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Research Paper

Regulation of astrocyte glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4) expression in a model of epilepsy

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ABSTRACT

Astrocytes regulate extracellular glutamate and water homeostasis through the astrocyte-specific membrane proteins glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4), respectively. The role of astrocytes and the regulation of GLT1 and AQP4 in epilepsy are not fully understood. In this study, we investigated the expression of GLT1 and AQP4 in the intrahippocampal kainic acid (IHKA) model of temporal lobe epilepsy (TLE). We used real-time polymerase chain reaction (RT-PCR), Western blot, and immunohistochemical analysis at 1, 4, 7, and 30 days after kainic acid-induced status epilepticus (SE) to determine hippocampal glial fibrillary acidic protein (GFAP, a marker for reactive astrocytes), GLT1, and AQP4 expression changes during the development of epilepsy (epileptogenesis). Following IHKA, all mice had SE and progressive increases in GFAP immunoreactivity and GFAP protein expression out to 30 days post-SE. A significant initial increase in dorsal hippocampal GLT1 immunoreactivity and protein levels were observed 1 day post SE and followed by a marked downregulation at 4 and 7 days post SE with a return to near control levels by 30 days post SE. AQP4 dorsal hippocampal protein expression was significantly downregulated at 1 day post SE and was followed by a gradual return to baseline levels with a significant increase in ipsilateral protein levels by 30 days post SE. Transient increases in GFAP and AQP4 mRNA were also observed. Our findings suggest that specific molecular changes in astrocyte glutamate transporters and water channels occur during epileptogenesis in this model, and suggest the novel therapeutic strategy of restoring glutamate and water homeostasis.

1. Introduction

Epilepsy is a group of disorders characterized by the unpredictable occurrence of seizures. It is a major public health concern and is estimated to afflict one in 26 people in their lifetime (Hesdorffer et al., 2011). Temporal lobe epilepsy (TLE), the most common form of epilepsy, affects over 40 million people worldwide alone (de Lanerolle et al., 2012). Approximately 30% of patients taking antiepileptic drugs (AEDs), however, do not become seizure-free with existing medications. Current AEDs primarily target neurons and often cause severe cognitive, developmental, and behavioral side effects. Therefore, new drugs based on non-neuronal targets are an appealing alternative approach with potentially fewer deleterious effects. Although it has been well established that increased neuronal excitability is a major contributor to epilepsy, increasing evidence suggests that changes in astrocytes contribute to the development of epilepsy (Hubbard et al., 2013).

Astrocytes, a critical component of the tripartite synapse (Araque et al., 1999), participate in ionic homeostasis, energy metabolism (Ransom and Ransom, 2012), the formation of synaptic networks (Ransom et al., 2003), and the modulation of synaptic transmission (Halassa and Haydon, 2010; Murphy-Royal et al., 2015; Volterra and Meldolesi, 2005). Striking changes in astrocytic shape and function are observed in "reactive" cells, often measured by levels of the intermediate filament glial fibrillary acidic protein (GFAP). Based on not only morphological alterations but also functional changes in expression of channels and receptors, reactive astrocytes may contribute to increased neuronal excitability and the development of epilepsy (Binder and Steinhäuser, 2006; Hubbard et al., 2013).

Extracellular glutamate levels determine the extent of neuronal excitability; therefore it is crucial to maintain low glutamate levels in the extracellular space (ECS). Glutamate transporters are responsible

Abbreviations: Antiepileptic drugs, (AEDs); aquaporin-4, (AQP4); central nervous system, (CNS); 4′,6-diamidino-2-phenylindole, (DAPI); electroencephalogram, (EEG); extracellular space, (ECS); glial fibrillary acidic protein, (GFAP); glutamate transporter-1, (GLT1); granule cell layer, (GCL); hippocampal sclerosis, (HS); kilodalton, (KDa); real-time polymerase chain reaction, (RT-PCR); status epilepticus, (SE); stratum lacunosum moleculare, (SLM).

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for removing glutamate from the ECS. The most prominently expressed glutamate transporter in the mammalian forebrain is glutamate transporter-1 (GLT1) (Danbolt, 2001), found almost exclusively on astrocytes. Deletion (Tanaka et al., 1997) or antisense oligonucleotide-mediated inhibition of synthesis (Rothstein et al., 1996) of GLT1 in rodents revealed that it is the major contributor to glutamate uptake from the ECS. GLT1-mediated glutamate removal from the tripartite synapse is conducted in an activity-regulated manner, thereby shaping synaptic transmission (Murphy-Royal et al., 2015). Furthermore, homozygous mice deficient in GLT1 develop lethal spontaneous seizures (Tanaka et al., 1997). Overexpression of GLT1, on the other hand, attenuated epileptogenesis and reduced seizure frequency in transgenic mice (Kong et al., 2012). It is widely accepted that delayed glutamate clearance from the ECS is implicated in seizures (Campbell and Hablitz, 2004; During and Spencer, 1993; Glass and Dragunow, 1995; Hubbard et al., 2013; Tanaka et al., 1997), but whether changes in GLT1 expression or function underlie the development of epilepsy is unknown.

The aquaporins (AQPs) are a family of small, hydrophobic membrane water channels that facilitate water transport in response to osmotic gradients (Agre et al., 2002; Amiry-Moghaddam and Ottersen, 2003; Binder et al., 2012; Verkman, 2005). Aquaporin-4 (AQP4) is the main water channel in the brain and spinal cord and is expressed by glial cells, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels and astrocyte membranes that ensheathe glutamatergic synapses (Nagelhus et al., 2004; Nielsen et al., 1997; Rash et al., 1998). The creation of AQP4 knockout mice in 1997 (Ma et al., 1997) helped elucidate the various functions of AQP4 in the brain. It is now known that AQP4 plays a role in potassium buffering (Binder et al., 2006), modulation of extracellular space diffusion (Binder et al., 2004), and even in synaptic plasticity and memory (Skucas et al., 2011; Szu and Binder, 2016). Mice deficient in AQP4 have increased seizure duration (Binder et al., 2006) and decreased AQP4 immunoreactivity during the early epileptogenic phase in mouse models of epilepsy (Alvestad et al., 2013; Lee et al., 2012b). AQP4 has been implicated in epilepsy (Binder et al., 2012; Binder et al., 2006; Eid et al., 2005; Lee et al., 2012a; Lee et al., 2012b), but the expression and regulation of AQP4 during epileptogenesis has not been fully characterized.

In this study, we used the well-established intrahippocampal kainic acid (IHKA) mouse model of temporal lobe epilepsy (Arabadzisz et al., 2005; Bouilleret et al., 1999; Riban et al., 2002) to fully examine GFAP, GLT1 and AQP4 regulation during epileptogenesis. We used real-time polymerase chain reaction (RT-PCR), Western blot, and immunohistochemical analysis at 1, 4, 7, and 30 days after IHKA-induced status epilepticus (SE) to determine hippocampal GFAP, GLT1, and AQP4 expression changes during the development of epilepsy. We found a significant initial increase in dorsal hippocampal GLT1 immunoreactivity and protein levels 1 day post SE and a significant downregulation by 7 days post SE. AQP4 dorsal hippocampal protein levels were downregulated at early time points after SE and were upregulated ipsilaterally at 30 days post SE. These results indicate significant downregulation of these critical glial transporters (GLT1 and AQP4) during the early epileptogenic period in this model.

2. Methods

2.1. Animals

All experiments were conducted in accordance with National Institutes of Health guidelines and were approved by the University of California, Riverside Institutional Animal Care and Use Committee (IACUC). Animals were housed under a 12 h light/12 h dark cycle with food and water provided *ad libitum*. 7 to 8-week-old CD1 male mice from Charles River were used for these experiments.

2.2. Surgery

We used intrahippocampal kainic acid (IHKA) injections to induce epileptogenesis (Arabadzisz et al., 2005; Bouilleret et al., 1999; Riban et al., 2002). Briefly, mice were anesthetized with an intraperitoneal (i.p.) injection of a mixture of 80 mg/kg ketamine and 10 mg/kg xylazine and mounted in a stereotaxic frame. An incision was made to expose the skull and bregma was located. Using stereotaxic coordinates of the hippocampus (Paxinos and Franklin, 2001), a 0.6 mm burr hole was made 1.8 mm posterior and 1.6 mm lateral from bregma with a high-speed drill (Drummond Scientific). Mice were injected with either 74 nL of a 20 mM solution of kainic acid or an equal volume of 0.9% saline over a period of 4 min using a microinjector (Nanoject, Drummond Scientific) into the right dorsal hippocampus (dorsoventral coordinate 1.9 mm).

2.3. Kainic acid status epilepticus

After kainic acid injections, all mice experienced status epilepticus (SE), defined by Racine scale stage 3–5 seizures (Racine, 1972) continuously for a period of 3 or more hours. In preliminary studies, we verified the presence of epileptiform activity in 100% of animals after IHKA injections by video-electroencephalogram (EEG) monitoring (Lee et al., 2012b) (Supplemental Fig. S1 in the online version at http://dx.doi.org/10.1016/j.expneurol.2016.05.003.). Within 30 min of IHKA injections, mice experienced SE for several hours, which spontaneously subsided. In this model, mice exhibit spontaneous recurrent seizures in both hippocampi (Supplemental Fig. S1 in the online version at http://dx.doi.org/10.1016/j.expneurol.2016.05.003.). Previous studies in the IHKA model (Arabadzisz et al., 2005; Bouilleret et al., 1999; Riban et al., 2002) demonstrated that the model reproduced morphological characteristics of mesial temporal sclerosis, including neuronal loss, gliosis, mossy fiber sprouting, and dentate granule cell dispersion. We have previously confirmed the pattern of neuronal loss in CA1 and progressive dentate granule cell dispersion as well as performed chronic video-EEG recording in this model (Lee et al., 2012b) (Supplemental Fig. S1 in the online version at http://dx.doi.org/10.1016/j.expneurol.2016.05.003.).

For the current studies, we wished to avoid electrode damage to the brain and therefore proceeded without the use of EEG implantation. Instead, we monitored animals for behavioral seizures. All animals experienced continuous (3 or more hours) Racine stage 3–5 seizures (Racine, 1972), characterized by forelimb and hindlimb clonus, rearing, and falling. Animals that died due to SE were excluded from the study. Otherwise, at each time point $n = 5$ animals were euthanized with fatal plus (Western Medical Supply), perfused, and processed for each experiment, unless otherwise specified.

2.4. Immunohistochemistry

Mice were perfused transcardially with ice-cold phosphate buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde, pH 7.4. Brains were quickly removed and postfixed in 4% paraformaldehyde overnight at 4 °C followed by two days of cryoprotection in 30% sucrose in PBS at 4 °C. Brains were then cut into 50 μm coronal sections using a cryostat (Leica CM 1950, Leica Microsytems, Bannockburn, IL) and stored in PBS at 4 °C. All slices were processed simultaneously. Endogenous peroxidase activity was quenched by incubating slices in 3% H_2O_2 for 1 h at room temperature. This was followed by a 1 h blocking step with 5% normal goat serum in 0.1 M PBS. Slices were then incubated with primary antibody to GLT1 (1:3000, AbCam AB41621) and GFAP (1:200, Millipore MAB360) in 0.3% Triton X-100 overnight at 4 °C. After washing slices with PBS, sections were incubated with species-specific secondary antibody conjugated with Alexa 488 or 594 and a tyramide signaling amplification (TSA) kit (Molecular Probes/Invitrogen) for visualization. Slices were mounted in Vectorshield with DAPI (Vector Laboratories)