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HIPPOCAMPAL TISSUE OF PATIENTS WITH REFRACTORY TEMPORAL LOBE EPILEPSY IS ASSOCIATED WITH ASTROCYTE ACTIVATION, INFLAMMATION, AND ALTERED EXPRESSION OF CHANNELS AND RECEPTORS

A. DAS,[a] G. C. WALLACE IV,[a] C. HOLMES,[a] M. L. MCDOWELL,[a] J. A. SMITH,[a] J. D. MARSHALL,[a] L. BONILHA,[a] J. C. EDWARDS,[a] S. S. GLAZIER,[a] S. K. RAY[b] AND N. L. BANIK[a]*

[a] *Department of Neurosciences (Divisions of Neurology and Neurosurgery), Medical University of South Carolina, Charleston, SC 29425, USA*

[b] *Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC 29209, USA*

Abstract—Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy. Previous research has demonstrated several trends in human tissue that, undoubtedly, contribute to the development and progression of TLE. In this study we examined resected human hippocampus tissue for a variety of changes including gliosis that might contribute to the development and presentation of TLE. The study subjects consisted of six TLE patients and three sudden-death controls. Clinicopathological characteristics were evaluated by H&E staining. Immunohistological staining and Western blotting methods were used to analyze the samples. Neuronal hypertrophy was observed in resected epileptic tissue. Immunohistological staining demonstrated that activation of astrocytes was significantly increased in epileptic tissue as compared to corresponding regions of the control group. The Western blot data also showed increased CX43 and AQP4 in the hippocampus and downregulation of Kir4.1, α-syntrophin, and dystrophin, the key constituents of AQP4 multi-molecular complex. These tissues also demonstrated changes in inflammatory factors (COX-2, TGF-β, NF-κB) suggesting that these molecules may play an important role in TLE pathogenesis. In addition we detected increases in metabotropic glutamate receptor (mGluR) 2/3, mGluR5 and kainic acid receptor subunits KA1 (Grik4) and KA2 (Grik5) in patients' hippocampi. We noted increased expression of the α1c subunit comprising class C L-type Ca^{2+} channels and calpain expression in these tissues, suggesting that these subunits might have an integral role in TLE pathogenesis. These changes found in the resected tissue suggest that they may contribute to TLE and that the kainic acid receptor (KAR) and deregulation of GluR2 receptor may play an important role in TLE development and disease course. This study identifies alterations in number of commonly studied molecular targets associated with astrogliosis, cellular hypertrophy, water homeostasis, inflammation, and modulation of excitatory neurotransmission in hippocampal tissues from TLE patients. Published by Elsevier Ltd. on behalf of IBRO.

Key words: temporal lobe epilepsy, gliosis, inflammatory factors, aquaporin, expression of channels and receptors.

BACKGROUND

Epilepsy is a chronic brain disorder, defined by spontaneous recurrent seizures. Temporal lobe epilepsy (TLE), the most common form in adults, is generally characterized by a unilateral temporal lobe seizure foci (Cascino, 2005; Sharma et al., 2007). Due to the lack of effective pharmacotherapies, a relatively large percentage of the TLE patients suffer from medically intractable seizures. As a result, invasive resection of the seizure focus is often recommended as a final alternative to improve quality of life. Analysis of hippocampal tissue resected from TLE patients and animal models of this disorder reveal a number of histopathological changes including neuronal loss in the CA1 and CA3 regions, sclerotic gliosis, and aberrant mossy fiber sprouting from dentate granule cells (de Lanerolle et al., 2003; Wieser, 2004; Cascino, 2005; Epsztein et al., 2005; Sharma et al., 2007; Bae et al., 2010; Melo et al., 2010; Yang et al., 2010). Despite intensive study, changes at the molecular level responsible for the histological aberrations observed in TLE patients are largely unknown. Further understanding of these molecular alterations may provide insight into disease pathology and novel therapeutic targets for the treatment of TLE.

Epileptogenesis is not strictly a result of neural pathway dysfunction, but may also occur due to aberrant glial cell function. However, the role of glial cells, particularly astrocytes, in TLE pathogenesis and progression remains understudied. Reactive astrogliosis generally serves as a non-specific marker of scarring in the central nervous system (CNS) and has been noted in animal models of epilepsy where seizures cause their activation and subsequent pro-inflammatory cytokine production (Ridet et al., 1997; Choi et al., 2009; Lauri and Taira, 2011; Ravizza

*Corresponding author. Address: Division of Neurology, Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425, USA. Tel: +1-(843)-792-3946; fax: +1-(843)-792-8626.
E-mail address: baniknl@musc.edu (N. L. Banik).

Abbreviations: AQP, aquaporin; BBB, blood–brain-barrier; COX-2, cyclooxegenase-2; CX43, connexin 43; DAPC, dystrophin-associated protein complex; GFAP, glial fibrilary acidic protein; Kir4.1, K^+ channel 4.1; mGluR, metabotropic glutamate receptor; NF-κB, nuclear factor kappa beta; TGF-β, transforming growth factor beta; TLE, temporal lobe epilepsy.

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et al., 2011). Astrocytic activation results in varying outcomes following seizure because inflammatory mediators may protect or damage CNS cells (Raivich et al., 1999). Therefore, a delicate balance between pro-inflammatory and anti-inflammatory processes may determine the extent of residual cellular damage in affected brain regions of TLE patients.

In addition to regulating inflammatory activity in the epileptic brain, astroglia also act to buffer extracellular ions and neurotransmitter levels thus maintaining synaptic homeostasis (Shao and McCarthy, 1994). Voltage-gated Ca^{2+} channels serve as one mechanism by which astrocytes respond to and regulate ion gradients across neuronal membranes (Dani et al., 1992; Papadopoulos et al., 2002). Manipulation of cell volume is another means by which astrocytes regulate both extracellular volume and ionic concentrations and has been shown to affect neuronal excitability (D'Ambrosio, 2006; Broberg et al., 2008). Aquaporin (AQP) 4, the predominant form of AQP expressed in astrocytes, is a membrane-associated water channel that plays an important role in maintaining the extracellular environment. Altered expression of AQP4 has been shown in response to a number of pathological conditions marked by astrocytic activation and/or blood–brain-barrier (BBB) disruption (Taniguchi et al., 2000; Theis et al., 2004; St. Hilarie et al., 2005). These observations suggest that astroglial AQP4 may modulate neuronal excitability by regulating the extraneuronal and extrasynaptic environments. Connexin 43 (CX43), a major component protein of astrocyte gap junctions, also aids in astrocytic regulation of the extracellular environment in the CNS (Wolburg et al., 2009). As osmolarity within the astrocyte increases, solutes (such as neurotransmitters) diffuse through CX43-expressing astrocytic gap junctions allowing for greater neurotransmitter clearance from the extracellular milieu.

There is also physiological evidence that coupling of the inwardly rectifying K^+ channel 4.1 (Kir4.1) to AQP4 may have a role in neuronal excitability. It is believed that Kir4.1 and AQP4 are key constituents of a multi-molecular complex in astrocytes that includes α-syntrophin, Dp71, dystrobrevin, α-dystroglycan, and β-dystroglycan. This protein complex, typically localized to the perivascular astrocyte endfoot, permits efficient uptake of K^+ and regulation of neuronal membrane potential (Aronica et al., 2000). Increased membrane expression of metabotropic glutamate receptors (mGluR)5 and mGluR2/3 has been demonstrated in reactive astrocytes of epileptic hippocampal tissue (Seifert et al., 2006). Activation of these glial mGluRs can regulate glial function and facilitate neuron–glia communication (Seifert et al., 2006). Furthermore, mGluR3 can be co-localized with AQP4, suggesting a role for astrocytic mGluRs in regulation of extracellular glutamate levels (Jankowsky and Patterson, 2001; Seifert et al., 2006).

Mounting evidence also suggests that other glutamate receptors including kainate receptor subunits KA1 and KA2 are contributors to neuronal pre- and post-synaptic activities through direct modulation of intracellular Ca^{2+} level (Darstein et al., 2003). KA1 and KA2 are differentially expressed in the hippocampus with KA1 mRNA expression restricted to the CA3 region whereas KA2 mRNA is widespread (Lauri and Taira, 2011). Altered levels of KA1 or KA2 expression could have serious functional consequences for synaptic homeostasis.

In the current study, we examined surgically resected hippocampal tissue from TLE patients for a variety of changes that may contribute to the development and progression of TLE. Glial fibrilary acidic protein (GFAP) immunoreactivity, generalized cellular swelling, and inflammatory mediators such as cyclooxegenase-2 (COX-2), transforming growth factor beta (TGF-β), nuclear factor kappa B (NF-κB) were determined as measures of astrocyte activation. Levels of several known regulators of extracellular environment and neuronal excitability (AQP4, CX43, Kir4.1, mGluRs, KA1/2, etc.) were also measured to determine their potential involvement in TLE pathology. Findings presented in this study suggest that refractory TLE may be characterized by aberrant expression of osmoregulatory proteins, mGluRs, and the Kir4.1 potassium channel. Furthermore, a number of associated pathological changes were noted, including increased GFAP immunoreactivity, cellular swelling, and production of pro-inflammatory mediators that might be indicative of generalized astrocyte activation in the epileptic hippocampus. Together, the biochemical and cellular alterations described in this study may serve both as potential disease markers and targets for therapeutic intervention in TLE patients.

EXPERIMENTAL PROCEDURES

Acquisition of human epileptic samples

Patient samples were collected following temporal lobe resection performed as treatment for refractory TLE. Patient consent was obtained for tissue use before surgery. All patients included in this study were diagnosed as epileptic according to the criteria defined by the International League Against Epilepsy (Commission of Classification and Terminology of the International League Against Epilepsy). Patients were evaluated based on clinical history, physical examination, ictal EEG recording, and MRI imaging. All patients were considered to have medication refractory TLE, and after careful review of the clinical information by the multi-disciplinary comprehensive epilepsy team at the Medical University of South Carolina, all patients were referred for anterior temporal lobectomy for treatment of epilepsy. All surgeries were performed by the same neurosurgeon (SG) and the surgical technique was equivalent for all cases. Tissue was collected and handled according to the Medical University of South Carolina procedures and with approval of the Institutional Review Board (IRB) of the Medical University. Postmortem blocks of tissue to be used as controls in this study were obtained from the Harvard Brain Research Center and Medical Research Council Sudden Death Brain & Tissue Bank in Edinburgh, Scotland. To the best of our knowledge, control patients were not taking neuroactive medication at the time of death. Hippocampal samples were surgically removed from deceased patients, and within 15 min were processed and stored at −80 °C.

H&E Staining

Sections of 8-μm thickness were taken using a Rechert-Jung cryostat (Leica, Deerfield, IL, USA) and processed for hematoxylin and eosin (H&E) staining by standard methods. Three slides