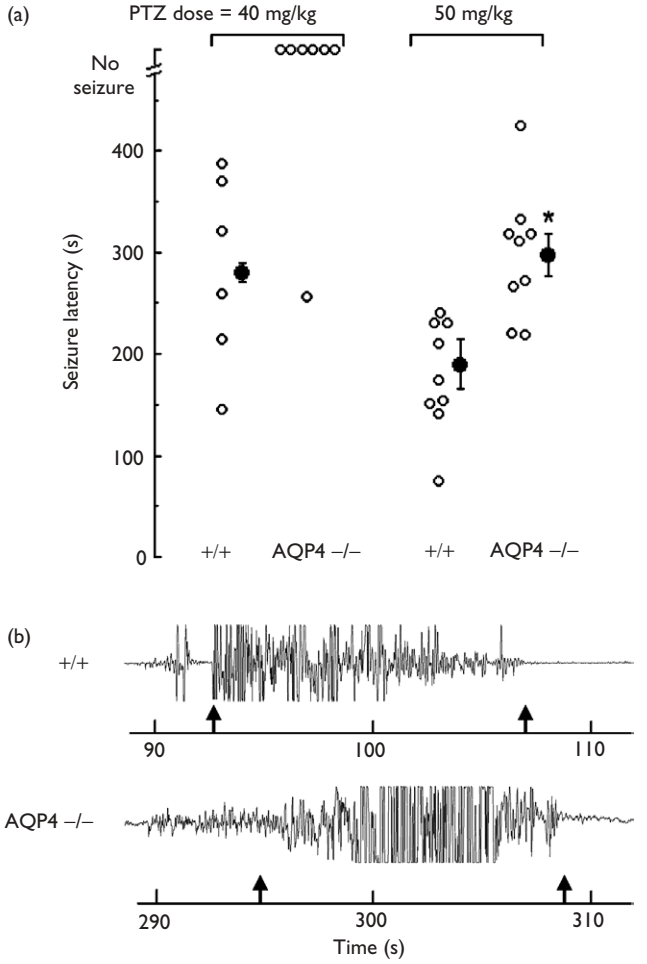

Fig. 2. Reduced seizure susceptibility of AQP4$^{-/-}$ mice. (**a**) Latency to generalized seizure in wild-type (+/+) and AQP4$^{-/-}$ mice at 40 and 50 mg/kg PTZ. Open circles represent individual mice; filled circles are mean ± s.e.m. *$p < 0.05$. (**b**) Representative electroencephalographic recordings. Generalized seizures in a wild-type mouse beginning 92 s after injection and in an AQP4$^{-/-}$ mouse beginning 295 s after injection are shown. Arrows denote onset and end of observed behavioral seizure activity.

that could be associated with water movement via aquaporin channels in response to physiological activity [16]. A recent model for the role of AQP4 comes from study of mice deficient in the gene dystrophin; in these mice there is mislocalization of the AQP4 protein, with a dramatic reduction of AQP4 in astroglial end-feet surrounding capillaries [17,18]. Dystrophin-deficient mice, like the AQP4$^{-/-}$ mice [13], demonstrate delayed brain edema in response to water intoxication [18]. Deletion of α-syntrophin, an adapter protein in the dystrophin-containing protein complex required for anchoring AQP4 to specialized membrane domains [19], also leads to attenuated brain edema in response to transient cerebral ischemia [20].

Although these studies support a role of AQP4 in brain water fluxes, they do not establish a mechanistic basis for the observation of decreased seizure susceptibility in AQP4$^{-/-}$ mice. Since brain tissue excitability is very sensitive to ECS volume, AQP4 deletion may alter ECS volume or composition at baseline and/or following neuronal activity. A larger ECS volume fraction prior to seizure-inducing stimuli and/or a blunted reduction in ECS volume during neuronal activity via abrogation of water influx through glial AQP4 may limit neuronal excitability and synchrony. Altered glial/neuronal water and K^+ recycling in response to neuronal activity has already been proposed to underlie impaired hearing in AQP4$^{-/-}$ mice [21]. Definitive elucidation of the role of AQP4 in modulating brain excitability will require determination of extracellular space volume, composition, and dynamics.

CONCLUSION

The increased seizure threshold and latency to generalized seizure in AQP4$^{-/-}$ mice suggests that water movement via glial aquaporin channels may modulate intrinsic brain excitability. These data add to a growing body of evidence implicating nonsynaptic mechanisms in seizure expression and propagation [22]. Inhibition or modulation of AQP4 by specific pharmacological agents may represent a novel anticonvulsant target.

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