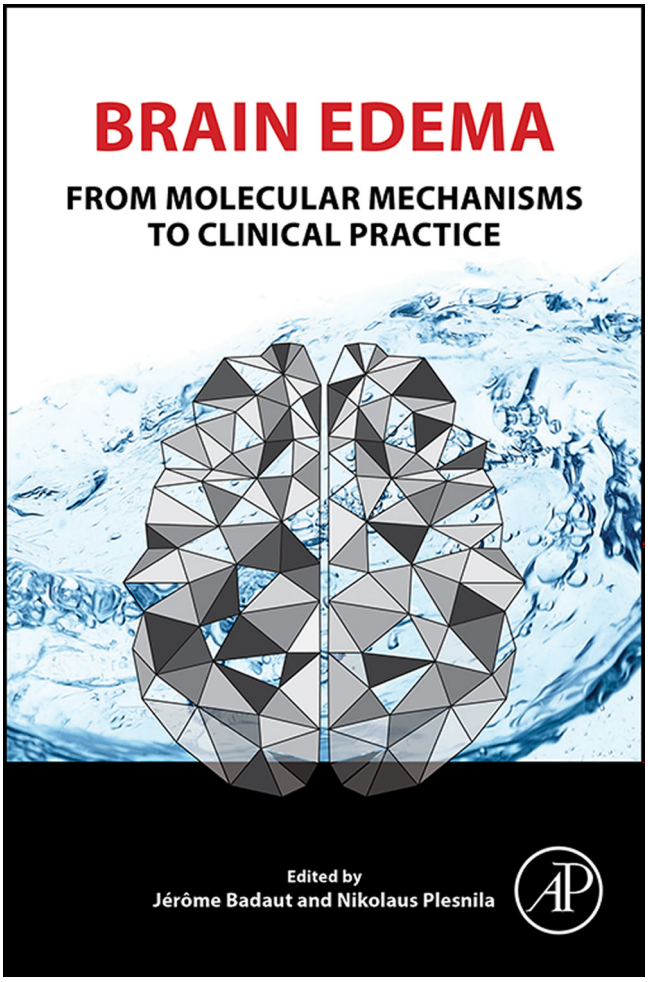

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CHAPTER

17

Water Homeostasis Dysfunction in Epilepsy

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OUTLINE

WATER AND ION HOMEOSTASIS AND EPILEPSY

Glial cells are involved in many important physiologic functions, such as sequestration and/or redistribution of K^+ during neural activity, neurotransmitter cycling, and provision of energy substrates to neurons.[1] Several recent lines of evidence strongly suggest that changes in glial cells potentially contribute to epilepsy.[2–4] First, many studies now link

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glial cells to modulation of synaptic transmission.[5–9] Second, functional alterations of specific glial membrane channels and receptors have been discovered in epileptic tissue.[10–15] Third, direct stimulation of astrocytes has been shown to be sufficient for neuronal synchronization in epilepsy models[9] (although see Fiacco et al., 2007[16]). Thus, if the cellular and molecular mechanisms by which glial cells (especially astrocytes) modulate excitability are better understood, specific antiepileptic therapies based on modulation of glial receptors and channels can be contemplated.[17] It is likely that therapies directed to glial cells would have fewer deleterious side effects than current therapies targeting neurons.

Alteration of water and K^+ homeostasis could dramatically affect seizure susceptibility. First, brain tissue excitability is exquisitely sensitive to osmolarity and the size of the extracellular space (ECS).[18] Decreasing ECS volume with hypoosmolar treatment produces hyperexcitability and enhanced epileptiform activity.[19–22] At least two mechanisms could be operative: decreasing ECS volume could increase extracellular neurotransmitter and ion concentrations and magnify interactions among neurons due to increased ECS resistance.[3] Conversely, increasing ECS volume with hyperosmolar treatment powerfully attenuates epileptiform activity.[20,22–24] These experimental data parallel extensive clinical experience indicating that hypoosmolar states such as hyponatremia lower seizure threshold while hyperosmolar states elevate seizure threshold.[25–27] Second, millimolar and even submillimolar increases in extracellular K^+ concentration powerfully enhance epileptiform activity in the hippocampus.[28–31] High-K^+ reliably induces epileptiform activity in hippocampal slices from human patients with intractable temporal lobe epilepsy (TLE).[32]

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.[33–35] The AQPs are small hydrophobic integral membrane proteins (~30 kDa monomer) that facilitate bi-directional water transport in response to osmotic gradients.[36] 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).[35,36]

Aquaporin-4 (AQP4)[37–39] is of particular interest in neuroscience as 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.[40–44] Activity-induced radial water fluxes in neocortex have been demonstrated that may represent water movement via AQP channels in

response to physiological activity.[45,46] Interestingly, AQP4 is a structural component of orthogonal arrays of particles (OAPs) seen in freeze-fracture electron micrographs[47–51] (for details see Chapter 11: Role of Matrix Metalloproteinases in Brain Edema).

AQP4$^{-/-}$ Mice

AQP4$^{-/-}$ mice were originally generated by targeted gene disruption in 1997,[52] and recently a glial conditional deletion of AQP4 has been generated.[53] AQP4$^{-/-}$ 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.[54–56] A mild hearing impairment has been recorded and the electroretinogram is perturbed.[57,58] Mice with AQP4 deletion 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.[52]

Interestingly, more detailed studies of brain tissue from AQP4$^{-/-}$ mice demonstrated reduced osmotic water permeability as measured in isolated membrane vesicles,[52] brain slices,[59] and intact brain.[60] Also, water permeability was sevenfold reduced in primary astrocyte cultures from AQP4$^{-/-}$ mice as measured by a calcein fluorescence quenching method,[61] and similar results were obtained with AQP4 RNAi knockdown experiments in wild-type astrocytes.[62] These data directly demonstrated 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[63] and impaired clearance of brain water in models of vasogenic edema.[56] Clearance of seizure-induced edema may also be AQP4-dependent.[64] Impaired water flux into (in the case of cytotoxic edema) and out of (in the case of vasogenic edema) the brain makes sense based on the bi-directional nature of water flux across the AQP4 membrane channel. The recently generated glial conditional AQP4 knockout mouse line demonstrates a 31% reduction in brain water uptake after systemic hypoosmotic stress.[53] Similarly, mice deficient in dystrophin or α-syntrophin, in which there is mislocalization of the AQP4 protein,[65–67] show attenuated cerebral edema.[66,68]

Altered Extracellular Space in AQP4$^{-/-}$ Mice

The ECS in brain comprises ~20% of brain tissue volume, consisting of a jelly-like matrix in which neurons, glia, and blood vessels are

embedded.[69] 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.[70] The size of the ECS is likely largely controlled by astrocytic mechanisms.[71–73]

The ECS of AQP4$^{-/-}$ mice was assessed using a cortical fluorescence recovery after photobleaching (cFRAP) method to measure the diffusion of fluorescently labeled macromolecules in the cortex.[74] ECS in mouse brain was labeled by exposure of the intact dura to fluorescein-dextrans (M_r 4, 70, and 500 kDa) after craniectomy, and fluorescein-dextran diffusion was detected by fluorescence recovery after laser-induced cortical photobleaching using confocal optics. The cFRAP method was applied to brain edema, seizure initiation, and AQP4 deficiency. In contrast to the slowed diffusion produced by brain edema and seizure activity, ECS diffusion was *faster* in AQP4$^{-/-}$ mice, indicating ECS expansion in AQP4 deficiency.[74] Similar results were obtained with follow-up studies using the TMA^+ method.[75]

Seizure Phenotype of AQP4$^{-/-}$ Mice

Seizure susceptibility of AQP4$^{-/-}$ mice was initially examined using the convulsant ($GABA_A$ antagonist) pentylenetetrazol (PTZ).[55] At 40 mg/kg 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 PTZ, both groups exhibited seizure activity; however, the latency to generalized (tonic-clonic) seizures was longer in AQP4$^{-/-}$ mice.[55] Since seizure propensity is exquisitely sensitive to ECS volume,[18] the expanded ECS in AQP4 deficiency is consistent with the increased seizure threshold. Thus, more intense stimuli (e.g., higher PTZ doses or a longer time after PTZ) may be required to overcome the expanded ECS of AQP4$^{-/-}$ mice in order to initiate a seizure.

In order to analyze the seizure phenotype of the AQP4$^{-/-}$ mice in greater detail, in vivo electroencephalographic (EEG) characterization with stimulation and recording was employed.[76] 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. AQP4$^{-/-}$ mice had a higher mean electrographic seizure threshold than wild-type controls, consistent with the prior PTZ studies.[55] However, AQP4$^{-/-}$ mice were also found to have remarkably prolonged stimulation-evoked seizures compared to wild-type controls.[55]

Altered K^+ Homeostasis in AQP4$^{-/-}$ Mice

Because of the colocalization of AQP4 and inwardly rectifying K^+ (K_{ir}) channels in glial endfeet, the hypothesis arose that AQP4 was indirectly

involved in K^+ reuptake.[40,77] 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.[10,28–30] Indeed, in addition to modulation of brain water transport, AQP4 and its known molecular partners have been hypothesized to modulate ion homeostasis.[78,79] During rapid neuronal firing, extracellular $[K^+]$ ($[K^+]_o$) 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.[69,80–82] Such K^+ reuptake into glial cells could be AQP4-dependent, as water influx coupled to K^+ influx is thought to underlie activity-induced glial cell swelling.[83,84] In support of this possibility was the known subcellular colocalization of AQP4 with the inwardly rectifying K^+ channel $K_{ir}4.1$ in the retina.[41,77,85] $K_{ir}4.1^{-/-}$ mice, like $AQP4^{-/-}$ mice,[57,58] demonstrate abnormal retinal and cochlear physiology presumably due to altered K^+ homeostasis.[86–89] $K_{ir}4.1$ is thought to contribute to K^+ reuptake and spatial K^+ buffering by glial cells,[90–93] and pharmacological or genetic inactivation of $K_{ir}4.1$ leads to impairment of extracellular K^+ regulation.[86,94–99]

To address the possibility that AQP4 deficiency was associated with a deficit in K^+ homeostasis, K^+ dynamics were examined in vivo in $AQP4^{-/-}$ mice.[76] Neither baseline $[K^+]_o$ nor the "Lux–Heinemann ceiling" level of activity-induced $[K^+]_o$ elevation (~12 mM)[80,81] were altered in AQP4 deficiency, indicating that basic K^+ homeostasis was intact. Based on K^+ measurements made from cortex with double-barreled K^+-sensitive microelectrodes, stimulation-induced rises in $[K^+]_o$ were quite different in $AQP4^{-/-}$ compared to wild-type mice; in particular, there was a markedly slower rise and decay time for poststimulus changes in $[K^+]_o$ in $AQP4^{-/-}$ mice.[76] A similar delay in K^+ kinetics was observed following cortical spreading depression in $AQP4^{-/-}$ mice using a fluorescent K^+ sensor.[100]

Slowed $[K^+]_o$ rise time is consistent with increased ECS volume fraction in $AQP4^{-/-}$ mice.[74] Slowed $[K^+]_o$ decay is possibly due to impaired K^+ reuptake into $AQP4^{-/-}$ astrocytes. Interestingly, there is no difference in expression of $K_{ir}4.1$ protein[76] or $K_{ir}4.1$ immunoreactivity[101] in $AQP4^{-/-}$ mice nor AQP4 immunoreactivity in $K_{ir}4.1^{-/-}$ mice.[101] In addition, no alterations were observed in membrane potential, barium-sensitive $K_{ir}4.1$ K^+ current, or current–voltage curves in $AQP4^{-/-}$ retinal Müller cells[102] or brain astrocytes.[103] Lack of alteration of K_{ir} channels in $AQP4^{-/-}$ mice suggests the interesting possibility that the slowed $[K^+]_o$ decay may be a secondary effect of slowed water extrusion (deswelling) following stimulation, and this has been modeled carefully[104] but not directly demonstrated.

Stroschein et al.[105] investigated the impact of AQP4 on stimulus-induced alterations of $[K^+]_o$ in hippocampal slices. Antidromic stimulation evoked smaller increases and slower recovery of $[K^+]_o$ in the stratum pyramidale of $AQP4^{-/-}$ mice, consistent with the previous in vivo studies

in cortex. Interestingly, astrocyte gap junction coupling as assessed with tracer filling during patch-clamp recording demonstrated enhanced tracer coupling in AQP4$^{-/-}$ mice, and laminar profiles indicated enhanced spatial redistribution of K^+.[105] The functional consequences of alterations in gap junctional coupling are not completely clear; however the complete absence of astrocytic gap junctions impairs K^+ homeostasis.[106] To further assess the direct link between AQP4 and K^+ homeostasis, Haj-Yasein et al. examined extracellular K^+ concentration in a distinct constitutive AQP4$^{-/-}$ mouse line[107] and found a layer-specific effect of AQP4 deletion on K^+ dynamics during synaptic stimulation.[108]

Anchoring of AQP4 by the Dystrophin-Associated Protein Complex (DAPC)

AQP4 in endfeet membranes is anchored to the DAPC. For example, endfoot expression of AQP4 is dependent on α-syntrophin, a dystrophin-associated protein.[67] Accordingly, mice deficient in α-syntrophin show marked loss of AQP4 from perivascular and subpial membranes as judged by quantitative immunogold electron microscopy.[68] Similarly, dystrophin-deficient (*mdx*) mice exhibit 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.[66] These studies clearly suggest that alterations in components of the DAPC may affect the subcellular targeting and function of AQP4. A recent study also demonstrates that dystrophin localization at the astrocytic endfoot is dependent on syntrophin.[109] Another study using the zero magnesium in vitro model of epilepsy demonstrated reduction in expression of a distinct member of the DAPC, β-dystroglycan, in cortical astrocytes following continuous seizures, which was accompanied by a decrease in AQP4 expression.[110] Astrocyte swelling is markedly impaired in response to hypoosmotic stress, oxygen-glucose deprivation or high K^+ in the cortex of α-syntrophin-negative glial fibrillary acidic protein (GFAP)/EGFP mice.[111]

What are the functional consequences to seizure susceptibility of loss of DAPC components? Amiry-Moghaddam et al. (2003) were the first to report a possible association between AQP4 and epilepsy by demonstrating an increased severity of hyperthermia-evoked seizures in α-syntrophin-deficient mice. These mice exhibited a deficit in extracellular K^+ clearance following evoked neuronal activity.[4] Dystrophin-deficient mice were found to have altered seizure susceptibility in response to various chemical convulsants; in particular, *mdx* mice showed enhanced seizure severity and a shorter latency in the development of chemical kindling produced by administration of PTZ.[112] It is interesting to note

that there is an increased incidence of epilepsy in forms of human muscular dystrophy in which the dystrophin complex is affected.[113] Taken together, these data suggest that AQP4 and its molecular partners together comprise a multifunctional "unit" responsible for clearance of K^+ and/or water following neural activity and that alterations in expression of this complex can lead to alterations in seizure susceptibility.

Human Tissue Studies

The most common pathology in patients with medically intractable TLE is mesial temporal sclerosis (MTS), characterized by marked neuronal cell loss in specific hippocampal areas, gliosis, and microvascular proliferation.[114] Emerging work also demonstrates dysregulation of water and K^+ homeostasis in patients with mesial TLE. First, imaging studies demonstrate abnormal T2 prolongation by magnetic resonance imaging (MRI) in the epileptic hippocampus, possibly partially due to increased water content.[115] This is accompanied by alterations in apparent diffusion coefficient (ADC) with diffusion-weighted MRI imaging[116] (see Chapter 4: Experimental Techniques to Investigate the Formation of Brain Edema In Vivo, for details on neuroimaging techniques). Second, the expression and subcellular localization of AQP4 have been shown 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.[117] However, using quantitative immunogold electron microscopy, the same group found that there was mislocalization of AQP4 in the human epileptic hippocampus, with reduction in perivascular membrane expression.[118] Thus, although there was an overall increase in AQP4 content by Western blot, RT-PCR, and gene chip analysis, the subcellular distribution of AQP4 in mesial TLE tissue had changed. Their hypothesis was that reduction in perivascular AQP4 expression would lead to water and K^+ dysregulation in the epileptic hippocampus, potentially contributing to hyperexcitability.

Medici et al. reported AQP4 expression in tissue from patients with focal cortical dysplasia (FCD) type IIB, normal-appearing (cryptogenic) epileptic cortex, and nonepileptic control tissue.[119] AQP4 expression and distribution in the cryptogenic cases were similar to control cases, i.e., with intact perivascular immunoreactivity. In the patients with FCD type IIB, the pattern was different, with strong AQP4 immunoreactivity around dysplastic neurons but with very weak immunoreactivity for AQP4 around blood vessels.[119]

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,[118] these authors also studied perivascular dystrophin

expression. Like AQP4 expression, perivascular dystrophin expression was also reduced in tissue from the sclerotic epileptic hippocampus.[118] The more recent study also demonstrated reduced perivascular dystrophin expression in FCD type IIB.[119] Thus, subcellular alteration in AQP4 expression may result secondarily from alterations in dystrophin and/or other members of the DAPC.

Dysfunction of astroglial K_{ir} channels has also been found in specimens from patients with TLE.[10,12] First, using ion-sensitive microelectrodes, Kivi et al. demonstrated an impairment of glial barium-sensitive K^+ uptake in the CA1 region of MTS specimens.[11] Second, using patch-clamp analysis, Hinterkeuser et al. demonstrated a dramatic reduction in astroglial K_{ir} currents in MTS tissue.[13] Reduction in K_{ir} currents would be expected to contribute to hyperexcitability; evidence for this comes from the finding of stress-induced seizures in conditional $K_{ir}4.1^{-/-}$ mice.[97]

Methodological Issues

In assessing the contribution of AQP4 and its molecular partners to epileptogenesis, it is critical 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 may show many molecular changes in glial cells including the reported altered distribution of AQP4,[117,118] but what is unclear from these studies is whether AQP4 dysregulation 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 molecular changes in glial cells during epileptogenesis[2] 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,[4] 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 electrophysiologic 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.

The studies discussed above suggest novel roles for AQP4 in control of seizure susceptibility,[4,55,76] K^+ homeostasis,[4,76,100] and ECS physiology.[74] These findings together with the changes in human epileptic tissue[117,118] lead to the unifying hypothesis that AQP4 and its molecular partners may play a functional role in epilepsy.[3,120] Thus, more recent studies have examined the pattern of expression, regulation, and role(s) of AQP4 in various animal models of epileptogenesis (development of epilepsy).

Expression and Regulation of AQP4 During Epileptogenesis

Early studies demonstrated the perivascular localization of AQP4[40,41,121,122] but did not examine region-specific expression of AQP4 in the brain in detail. A recent study examined subregional expression pattern of AQP4 within the mouse hippocampus, a structure critical to epilepsy.[101] AQP4 immunohistochemistry revealed a developmentally regulated and laminar-specific pattern, with highest expression in the CA1 stratum lacunosum-moleculare (SLM) and the molecular layer (ML) of the dentate gyrus (DG). AQP4 was also ubiquitously expressed in astrocytic endfeet around blood vessels.

This study also addressed the cell type-specificity of AQP4 expression in the hippocampus. The description of novel classes of hippocampal glial cells (classical astrocytes vs NG2 cells) with different morphology and functional properties[123–126] makes it particularly important to clearly identify which "glial cell" expresses AQP4. While AQP4 is thought to be expressed predominantly by "classical" astrocytes, some have reported expression in other cell types, such as activated microglia.[127] However, a recent study found no AQP4 expression in resting microglia of the neocortex[128] and conditional AQP4 deletion driven by the human *GFAP* promoter completely removed AQP4 from brain.[53] In line with the latter studies, AQP4 was found to colocalize with the astrocyte markers GFAP and S100β in the hippocampus, and electrophysiological and postrecording RT-PCR analyses of individual cells revealed that AQP4 and $K_{ir}4.1$ were coexpressed in nearly all CA1 "classical astrocytes." In NG2 cells, AQP4 was detected at the transcript level but not with immunohistochemistry.[101]

Seizure activity is accompanied by a variety of changes in gene expression, and repeated or prolonged seizure activity leads to glial changes, ultimately including the proliferation of "reactive" astrocytes (gliosis). The human studies indicated an upregulation and altered distribution of AQP4 protein in sclerotic tissue resected from patients with mesial TLE.[117,118] 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, or gliosis.[129] AQP4 is known to be dramatically upregulated in reactive astrocytes following injury[130–132] but may also be regulated by physiologic stimuli.[133]

Of course, whether AQP4 dysregulation *precedes* or *follows* epileptogenesis impacts its functional role as cause or consequence. To address this question will require studies of AQP4 regulation with concurrent video-EEG monitoring during epileptogenesis to determine the exact timing of onset of spontaneous seizures relative to changes in AQP4 and its molecular partners. Three studies so far address this key issue. First, Kim et al. studied regulation of various AQPs in the rat pilocarpine model of epilepsy.[134,135] In control animals, AQP4 immunoreactivity was detected

diffusely in the piriform cortex and hippocampus, with greatest expression at astrocyte endfeet. Following status epilepticus in this model the authors describe an "AQP4-deleted area" in the piriform cortex, associated with decreases in immunoreactivity for dystrophin and α-syntrophin, members of the DAPC.[135] However, EEG analysis for timing of spontaneous seizure onset was not investigated.

Second, Lee et al.[136] studied AQP4 distribution and regulation in the intrahippocampal model of epileptogenesis. In this model, stereotactic microinjection of kainic acid into the mouse dorsal hippocampus leads to limbic status epilepticus followed by a "latent" period of several days (usually about 7 days) prior to the occurrence of spontaneous recurrent seizures (epilepsy) recorded by chronic video-EEG monitoring. Lee et al. found dramatic downregulation of AQP4 in multiple hippocampal subregions/laminae during epileptogenesis in this model. Importantly, hippocampal AQP4 immunoreactivity was markedly downregulated during the latent phase prior to the occurrence of spontaneous seizures as well as during the chronic epileptic phase, with partial recovery in some hippocampal laminae. While marked reactive astrocytosis was observed, the reactive astrocytes in this model were largely AQP4-negative. Interestingly, in this study, $K_{ir}4.1$ expression on reactive astrocytes was preserved.[136]

Third, Alvestad et al.[137] characterized EM localization of AQP4 in the latent and chronic phase in the intraperitoneal kainic acid rat model. Immunogold electron microscopic analysis revealed that adluminal AQP4 expression in astrocyte endfoot membranes was decreased in KA-treated rats during the latent phase. There was an accompanying reduction in adluminal alpha-syntrophin expression.[137] These data together with those of Kim et al.[134,135] and Lee et al.[136] suggest downregulation and/or mislocalization of AQP4 during early epileptogenesis. Thus, AQP4 dysregulation may contribute a critical role to loss of ion and water homeostasis leading to excitability and decreased seizure threshold during epileptogenesis.

Further studies will need to address the coordinate regulation of AQP4 and other relevant astrocyte molecules such as $K_{ir}4.1$ in these models. Finally, distinct epilepsy models in which epileptogenesis occurs in the *absence* of detectable cell death would prove useful in determining the threshold for AQP4 regulation.[138,139]

Subcellular Alteration in AQP4 Distribution: A Common Disease Mechanism?

The hallmark of AQP4 expression in the CNS is its polarized expression at astrocyte endfeet ensheathing blood vessels.[40,41,140] Loss of such polarization is associated with pathology in mesial TLE as discussed above.[118]

During seizures, there is focal swelling in the area of the seizure focus[23,74]; and the putative effect of loss of the perivascular pool of AQP4 would be to slow water egress from astrocyte to capillary leading to local astrocyte swelling, ECS constriction, and increased excitability.[3]

Interestingly, a similar loss of AQP4 polarization has been observed in distinct models of neurological diseases. For example, in a mouse model of Alzheimer's disease, loss of AQP4 from endfoot membranes at sites of perivascular amyloid deposits was observed.[141] Such subcellular alterations could lead to perturbation of local water and K^+ homeostasis in affected brain regions, and thus contribute to cognitive decline and seizure susceptibility. Similarly, Badaut et al. found loss of polarization of AQP4 expression on astrocyte endfeet following subarachnoid hemorrhage (SAH) and in peritumoral tissue[130]; thus, posttraumatic epilepsy and tumor-associated epilepsy[142] could be associated with alterations in AQP4 expression and distribution. Further studies will require careful determination of perivascular/perisynaptic AQP4 expression ratios in a variety of conditions.

AQP4 and the Gliovascular Junction

Another area of astrocyte biology of potential importance for epilepsy is the "gliovascular junction."[78] Recent studies have shown the close relationship between astrocyte endfeet ensheathing blood vessels, the targeted expression of AQP4 and $K_{ir}4.1$ on astrocyte endfeet, and the role of astrocytes in blood–brain barrier permeability[143] and control of cerebrovascular tone.[144–148] Molecular and cellular alterations in the gliovascular junction could perturb blood flow, water, and K^+ regulation, and therefore local tissue excitability. Interestingly, recent studies have shown that transient opening of the blood–brain barrier is sufficient for focal epileptogenesis.[149,150]

Just as alterations in the perivascular pool of AQP4 on existing blood vessels could affect local water and K^+ regulation, it is also the case that many new blood vessels can be formed in epileptic tissue. MTS is characterized by neuronal cell loss in specific hippocampal areas, gliosis, synaptic reorganization, but also significant microvascular proliferation.[114] How does the regulation of AQP4 relate to angiogenesis during epileptogenesis? The increased number of microvessels in epileptic tissue may in part be due to upregulation of vascular endothelial growth factor (VEGF) following seizures.[151–153] VEGF administration itself has been shown to upregulate AQP4,[154] but it is unclear whether AQP4 distribution and/or function may be unique in "epileptic vasculature." Alterations in cerebrovascular tone in "epileptic vasculature" would be another interesting topic of investigation.

A Role for AQP4 at the Synapse?

It was clear from early immunogold studies of AQP4 localization that while the most abundant pool was localized at astrocyte endfeet, there was also a significant concentration in nonendfeet membranes, including those astrocyte membranes that ensheath glutamatergic synapses.[40,41] Given the role of AQP4 in rapid water fluxes and K^+ homeostasis, more specific roles of AQP4 in synaptic transmission, plasticity, and behavior have begun to be tested.

Skucas et al. evaluated long-term potentiation (LTP) and long-term depression (LTD) in hippocampal slices from AQP4$^{-/-}$ mice.[155] Interestingly, AQP4$^{-/-}$ mice exhibited a selective defect in LTP and LTD without a change in basal synaptic transmission or short-term plasticity. The impairment in LTP in AQP4$^{-/-}$ mice was specific for the type of LTP that depends on the neurotrophin brain-derived neurotrophic factor (BDNF), which is induced by stimulation at theta rhythm [theta-burst stimulation (TBS)-LTP], but there was no impairment in a form of LTP that is BDNF-independent, induced by high-frequency stimulation. LTD was also impaired in AQP4$^{-/-}$ mice, which was rescued by a scavenger of BDNF or a blockade of Trk receptors. The AQP4$^{-/-}$ mice also exhibited a cognitive defect in location-specific object memory but not Morris water maze or contextual fear conditioning. These results suggest that AQP4 channels in astrocytes may play an unanticipated role in neurotrophin-dependent plasticity and influence behavior.

Based on these results, downregulation of AQP4 may not only lead to increased neural excitability due to abnormalities of water and potassium homeostasis but may also lead directly to abnormalities in synaptic plasticity (both LTP and LTD). This provides a potential explanation for the way that astrocytic changes in epilepsy may contribute not only to seizures but also to cognitive deficits. Cognitive impairment is very important because patients with TLE have many alterations in cognitive function and in particular hippocampal-dependent tasks such as spatial memory.[156–159] In addition, many other forms of plasticity are operative in the epileptic brain such as potentiation of synapses, reorganization of neuronal circuitry, and alteration in postnatal neurogenesis.[160–162] More experiments are necessary to elucidate the role of AQP4 in modulation of these processes underlying epileptogenesis.

Genetic Studies of AQP4 and Epilepsy

Based on the idea that AQP4 and $K_{ir}4.1$ act in concert to regulate water and K^+ homeostasis in the brain, the hypothesis arose that variants of the *AQP4* and *KCNJ10* (*K_{ir}4.1*) genes may be associated with forms of epilepsy. In a study of 218 Norwegian patients with TLE and 181 controls,

Heuser et al. found several single nucleotide polymorphisms (SNPs) in the *KCNJ10* and *AQP4* genes associated with TLE.[163] In a mouse model with glial-specific deletion of *KCNJ10*, delayed stimulation-associated K^+ clearance in CA1 stratum radiatum was observed.[99]

Restoration of Water and Ion Homeostasis

The above considerations lead to a new potential therapeutic opportunity: restoration of water and K^+ homeostasis by regulation of AQP4 targeting and distribution. Whether such a therapeutic approach in epilepsy may be reasonable is an open question. Might AQP4 upregulation be therapeutically feasible? So far there is no known pharmacological method to upregulate AQP4. However, increasing understanding of the molecular partners involved (such as the DAPC) could lead to such approaches. For example, the extracellular matrix protein agrin, a heparin sulfate proteoglycan, may be a critical regulator of AQP4 membrane trafficking and polarity.[164] Astrocytes cultured with the neuronal agrin isoform A4B8 but not with the endothelial and meningeal isoform A0B0 demonstrated increases in the M23 splice variant of AQP4, increased water transport capacity, and increased membrane density of OAPs.[164] The glucocorticoid triamcinolone acetonide (TA) was shown to induce AQP4 downregulation in the normal retina but increased expression in the inflamed retina.[165] Targeting the DAPC for increased expression has the potential to be a novel therapeutic target, but a way to modulate its expression in the brain is still unknown. Current studies focus on the role of dystrophin in Duchenne muscular dystrophy and a few have found ways to upregulate the anchoring protein,[166,167] but the expression changes in brain tissue and effects on AQP4 expression have not yet been examined.

CONCLUSIONS

Compelling evidence indicates that the glial water channel AQP4 plays 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 be responsible for water and K^+ homeostasis during neural activity. Because osmolarity and K^+ have powerful effects on seizure susceptibility, AQP4 and its molecular partners may represent novel therapeutic targets for control of seizures. $AQP4^{-/-}$ mice have significantly prolonged seizure duration, associated with alterations in extracellular K^+ clearance and gap junctional coupling. Dysfunctional K^+ homeostasis and upregulation and altered subcellular distribution of AQP4 have been observed in human epileptic tissue. The relevance of these findings to hippocampal epileptogenesis and also 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 astroglial processes investing synapses, AQP4 may be involved in glio-neuronal interaction of water and ion fluxes and also in gliovascular interactions across the blood–brain barrier and thus play a critical role both during active tissue metabolism and in pathophysiologic states.

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