Neuroscience 248 (2013) 602–619

STATUS EPILEPTICUS TRIGGERS EARLY AND LATE ALTERATIONS IN BRAIN-DERIVED NEUROTROPHIC FACTOR AND NMDA GLUTAMATE RECEPTOR *GRIN2B* DNA METHYLATION LEVELS IN THE HIPPOCAMPUS

R. RYLEY PARRISH, A. J. ALBERTSON, S. C. BUCKINGHAM, J. J. HABLITZ, K. L. MASCIA, W. DAVIS HASELDEN AND F. D. LUBIN *

Evelyn F. McKnight Brain Institute, Department of Neurobiology, University of Alabama at Birmingham, 1825 University Boulevard, Birmingham, AL 35294, USA

Abstract—Status epilepticus (SE) triggers abnormal expression of genes in the hippocampus, such as glutamate receptor subunit epsilon-2 (*Grin2b*/*Nr2b*) and brain-derived neurotrophic factor (*Bdnf*), that is thought to occur in temporal lobe epilepsy (TLE). We examined the underlying DNA methylation mechanisms and investigated whether these mechanisms contribute to the expression of these gene targets in the epileptic hippocampus. Experimental TLE was provoked by kainic acid-induced SE. Bisulfite sequencing analysis revealed increased *Grin2b*/*Nr2b* and decreased *Bdnf* DNA methylation levels that corresponded to decreased *Grin2b*/*Nr2b* and increased *Bdnf* mRNA and protein expression in the epileptic hippocampus. Blockade of DNA methyltransferase (DNMT) activity with zebularine decreased global DNA methylation levels and reduced *Grin2b*/*Nr2b*, but not *Bdnf*, DNA methylation levels. Interestingly, we found that DNMT blockade further decreased *Grin2b*/*Nr2b* mRNA expression whereas GRIN2B protein expression increased in the epileptic hippocampus, suggesting that a posttranscriptional mechanism may be involved. Using chromatin immunoprecipitation analysis we found that DNMT inhibition restored the decreases in AP2alpha transcription factor levels at the *Grin2b*/*Nr2b* promoter in the epileptic hippocampus. DNMT inhibition increased field excitatory postsynaptic potential in hippocampal slices isolated from epileptic rats. Electroencephalography (EEG) monitoring confirmed that DNMT inhibition did not significantly alter the disease course, but promoted the latency to seizure onset or SE. Thus, DNA methylation may be an early event triggered by SE that persists late into the epileptic hippocampus to contribute to gene expression changes in TLE. Published by Elsevier Ltd. on behalf of IBRO.

Key words: epigenetics, *Bdnf*, *Grin2b*/*Nr2b*, epilepsy, seizures, DNA demethylation.

INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures. In humans, temporal lobe epilepsy (TLE) is the most common adult form of epilepsy and can be triggered by an insult such as status epilepticus (SE) or prolonged seizure activity. SE results in changes in gene expression that are thought to subsequently lead to molecular and structural changes to produce the epileptic phenotype (Jones et al., 2001; Ma et al., 2009b; Kobow and Blumcke, 2011). However, to date, the underlying transcriptional mechanisms that orchestrate aberrant steady-state gene expression changes in TLE are still uncertain. Investigation of these underlying mechanisms is important because targeting them may help to mitigate or disrupt the epileptic phenotype.

DNA methylation is a potent epigenetic regulator of chromatin structure that controls persistent gene expression in the central nervous system (CNS) (Jiang et al., 2008; Lubin et al., 2008). DNA methylation in the CNS exists in two methylation forms: 5-methylcytosine (5-mC) formation is catalyzed by DNA methyltransferases (DNMTs) from 5-cytosine (5-C), and 5-hydroxymethylcytosine (5-hmC) formation is catalyzed by TET1 from 5-mC (Kriaucionis and Heintz, 2009; Munzel et al., 2010; Guo et al., 2011). DNA methylation is thought to occur primarily during neuronal development and differentiation, and to remain static thereafter. However, a number of studies have challenged this idea and recent evidence suggests that DNA methylation is in fact both a dynamic and persistent molecular process controlling gene transcription in post-mitotic neurons in the adult CNS (Levenson et al., 2006; Jiang et al., 2008; Nelson et al., 2008; Feng and Fan, 2009; Feng et al., 2010).

*Corresponding author. Tel: +1-205-996-6084; fax: +1-205-934-6571.
E-mail addresses: rparris1@uab.edu (R. Ryley Parrish), asher693@uab.edu (A. J. Albertson), sbucking@uab.edu (S. C. Buckingham), Hablitz@nrc.uab.edu (J. J. Hablitz), Kmascia@uab.edu (K. L. Mascia), haselden@uab.edu (W. Davis Haselden), flubin@uab.edu (F. D. Lubin).

Abbreviations: 5-C, 5-cytosine; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; *Bdnf*, brain-derived neurotrophic factor; ChIP, chromatin immunoprecipitation; CpG, cytosine phosphodiester guanine; CREB, cAMP response element-binding; DG, dentate gyrus; DMSO, dimethyl sulfoxide; DNMT, DNA methyltransferase; EDTA, ethylenediamine tetraacetic acid; EEG, electroencephalography; fEPSP, field excitatory postsynaptic potential; *Grin2b*/*Nr2b*, glutamate receptor subunit epsilon-2; H/KDAC, histone/lysine deacetylase; ICV, intracerebralventricular; IP, intraperitoneally; KA, kainic acid; PBS, phosphate-buffered saline; RT-PCR, real-time polymerase chain reaction; SE, status epilepticus; TE, Tris–EDTA; TLE, temporal lobe epilepsy.

0306-4522/13 $36.00 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2013.06.029

Additionally, numerous studies have linked dysregulation of DNA methylation to several neurological disorders, including Rett syndrome, schizophrenia, and depression (Amir et al., 1999; Connor and Akbarian, 2008; Feng and Fan, 2009; Higuchi et al., 2011; Sales et al., 2011). Despite the link between DNA methylation and other neurological disorders, its role in aberrant transcriptional gene regulation in epilepsy disorders has not been fully explored (reviewed in Lubin, 2012). Therefore, we used kainic acid (KA)-induced SE as an experimental TLE model system to investigate the contribution of DNA methylation to gene expression changes in TLE. Specifically, we focused on a potential role for DNA methylation in the abnormal transcriptional regulation of the glutamate receptor subunit epsilon-2 also known as *N*-methyl-D-aspartate receptor subtype 2B (*Grin2b*/*Nr2b*) and brain-derived neurotrophic factor (*Bdnf*) genes in the epileptic hippocampus, both of which have been reported to accompany, and perhaps contribute to TLE (Mathern et al., 1998; Tongiorgi et al., 2004; Bovolenta et al., 2010; Ghasemi and Schachter, 2011).

The present study was undertaken to investigate whether DNA methylation contributes to *Grin2b* and *Bdnf* expression during the epileptogenic process triggered by SE. We found that SE triggered increases in DNA methylation levels at the *Grin2b*/*Nr2b* promoter and decreased DNA methylation levels at the *Bdnf* promoter with a positive correlation on *Grin2b*/*Nr2b* and *Bdnf* gene and protein expression levels in the epileptic hippocampus. We found that DNMT blockade had no effect on *Bdnf* DNA methylation. However, DNMT inhibition attenuated both global DNA methylation and *Grin2b*/*Nr2b* gene-specific DNA methylation levels, corresponding with increased binding of the AP2alpha transcription factor at the *Grin2b*/*Nr2b* promoter and increased GRIN2B/NR2B protein expression in the zebularine-treated epileptic hippocampus. Intriguingly, inhibiting DNMT activity during the initial SE insult further increased field excitatory postsynaptic potentials (fEPSPs) in the zebularine-treated epileptic hippocampus. Results suggest that alterations of methylating and *de*methylating enzymes that facilitate DNA methylation mechanisms appear to be early and late events triggered by SE that serve to control hippocampal gene expression and subsequent protein expression in an experimental model of TLE.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (150–200 g) were used for all experiments. Animals were housed in a 12-h light/dark cycle and allowed access to food and water *ad libitum*. Animals were handled for 3–5 days before use in experiments. All procedures were performed with the approval of the University of Alabama at Birmingham Institutional Animal Care and Use Committee and according to national guidelines and policies.

Kainate treatment

Animals were injected with KA [15 mg/kg; (Tocris Cookson Inc., Ellisville, MO, USA)] or saline (vehicle) intraperitoneally (IP). The severity of behavioral seizures following KA injection was scored according to the Racine scale (Racine, 1972): score 1, mouth and face clonus and head nodding; score 2, clonic jerks of one forelimb; score 3, bilateral forelimb clonus; score 4, forelimb clonus and rearing; score 5, forelimb clonus with rearing and falling. The onset of SE was defined as the time from KA injection to the occurrence of continuous seizure activity (scores 4 or 5 in the Racine scale). One cohort of animals was sacrificed at 1 h after the onset of SE while another cohort was sacrificed 6 weeks after the onset of SE with the control animals being sacrificed in parallel. A 3rd cohort of animals was sacrificed at 14 days after onset of SE. All control animals were handled in the same manner as the kainate-treated animals, except for KA administration. All kainate-treated animals used at the 14-day or 6-week time point had observable seizures. For tissue collection, the hippocampus was removed, oxygenated (95%/5% O_2/CO_2) in ice cold cutting solution (110 mM sucrose, 60 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 28 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 5 mM glucose, 0.6 mM ascorbate), and then areas CA1, CA3, and the dentate gyrus (DG) region were microdissected and frozen immediately on dry ice. The microdissection was performed with the aid of a dissecting microscope. The hippocampus was bisected with the dorsomedial half being divided into four pieces. Using anatomic landmarks, each piece was dissected into CA1, CA3, and the DG region. CA3 was dissected with a cut connecting the ends of the inner and outer blade of the DG. The DG and CA1 were dissected with a cut along the hippocampal fissure. The tissue was then stored in a −80 °C freezer until RNA and DNA extractions were performed.

Intracerebralventricular (ICV) cannula implantation and drug treatment

For administration of the drugs to the entire brain, a unilateral 23-gauge single guide cannula (Plastics One, Roanoke, VA, USA) was implanted in each rat. Guide implantation was alternated between the left and right ventricles. The injector extended 1.5 mm beyond the guide. The stereotaxic coordinates used were: anteroposterior, −1.0 mm from bregma, ±1.2 mm lateral from the midline, and −3.5 mm from dura measured from the tip of the cannula guide (Lei et al., 2004; Lubin et al., 2007; Lubin and Sweatt, 2007). Animals were habituated to the dummy cannula removal and were allowed 5 days to recover and be handled before the start of experimentation. Animals were then infused with 5 μL of zebularine (600 ng per μL in 10% dimethyl sulfoxide (DMSO)) or vehicle (saline in 10% DMSO) 1 h before KA injection. KA injections were performed as described previously. Animals were monitored throughout the process of entry into SE using the Racine scale as described previously.