Decreased expression of the glial water channel aquaporin-4 in the intrahippocampal kainic acid model of epileptogenesis

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A B S T R A C T

Recent evidence suggests that astrocytes may be a potential new target for the treatment of epilepsy. The glial water channel aquaporin-4 (AQP4) is expressed in astrocytes, and along with the inwardly-rectifying K+ channel K+4.1 is thought to underlie the reuptake of H2O and K+ into glial cells during neural activity. Previous studies have demonstrated increased seizure duration and slowed potassium kinetics in AQP4−/− mice, and redistribution of AQP4 in hippocampal specimens from patients with chronic epilepsy. However, the regulation and role of AQP4 during epileptogenesis remain to be defined. In this study, we examined the expression of AQP4 and other glial molecules (GFAP, K+4.1, glutamine synthetase) in the intrahippocampal kainic acid (KA) model of epilepsy and compared behavioral and histologic outcomes in wild-type mice vs. AQP4−/− mice. Marked and prolonged reduction in AQP4 immunoreactivity on both astrocytic processes and endfeet was observed following KA status epilepticus in multiple hippocampal layers. In addition, AQP4−/− mice had more spontaneous recurrent seizures than wild-type mice during the first week after KA SE as assessed by chronic video-EEG monitoring and blinded EEG analysis. While both genotypes exhibited similar reactive astrocytic changes, granule cell dispersion and CA1 pyramidal neuron loss, there were an increased number of fluorojade-positive cells early after KA SE in AQP4−/− mice. These results indicate a marked reduction of AQP4 following KA SE and suggest that dysregulation of water and potassium homeostasis occurs during early epileptogenesis. Restoration of astrocytic water and ion homeostasis may represent a novel therapeutic strategy.

Introduction

Recent evidence suggests that glial cells may play a role in epilepsy (Binder and Steinhäuser, 2006). First, many studies now link glial cells to modulation of synaptic transmission (Halassa and Haydon, 2010; Volterra and Meldolesi, 2005). Second, functional alterations of specific glial membrane channels and receptors have been discovered in epileptic tissue (Seifert et al., 2006; Steinhäuser and Seifert, 2002). Third, direct stimulation of astrocytes has been shown to be sufficient for neuronal synchronization in epilepsy models (Tian et al., 2005).

The aquaporins (AQPs) are a family of membrane protein water channels expressed in many cell types and tissues that facilitate bidirectional water transport in response to osmotic gradients (Verkman, 2002, 2005). Aquaporin-4 (AQP4) is expressed by glial cells, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels and astrocyte membranes that ensheath glutamatergic synapses (Nagelhus et al., 2004; Nielsen et al., 1997).

Modulation of water and potassium homeostasis by AQP4 could dramatically affect seizure susceptibility. Brain tissue excitability is exquisitely sensitive to osmolarity and the size of the extracellular space (ECS) (Schwartzkroin et al., 1998). Decreasing ECS volume produces hyperexcitability and enhanced epileptiform activity (Dudek et al., 1990; Roper et al., 1992); conversely, increasing ECS volume with hyperosmolar medium attenuates epileptiform activity (Dudek et al., 1990; Traynelis and Dingledine, 1989). These experimental data parallel extensive clinical experience indicating that hyposmolar states lower seizure threshold while hyperosmolar states elevate seizure threshold (Andrew et al., 1989). Second, millimolar and even submillimolar increases in extracellular potassium concentration powerfully enhance epileptiform activity in the hippocampus (Feng and Durand, 2006; Traynelis and Dingledine, 1988).

Emerging work indeed demonstrates dysregulation of water and potassium homeostasis in patients with mesial temporal lobe epilepsy (Binder and Steinhäuser, 2006). Imaging studies demonstrate abnormal
injected using a microinjector (Nanoject, Drummond Scientific Inc., Santa Barbara, CA).

EEGs were continuously recorded with a digital acquisition system (MP150 with EEG100C amplifiers, Biopac Systems Inc., Santa Barbara, CA). EEGs were continuously recorded with a digital video camera (Sony HDR-HC5, Sony Electronics, San Diego, CA).

Animals and surgery

Animals were freely moving and continuously monitored 24 h/day up to 14 days using a digital video camera (Sony HDR-HC5, Sony Electronics, San Diego, CA). EEGs were continuously recorded with a digital acquisition system (MP150 with EEG100C amplifiers, Biopac Systems Inc., Santa Barbara, CA).

Materials and methods

Animals were freely moving and continuously monitored 24 h/day up to 14 days using a digital video camera (Sony HDR-HC5, Sony Electronics, San Diego, CA).

EEG analysis

Only mice that experienced status epilepticus (SE) were evaluated in this study. SE was defined as continuous tonic–clonic seizure activity for at least 3 h. Spontaneous seizures during the post-SE period of epileptogenesis were defined as spiking epileptiform burst activity lasting for at least 5 s at a frequency of 3 Hz. Seizure analysis was performed blinded to genotype and motion artifacts were excluded from analysis.

Immunohistochemistry

Histological analysis was performed on control (AQ4+/+ and AQ4−/−) and experimental (AQ4+/+ and AQ4−/−) mice at 1 day, 4 days, 7 days, 14 days and 30 days after SE. Mice were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (200 mg/kg) and perfused transcardially with ice-cold phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde, pH 7.4. Brains were postfixed overnight in 4% paraformaldehyde at 4 °C followed by cryoprotection in 30% sucrose in PBS at 4 °C. Coronal 50 μm thick sections were cut from frozen blocks with a cryostat (Leica 1900, Leica Microsystems, Bannockburn, IL). Sections were stored in PBS at 4 °C. Sections were blocked with 5% normal goat serum in 0.1 M PBS, then incubated with primary antibody to AQ4 (1:200; Millipore, Temecula, CA), GFAP (1:100; Millipore, Temecula, CA), neuronal-specific nuclear protein (NeuN; 1:100; Millipore, Temecula, CA), Kir4.1 (1:100, Alomone, Jerusalem, Israel), and glucose synthetase (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) in 0.3% Triton X-100 at 4 °C overnight. After rinsing with PBS, sections were incubated with a species-specific secondary antibody conjugated with Alexa 488, 594, 647 or a TSA kit (Molecular Probes/Invitrogen, Carlsbad, CA) for visualization and then mounted in Vectorshield (Vector Laboratories, Burlingame, CA). Images were obtained on a fluorescence microscope (BX51; Olympus, San Diego, CA). Images and analysis of sections presented are of the hemisphere contralateral to the side of injection, except where otherwise noted (NeuN).

Quantification of immunoreactivity

Quantitative analysis of staining intensity was performed using densitometry. Fluorescent images of the immunoreactivity of various stains within the hippocampus were captured and analyzed using Slidebook (Intelligent Imaging Innovations, Inc, Santa Monica, CA). A square box delineating the region of interest with the width of the pyramidal cell layer of the hippocampus was placed in each of the layers of the hippocampus; stratum oriens (SO), stratum pyramidale (SP), stratum radiatum (SR), stratum lacunosum moleculare (SLM), dentate gyrus molecular layer (ML), dentate gyrus granule cell layer (GC), and hilus of the dentate gyrus (DG). Intensity per unit area was calculated and analyzed.

Fluoro-jade B histochemistry

Neuronal degeneration was characterized using fluoro-jade B (FJ-B) staining as described (Schmued et al., 1997). Sections were mounted onto slides, dehydrated, immersed in a solution containing 1% sodium hydroxide in 80% ethanol, rehydrated in 70% alcohol, rinsed with PBS, transferred to a solution of 0.06% potassium permanganate and blocked for 30 min with agitation. Following the blocking process, slides were then washed with PBS to remove excess potassium permanganate. The staining solution was prepared by diluting a stock solution of 0.1% stock solution of FJ-B (Histo-Chem Inc., Jefferson, AR) 1:10 in 0.1% acetic acid solution. Slides were allowed to incubate at room temperature for 30 min in the 0.01%FJ-B/0.1% acetic acid solution. After the incubation, sections were washed 2–3 times with PBS and once with distilled water.
water. Sections were left to dry overnight prior to imaging. Images of each section were captured using a fluorescent microscope (BX51; Olympus, San Diego, CA) and the number of FJ-B-positive cells in the hippocampus was counted.

**Results**

**Seizure analysis**

After injection of intra-hippocampal kainic acid, status epilepticus was induced in both AQP4+/+ and AQP4−/− mice as evidenced electrographically (Fig. 1A) and behaviorally (data not shown). Spontaneous seizures developed during post-status epilepticus (SE) days 2–11 (3.0 ± 0.9 days) in AQP4+/+ animals and post-SE days 2–3 (2.1 ± 0.1 days) in AQP4−/− animals (Figs. 1B and C, respectively). While spontaneous seizures developed in all experimental animals, AQP4−/− mice exhibited more seizures during the first week after injection (early epileptogenic period, Fig. 2). Specifically, AQP4−/− mice had a higher mean # of seizures per day during post-SE days 1–7 than AQP4+/+ mice (14.8 ± 2.1 vs. 6.5 ± 1.1, respectively; p < 0.05, t-test). In addition, there was a trend toward greater total seizure duration per day between AQP4−/− mice (282.8 ± 41.2 s) and AQP4+/+ mice (178.6 ± 28.0 s) but this did not reach statistical significance (p > 0.05). After post-SE day 7 through the end of the recording period (day 14), there were no significant differences in frequency or seizure duration between genotypes. No animals that were injected with saline exhibited more seizures during the first week after injection (early epileptogenic period, Fig. 2). Specifically, AQP4−/− mice had a higher # of seizures per day during post-SE days 1–7 than AQP4+/+ mice (14.8 ± 2.1 vs. 6.5 ± 1.1, respectively; p < 0.05, 2-way RM ANOVA) and a greater number of seizures overall during the first 7 days (103.5 ± 23.8 vs. 45.5 ± 14.0, respectively; p < 0.05, t-test). In addition, there was a trend toward greater total seizure duration per day between AQP4−/− mice (282.8 ± 41.2 s) and AQP4+/+ mice (178.6 ± 28.0 s) but this did not reach statistical significance (p > 0.05). After post-SE day 7 through the end of the recording period (day 14), there were no significant differences in frequency or seizure duration between genotypes. No animals that were injected with saline developed status epilepticus or spontaneous seizures.

In order to verify that seizures propagated to the contralateral hippocampus in the epileptogenic period, a subset of mice (n = 4) were implanted with bilateral hippocampal electrodes. These experiments confirmed that, similar to previous results (Arabadzisz et al., 2005), following unilateral KA injection seizures occurred in both hippocampi (Supplemental Fig. 1). There were fewer seizures in the hippocampus contralateral to the injection in both AQP4+/+ and AQP4−/− mice (data not shown).

**AQP4 immunoreactivity**

In this group of studies, we used a sensitive and specific AQP4 immunohistochemical protocol that has been previously validated in detail using AQP4−/− tissue (Hsu et al., 2011). No specific immunoreactivity was observed in the absence of anti-AQP4 primary antibody. Compared to saline controls, AQP4 immunoreactivity was significantly decreased on post-SE day 1 (saline control: 730.9 ± 59.5 vs. post-SE day 1: 406.2 ± 25.8 ADU/mm², p < 0.05). This initial decrease was followed by a slow return to near-normal levels over the next 30 days (Fig. 3A). Saline controls did not demonstrate any significant differences in AQP4 levels at any time point (data not shown). When analyzing the hippocampal layers individually, there were additional significant differences in various hippocampal layers and time points (Figs. 3B–H) relative to control. Specifically, there were no significant differences between saline control and experimental animals in stratum oriens at any time point. Relative to saline controls, stratum pyramidale demonstrated decrease at post-SE day 1 (969.3 ± 137.4 vs. 463.1 ± 25.1, respectively; p < 0.05) and post-SE day 4 (969.3 ± 137.4 vs. 498.2 ± 30.7, respectively; p < 0.05). Stratum radiatum demonstrated decrease at post-SE day 1 (3942 ± 345 vs. 1248.9 ± 205.0, p < 0.05), post-SE day 4 (428.3 ± 35.7 vs. 1248.9 ± 205.0, p < 0.05) and post-SE day 7 (738.3 ± 76.4 vs. 1248.9 ± 205.0, p < 0.05). The dentate granule cell layer demonstrated AQP4 decrease at all time points except for post-SE day 14. Decreased AQP4 immunoreactivity was noted at all time points post-SE in stratum lacunosum moleculare, the molecular layer and the hilus of the dentate gyrus (Figs. 3B–H).

**GFAP immunoreactivity**

In both AQP4+/+ and AQP4−/− animals, marked increases in GFAP immunoreactivity were detected on post-SE days 4, 7, and 14 in all layers of the hippocampus, followed by a slight decrease at post-SE day 30 (Fig. 4). No significant differences were observed in GFAP immunoreactivity between AQP4+/+ (Figs. 4A and C) and AQP4−/− (Figs. 4B and D) mice; in both genotypes, astrocyte morphology became reactive with ramified with thickened processes (Figs. 4 and 5).

**Ko4.1 immunoreactivity**

Overall Ko4.1 immunoreactivity in post-SE animals did not differ significantly from control AQP4+/+ mice (Fig. 6A). In addition, there were also no significant differences in overall Ko4.1 levels between post-SE AQP4+/+ and post-SE AQP4−/− mice for all time points in this study (data not shown). However, significant focal Ko4.1 immunoreactivity was detected on reactive astrocytes of CA1 stratum radiatum and stratum lacunosum moleculare on post-SE days 4 and
Fig. 3. AQP4 immunoreactivity following kainic acid status epilepticus. Significant reduction in hippocampal AQP4 immunoreactivity was observed with delayed partial recovery. A. 4× images at indicated time points after SE. Scale bar = 200 μm. Laminar-specific analysis of AQP4 immunoreactivity after SE demonstrates decreased AQP4 immunoreactivity detected in various layers of the hippocampus throughout the study period (B–H). The initial decrease in AQP4 immunoreactivity is followed by a gradual increase. Persistent down-regulation was observed in the SLM, ML, granule cell layer and hilus. ADU = arbitrary density units. **, p < 0.01 compared to saline control.
Fig. 4. Immunohistochemical staining of GFAP. Increased GFAP immunoreactivity and astrocyte ramification occurs following SE in both AQP4+/+ (A, C) and AQP4−/− (B, D) mice. Scale bar = 200 μm.

Fig. 5. Comparison of AQP4 and Kir4.1 labeling of reactive astrocytes. A. Confocal 40× image in CA1 stratum lacunosum moleculare 7 days post-SE. Note marked reactive phenotype of GFAP-positive astrocytes (center) but lack of strong AQP4 immunoreactivity. Scale bar = 50 μm. B. Confocal 63× image of reactive astrocytes in CA1 stratum radiatum 14 days post-SE. Note strong Kir4.1 immunoreactivity on reactive astrocytes. Scale bar = 20 μm.
Reactive astrocytes exhibited an upregulation of Kir4.1 and GFAP expression as well as thicker processes (Fig. 5).

Glutamine synthetase (GS) immunoreactivity

A modest but statistically significant increase in GS immunoreactivity was observed at post-SE days 4 and 7 in AQP4+/+ mice when compared to saline controls (Fig. 6B). No significant differences were seen at longer time points (14 and 30 days post-SE). In addition, as for Kir4.1, there were no significant differences in GS immunoreactivity between AQP4−/− and AQP4+/+ mice (data not shown).

NeuN immunoreactivity

After KA-induced SE, a dispersion of the cells of the granule cell layer of the dentate gyrus was noted in both AQP4+/+ and AQP4−/− mice (Fig. 7). The dispersion occurred only in the hippocampus ipsilateral to the injection. Significant progressive loss of CA1 pyramidal cell layer NeuN immunoreactivity was observed, greatest at post-SE day 30 (Fig. 7).

Fluoro-jade B histochemistry

From post-SE day 1 to post-SE day 14, sections from both genotypes exhibited Fj-B-positive cells. These cells were mostly observed in CA3 and hilus of the dentate gyrus in the hippocampus ipsilateral to the injection (Fig. 8). Following blinded analysis, there were significantly more Fj-B-positive cells in the AQP4−/− mice relative to AQP4+/+ mice on post-SE day 1 (173.1±33.4 vs. 54.6±30.8, p<0.05) and post-SE day 4 (94.0±18.6 vs. 40.4±9.0, p<0.05) (Fig. 8B). Fewer Fj-B-positive cells and no significant genotypic differences were observed at longer time points (Fig. 8B).

Discussion

In this study, we have used the intrahippocampal kainic acid model to examine changes in glial cell molecules early during the process of epileptogenesis. This led to several novel findings: first, AQP4−/− mice had more spontaneous recurrent seizures than wild-type mice during the first week after SE; second, there is a dramatic and prolonged downregulation of AQP4 immunoreactivity in the hippocampus following KA SE; third, while both genotypes exhibited similar reactive astrocytic changes, granule cell dispersion and CA1 pyramidal neuron loss, AQP4−/− mice had an increased number of...
fluoro-jade positive cells early after KA SE. These results support the hypothesis that intense seizures regulate AQP4 expression and may lead to a functionally relevant dysregulation of water and potassium homeostasis during epileptogenesis.

Increased seizure frequency in AQP4−/− mice

Previous results in acute seizure models demonstrated that AQP4−/− mice have a higher seizure threshold (Binder et al., 2004, 2006) but that seizures once elicited demonstrated a prolonged duration, presumably due in part to impaired K+ reuptake (Binder et al., 2006). Using chronic video-EEG recording for the first two weeks after KA SE, we did not find a statistically significant difference in total seizure duration between AQP4+/+ and AQP4−/− mice in the intrahippocampal KA model, but rather found an increase in the frequency of seizures in the AQP4−/− animals during the first week after KA SE (Fig. 2). Of course, in this chronic model of epileptogenesis, the initiation of SRS depends on multiple factors. For example, recent studies have indicated that following KA SE, reactive astrocytes exhibit alterations in glutamate transporter-dependent currents and also substantially increased dye coupling (Takahashi et al., 2010), although interestingly this group did not observe a change in astrocyte K+ currents early after SE. Impaired K+ reuptake in the AQP4−/− mice as previously described (Binder et al., 2006) would presumably lead to an increased excitability at least during the early phase of epileptogenesis which is consistent with the

**Fig. 7.** Granule cell dispersion and CA1 pyramidal cell loss following intrahippocampal KA administration. After KA-induced SE, granule cell dispersion was noted in both AQP4+/+ (A) and AQP4−/− (B) mice as early as post-SE day 7. Granule cell dispersion was observed at post-SE day 14 and 30, and is accompanied by loss of NeuN immunoreactivity in the CA1 pyramidal cell layer. Scale bar = 200 μm.
increased seizure frequency observed. Our novel finding of slightly increased cell death at 1 and 4 days following SE in the AQP4−/− mice (Fig. 8) may also contribute to an increased excitability after SE. Interestingly, since there is downregulation of AQP4 in the wild-type mice during this period, the wild-type mice may thus develop a “functional knockdown” of AQP4 and this may limit difference between the genotypes thereafter. Acquired dysregulation of AQP4 could lead to hyperexcitability by both astrocyte swelling-induced ephaptic interactions and impaired K⁺ homeostasis (Hsu et al., 2007; Wetherington et al., 2008).

Previous studies demonstrated alteration in the expression and subcellular localization of AQP4 in sclerotic hippocampi obtained from patients with MTS (Lee et al., 2004), in particular a reduction in perivascular membrane expression (Eid et al., 2005).

Here, using a validated AQP4 immunohistochemical protocol (Hsu et al., 2011), we report a marked, immediate and prolonged decrease in AQP4 immunoreactivity following KA SE. Compared to controls, AQP4 immunoreactivity was decreased in stratum lacunosum moleculare, the molecular layer and the dentate gyrus for all the time points in this study, and in a number of other layers of the hippocampus in the initial days following SE. The loss of AQP4 immunoreactivity occurred on both the endfeet and fine processes of astrocytes throughout the hippocampal layers. The mechanism of this decrease remains unclear: AQP4 could be downregulated or internalized from the cell surface. Also, it is not clear whether AQP4 downregulation is due entirely to the initial episode of SE or whether SRS maintain AQP4 downregulation. Further studies would be required to determine whether brief or shorter seizures have the capacity to downregulate AQP4.

Decrease in AQP4 immunoreactivity following status epilepticus

In studies in human patients with mesial temporal sclerosis (MTS), it was noted that hippocampal tissue sections have increased AQP4 expression (Lee et al., 2004). However, these studies were conducted in chronic epileptic tissue and not much is known about the expression of AQP4 during the development of epilepsy.

Fig. 8. Fluoro-jade-B histochemistry following SE. A. Representative examples of FJ-B-positive cells in the CA3 region and hilus of the dentate gyrus in the hippocampus ipsilateral to the injection in AQP4+/+ and AQP4−/− mice. Scale bar = 100 μm. B. There were significantly more FJ-B-positive cells within AQP4−/− hippocampi relative to AQP4+/+ hippocampi on post-SE day 1 (2-way ANOVA with post-hoc Bonferroni test, p < 0.001). 

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Kv4.1 immunoreactivity following status epilepticus

The inwardly-rectifying potassium channel Kv4.1 exhibits heterogeneous expression throughout the brain (Poopalasundaram et al., 2000), but is the predominant Kv subtype within the hippocampus (Seifert et al., 2009). Kv4.1 is thought to contribute to K⁺ reuptake and spatial K⁺ buffering by glial cells (Newman, 1986; Newman et al., 1984), and pharmacological or genetic inactivation of Kv4.1 leads to impairment of extracellular K⁺ regulation (Ballanyi et al., 1987; Djukic et al., 2007; Kofuji and Newman, 2004; Kofuji et al., 2000; Neusch et al., 2006). Dysfunction of astroglial Kv channels has also been found in specimens from patients with temporal lobe epilepsy (Hinterkeuser et al., 2000; Kivi et al., 2000) and impaired K⁺ uptake by astrocytes through Kv channels may contribute to epileptogenesis in a mouse model of TSC (Jansen et al., 2005).

Based on its demonstrated importance to K⁺ homeostasis, we also examined Kv4.1 immunoreactivity in the KA SE model. Interestingly, in contrast to AQP4, we did not observe downregulation of Kv4.1 at the time points studied. Rather, Kv4.1 was observed upregulated specifically on reactive astrocytes, especially in CA1 SR and SLM (Fig. 5B), on which AQP4 was absent (Fig. 5A). Thus, there appears to be distinct regulation of AQP4 and Kv4.1. While initial studies indicated a subcellular co-localization of AQP4 with Kv4.1 in the retina (Connors et al., 2004; Nagelhus et al., 2004), more recent studies indicate a lack of interaction between the two proteins (Zhang and Verkman, 2008).

Changes in glutamine synthetase and GFAP immunoreactivity following SE

Glutamine synthetase (GS) catalyzes the conversion of glutamate to glutamine in astrocytes and has been found to be decreased in the sclerotic hippocampus of temporal lobe epilepsy patients (Eid et al., 2004; van der Hel et al., 2005). In our study, we observed a transient increase in GS immunoreactivity on post SE days 1 and 4 (Figs. 6B and D). This is similar to that which was observed in rats during the latent period following initial seizure insult prior to the onset of spontaneous seizures (Hammer et al., 2008). In that study, GS was increased in the latent phase compared with controls, as assessed by Western blots of whole hippocampal formation and subregions. Compared with the latent phase, the chronic phase revealed a lower level of hippocampal GS content (Hammer et al., 2008). Studies in other models have demonstrated that newly generated astrocytes after SE may have lower levels of GS (Kang et al., 2006).

Like many previous studies, we observed upregulation of GFAP on reactive astrocytes following KA SE (Fig. 6). No significant differences were observed in GFAP immunoreactivity between wild-type and AQP4−/− mice at any time point. The remarkable congruity of GFAP upregulation and similar morphology of reactive astrocytes regardless of genotype suggests that AQP4 does not directly affect astrocyte cytoskeletal reactivity and reactive astrocytosis. This is interesting in light of previous studies which demonstrated impaired migration of AQP4-deficient cells to the site of stab injury in cortex (Saadoun et al., 2005). Taken together, these data indicate an effect of AQP4 on cell migration but not on cytoskeletal changes associated with glial scar formation.

Changes in neuronal histology in IH KA model

Previous studies in the IH KA model (Arabadzisz et al., 2005; Bouilleret et al., 1999; Riban et al., 2002) demonstrated that the model reproduces morphological characteristics of mesial temporal sclerosis, including neuronal loss, gliosis, reorganization of neurotransmitter receptors, mossy fiber sprouting, and granule cell dispersion. We confirmed the pattern of neuronal loss in CA1 and progressive dentate granule cell dispersion as assessed by NeuN immunoreactivity and found that the pattern was not significantly different in AQP4−/− mice (Fig. 7). Compared with previous papers, we contribute the finding that dentate granule cell dispersion occurs as early as 7 days after SE in this model (Fig. 7). Granule cell dispersion is thought to be neurogenesis-independent (Nitta et al., 2008) and caused by reelin deficiency (Haas and Frotscher, 2010; Haas et al., 2002) and a recent study in the IH KA model demonstrates that it can be inhibited by hippocampal deafferentation (Pallud et al., 2010).

Despite the overall lack of difference between genotypes in chronic histologic changes described above, FJ-B histochemistry demonstrated an increased number of degenerating cells in AQP4−/− mice on post-SE days 1 (Fig. 8). The location of these cells was in the hilus (Fig. 8), CA3 and CA1 regions (not shown). The mechanism of slightly increased cell death in AQP4−/− mice is unclear but again may relate to impaired K⁺ and possibly glutamate homeostasis. Another possibility is that the actual KA SE was more intense or of longer duration in the AQP4−/− mice thus constituting a greater insult; however, great care was taken to observe and quantify the SE with video-EEG and no difference in duration or intensity of the initial SE was observed between genotypes. These data are the first to indicate a possible neuroprotective role of AQP4 aside from the well-known role of AQP4 in brain water balance (Manley et al., 2004; Verkman, 2005).

Conclusion

Future studies will need to examine the mechanisms of AQP4 regulation in greater detail at miRNA and protein levels. In addition to previously described roles in K⁺ regulation, the lack of AQP4 also appears to promote hyperexcitability and neurotoxicity early after SE. Still undetermined is the contribution of astrocytic cell swelling itself to excitotoxicity and seizure generation. Since astrocytes release glutamate under hypoxic stress through a calcium-independent pathway (Fiacco et al., 2007), swollen AQP4-deficient astrocytes could potentially contribute directly to excitability and excitotoxicity by increased release of glutamate, in addition to the more established contribution of loss of K⁺ buffering. It will be interesting to elucidate the role of astrocytic swelling and astrocytic swelling-induced glutamate release in acute seizures and during epileptogenesis, as well as to develop novel methods to upregulate AQP4 as a possible therapy to restore water and potassium homeostasis.

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References


