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SHORT COMMUNICATION

Aquaporin-4-dependent edema clearance following status epilepticus

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Summary We investigated the role of aquaporin-4 in the development of cerebral edema following kainic acid-induced status epilepticus (SE) using specific gravimetry and T2 MRI techniques at 6 h, 1 day, 4 days and 7 days after SE. Our results indicate significantly greater tissue edema and T2 MRI changes in AQP4$^{-/-}$ compared to AQP4$^{+/+}$ mice that peaks at about 1 day after SE (greater in hippocampus relative to cortex). These results have implications for the mechanisms of edema formation and clearance following intense seizure activity.

Introduction

While there have been many MRI studies demonstrating changes in T2 or apparent diffusion coefficient following status epilepticus, previous studies have not directly characterized changes in tissue water content. In addition, the mechanisms of seizure-induced edema formation and clearance are unclear. The availability of mice deficient in the glial water channel aquaporin-4 (AQP4) (Ma et al., 1997) allows study of the role of AQP4 in seizure-induced water movement in the brain. AQP4-null mice have reduced brain swelling and improved neurological outcome in models of (cellular) cytotoxic edema (Verkman et al., 2006). Conversely, AQP4-null mice have worsened outcome in models of vasogenic edema, presumably due to impaired clearance of tissue water (Papadopoulos et al., 2004).

Water content can be estimated by sensitive and accurate measurements of tissue specific gravity using bromobenzene—kerosene gravimetry (Marmarou et al., 1978). Using this technique, we analyzed hippocampal and cortical tissue samples at various time points after kainic acid SE in wild type (WT) and AQP4-null mice. Finally, we obtained coronal T2 MRI images at time points corresponding to the water content data to compare T2 signal changes with water content changes.

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Methods

Kainic acid status epilepticus

Kainic acid (30 mg/kg, s.c.) was administered to 6—8-week-old $AQP4^{+/+}$ and $AQP4^{-/-}$ male mice to induce status epilepticus (SE) (Ferraro et al., 1995). Mice were sacrificed at 6 h, 1 day, 4 days and 7 days after the initial SE. Saline-injected controls were used for each genotype ($n = 4$ controls and $n = 7$—9 experimental mice of each genotype per time point).

Specific gravity and percent difference in water content

Bilateral hippocampal and cortical microdissections were performed at the above time points. Specific gravities (SGs) of the hippocampal and cortical biopsies were obtained using an organic (bromobenzene—kerosene) density gradient column (Supplemental Fig. 1). Hippocampal and cortical SGs at each time point were compared with saline controls, and compared between $AQP4^{+/+}$ and $AQP4^{-/-}$ mice. Specific gravities were then converted to percent difference in water content (Emerson et al., 1999).

Statistical analysis was performed using a two-way ANOVA with *post-hoc* Bonferroni multiple comparison tests.

Magnetic resonance imaging

Magnetic resonance imaging (7.0 Tesla, TR = 3 s, TE = 60 ms, 0.7 mm slice thickness, 15 cm bore, MR Research Systems MR3330 magnet, Resonance Research actively shielded and water cooled gradient coil Model BFG-150/90-S) was performed on a separate group of $AQP4^{+/+}$ and $AQP4^{-/-}$ mice distinct from the water content analysis with water references at baseline and after SE at 6 h, 1 day, 4 days and 7 days. T2 intensities of the hippocampus were measured on multiple MRI slices, sampling left and right cortex and left and right dorsal and ventral hippocampi (circular sample, 0.5 mm diameter). Mean T2 intensities for each hippocampal and cortical sample were normalized to an imaged water reference.

Statistical analysis was performed using a two-way repeated measures ANOVA with *post-hoc* Bonferroni multiple comparison tests.

Results

Specific gravity and water content

Baseline specific gravities (SG) of $AQP4^{+/+}$ hippocampal and cortical tissue samples were slightly higher than those of $AQP4^{-/-}$ mice (hippocampus: 1.04411 ± 0.00018 versus 1.04294 ± 0.00017, $p < 0.0001$ and cortex: 1.04552 ± 0.00022 versus 1.04470 ± 0.00016, $p < 0.005$). The baseline percent difference in water content was 2.56% higher in $AQP4^{-/-}$ hippocampal samples and 1.84% higher in cortical samples compared to WT samples.

At 6 h after the initial SE, there were no significant changes in hippocampal or cortical SGs in both $AQP4^{+/+}$ (hippocampus experimental: 1.04319 ± 0.00120 versus control: 1.04305 ± 0.00050, $p > 0.05$) and $AQP4^{-/-}$ (hippocampus experimental: 1.04120 ± 0.00121 versus control: 1.04227 ± 0.00050, $p > 0.05$) mice relative to saline controls. Both $AQP4^{+/+}$ and $AQP4^{-/-}$ mice exhibited a trend toward increased water content at 6 h ($2.78 \pm 0.81\%$ and $2.64 \pm 1.61\%$, respectively, $p > 0.05$); however, $AQP4^{-/-}$ mice following SE had a significantly higher water content than $AQP4^{+/+}$ mice following SE ($p < 0.05$) (Fig. 1). At 1 day after SE, there was a significant reduction in hippocampal SG in $AQP4^{-/-}$ mice ($AQP4^{-/-}$ hippocampus experimental SG: 1.04093 ± 0.00057 versus control 1.04411 ± 0.00026, $p < 0.01$). At the same time point, there was a nonsignificant trend toward reduction in hippocampal SG in $AQP4^{+/+}$ mice ($AQP4^{+/+}$ hippocampus experimental SG: 1.04408 ± 0.00018 versus control 1.04575 ± 0.00019, $p > 0.05$). The difference between $AQP4^{+/+}$ and $AQP4^{-/-}$ mice was statistically significant ($p < 0.05$), demonstrating a higher water content in $AQP4^{-/-}$ mice. Expressed as calculated % water content, $AQP4^{-/-}$ mice demonstrated a significant increase in hippocampal % water content compared to saline-injected genotype controls ($8.75 \pm 1.56\%$, $p < 0.01$); $AQP4^{+/+}$ mice showed a trend toward increased hippocampal water content compared to saline-injected genotype controls ($3.81 \pm 0.43\%$, $p > 0.05$). The percent difference in water content relative to saline controls was significantly greater in $AQP4^{-/-}$ mice relative to $AQP4^{+/+}$ mice ($p < 0.01$, Fig. 1).

By day 4 after SE, $AQP4^{+/+}$ hippocampal SG had recovered to baseline and was actually higher than saline controls (experimental: 1.04509 ± 0.00016 versus control: 1.04394 ± 0.00034, $p < 0.05$). Consequently, percent difference in water content relative to saline controls decreased by $2.54 \pm 0.34\%$. By contrast, $AQP4^{-/-}$ hippocampal SG was no different than baseline (experimental: 1.04412 ± 0.00063 versus control: 1.04341 ± 0.00029, $p > 0.05$). On day 7 after SE, $AQP4^{+/+}$ and $AQP4^{-/-}$ hippocampal SG had returned to baseline, with no significant changes in percent water content relative to saline-injected controls. $AQP4^{-/-}$ mice again had higher water content (lower SG) than $AQP4^{+/+}$ mice at this time point ($p < 0.05$, Supplemental Fig. 2).

We separately analyzed cortical tissue samples at the identical time points. At 1 day after SE, there was a trend toward increased cortical tissue water content in $AQP4^{-/-}$ mice compared to saline-injected genotype controls ($5.03 \pm 1.71\%$). However, at all time points there was no significant difference in cortical percent water content relative to controls in either genotype (Fig. 1B).

T2 MRI changes after status epilepticus

Analysis of T2 MR images (Fig. 2 and Supplemental Fig. 3) demonstrated that $AQP4^{+/+}$ mice had significantly greater hippocampal T2 intensities compared to saline-injected controls at 6 h after SE (experimental: 1.108 ± 0.036 versus control: 0.853 ± 0.028, $p < 0.01$); however, no significant differences were noted in the $AQP4^{-/-}$ mice relative to control $AQP4^{-/-}$ mice at the same time point (experimental: 1.126 ± 0.060 versus control: 1.051 ± 0.051, $p > 0.05$). At 24 h after SE, $AQP4^{+/+}$ mice were no different than control animals (experimental: 1.069 ± 0.037 versus control: 0.893 ± 0.037, $p > 0.05$); however, at 24 h after SE, $AQP4^{-/-}$ mice had significantly greater T2 intensities than control $AQP4^{-/-}$ mice (experimental: 1.215 ± 0.0446 versus control: 0.986 ± 0.088, $p < 0.05$). At 4 days and 7 days after status epilepticus, there was no significant difference in T2 intensities relative to baseline in either genotype.

A separate analysis of cortical T2 intensities demonstrated no significant difference between $AQP4^{+/+}$ and $AQP4^{-/-}$ mice after SE relative to control mice (data not shown).

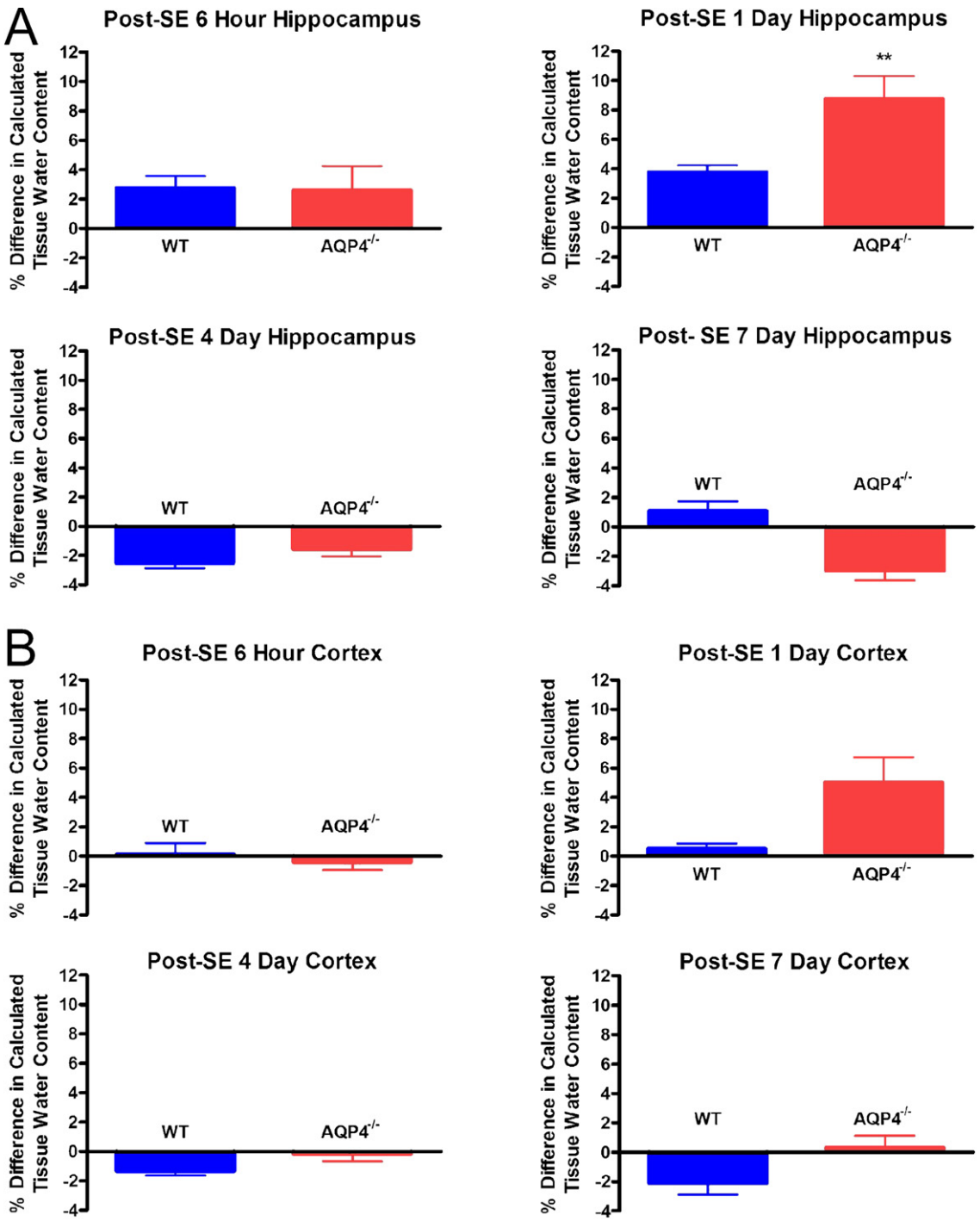

Figure 1 Hippocampal and cortical water content following status epilepticus. (A) Percent difference in hippocampal water content (derived from specific gravity measurements) compared to saline-injected controls at 6 h, 1 day, 4 days, and 7 days after kainic acid-induced SE. At 6 h after SE, there is a trend toward increased hippocampal water content in both genotypes (A, $p > 0.05$). At 1 day after SE, there is a marked increase in hippocampal water content in AQP4$^{-/-}$ but not in WT mice ($p < 0.01$). At 4 and 7 days post-SE, there is no significant difference in percent water content relative to baseline in either genotype. (B) Percent difference in cortical water content compared to saline-injected controls after kainic acid-induced SE. At 1 day after SE, there is a trend toward increased cortical tissue water content in AQP4$^{-/-}$ mice. However, at all time points there is no significant difference in cortical percent water content relative to baseline in either genotype.

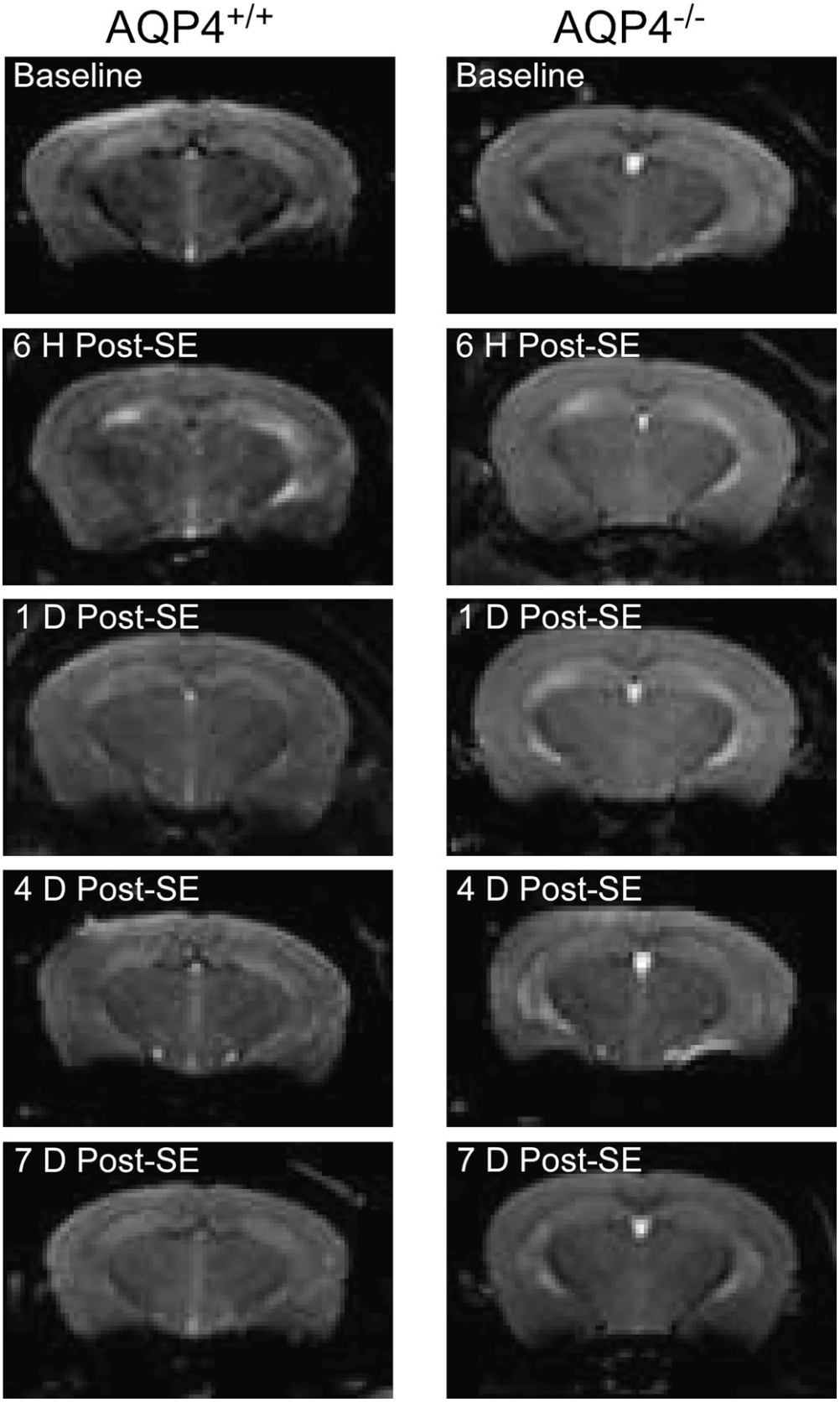

Figure 2 T2 MRI images following status epilepticus. Representative coronal T2 images at indicated time points at baseline and following SE in AQP4$^{+/+}$ (left) and AQP4$^{-/-}$ (right) mice. Note the increase in T2 signal intensity in the hippocampi at 6 h post-SE in both genotypes. Also note the marked bilateral increase in T2 signal intensity at 1 day post-SE in both dorsal and ventral hippocampi in the AQP4$^{-/-}$ but not WT mice.

Discussion

Using bromobenzene—kerosene gravimetry, we studied changes in tissue water content following SE. First, we found that significant changes in hippocampal water content occur after kainic acid SE. Second, there is a greater magnitude of increase in hippocampal water content in AQP4$^{-/-}$ mice 1 day post-SE. Third, these changes in water content coincide with increased T2 MRI signal during the same time course. Fourth, both the water content and T2 changes were found in hippocampus but not in cortex.

The marked increase in seizure-induced tissue water content in AQP4$^{-/-}$ mice suggests impaired clearance of tissue water. These results are quite similar to previous results in AQP4$^{-/-}$ mice indicating impaired clearance of vasogenic edema fluid in models of brain tumor, saline infusion, and brain abscess (Bloch et al., 2005; Papadopoulos et al., 2004). Previous research supports a transient increase in water content (24—48 h post-kainic acid) following status epilepticus (Kim et al., 2010; Nelson and Olson, 1987). Taken together with previous results indicating a breakdown of the blood—brain barrier following intense seizure activity, our results are therefore most consistent for a role of AQP4 in the clearance of water that entered the brain due to breakdown of the BBB during the episode of SE (i.e. vasogenic edema).

Two of our findings also suggest that indeed increases in T2 MRI intensity reflect increased tissue water content early after SE: (1) the similar time course of water content changes and T2 MRI changes; (2) the shared anatomic specificity of changes in this model in hippocampus but not cortex. Previous data has suggested that T2 signal intensity may increase and persist in the context of brain edema and seizures (Briellmann et al., 2005); however, in our model, T2 signal intensity appears to peak at specific time periods associated with the greatest differences in percent water content.

In summary, our results show that hippocampal but not cortical edema occurs after kainic acid SE in mice, and that AQP4 appears to be involved in regulation of tissue water content after status epilepticus. Interestingly, edema itself may contribute to epileptogenesis, as hypoosmolarity and reduction of extracellular space volume potently affect excitability (Schwartzkroin et al., 1998; Traynelis and Dingledine, 1989), reduction of edema by administration of mannitol may reduce seizure-induced histological damage (Nelson and Olson, 1987). Furthermore, changes in AQP4 distribution in epileptic hippocampi (Eid et al., 2005) may affect the ability of the tissue to clear water following repeated seizure activity. Further studies will need to delineate the therapeutic window for reduction of seizure-induced edema and determine whether treatments to reduce edema or restore water homeostasis may lead to improved outcome.

Conflicts of interest

The authors have no conflicts of interest to report related to this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eplepsyres.2011.09.016.

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