Neurobiology of Disease 108 (2017) 100–114

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Structural alterations in fast-spiking GABAergic interneurons in a model of posttraumatic neocortical epileptogenesis☆

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ARTICLE INFO

Article history:
Received 6 April 2017
Revised 11 August 2017
Accepted 16 August 2017
Available online 18 August 2017

Keywords:
Epilepsy
Parvalbumin
Axonal boutons
BDNF
TrkB
Trophic
GABA
Traumatic injury
Inhibitory synapses

ABSTRACT

Electrophysiological experiments in the partial cortical isolation ("undercut" or "UC") model of injury-induced neocortical epileptogenesis have shown alterations in GABAergic synaptic transmission attributable to abnormalities in presynaptic terminals. To determine whether the decreased inhibition was associated with structural abnormalities in GABAergic interneurons, we used immunocytochemical techniques, confocal microscopy and EM in UC and control sensorimotor rat cortex to analyze structural alterations in fast-spiking parvalbumin-containing interneurons and pyramidal (Pyr) cells of layer V. Principle findings were: 1) there were no decreases in counts of parvalbumin (PV)- or GABA-immunoreactive interneurons in UC cortex, however there were significant reductions in expression of VGAT and GAD-65 and -67 in halos of GABAergic terminals around Pyr somata in layer V. 2) Consistent with previous results, somatic size and density of Pyr cells was decreased in infragranular layers of UC cortex. 3) Dendrites of biocytin-filled FS interneurons were significantly decreased in volume. 4) There were decreases in the size and VGAT content of GABAergic boutons in axons of biocytin-filled FS cells in the UC, together with a decrease in colocalization with postsynaptic gephyrin, suggesting a reduction in GABAergic synapses. Quantitative EM of layer V Pyr somata confirmed the reduction in inhibitory synapses. 5) There were marked and lasting reductions in brain derived neurotrophic factor (BDNF)-IR and -mRNA in Pyr cells and decreased TrkB-IR on PV cells in UC cortex. 6) Results lead to the hypothesis that reduction in trophic support by BDNF derived from Pyr cells may contribute to the regressive changes in axonal terminals and dendrites of FS cells in the UC cortex and decreased GABAergic inhibition.

Significance: Injury to cortical structures is a major cause of epilepsy, accounting for about 20% of cases in the general population, with an incidence as high as ~50% among brain-injured personnel in wartime. Loss of GABAergic inhibitory interneurons is a significant pathophysiological factor associated with epileptogenesis following brain trauma and other etiologies. Results of these experiments show that the largest population of cortical interneurons, the parvalbumin-containing fast-spiking (FS) interneurons, are preserved in the partial neocortical isolation model of partial epilepsy. However, axonal terminals of these cells are structurally abnormal, have decreased content of GABA synthetic enzymes and vesicular GABA transporter and make fewer synapses onto pyramidal neurons. These structural abnormalities underlie defects in GABAergic neurotransmission that are a key pathophysiological factor in epileptogenesis found in electrophysiological experiments. BDNF, and its TrkB receptor, key factors for maintenance of interneurons and pyramidal neurons, are decreased in the injured cortex. Results suggest that supplying BDNF to the injured epileptogenic brain may reverse the structural and functional abnormalities in the parvalbumin FS interneurons and provide an antiepileptogenic therapy.

Abbreviations: GABA, gamma-amino-butyric acid; VGAT, vesicular GABA transporter; GAD-65, -67, glutamic acid decarboxylase 65, 67; UC, undercut or partial neocortical isolation; FS, fast-spiking; BDNF, brain derived neurotrophic factor; PV, parvalbumin; TrkB, tropomyosin receptor kinase B.

☆ Authors contributions: IP, FS, DAP planned the experiments; IP, FS, JL, KS obtained and analyzed ICC images; JW, PS performed and analyzed EM; KG, RMT obtained cell counts; AB, FG identified and labeled interneurons; DAP, FG, IP wrote the manuscript.

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2017.08.008
0969-9961/

1. Introduction

Structural and functional abnormalities in GABAergic neurons and inhibitory circuits are prominent in animal models of epileptogenesis (Ribak and Reiffenstein, 1982; Ribak, 1985; Houser et al., 1986; Li and Prince, 2002; Chen and Roper, 2003; Magloczky and Freund, 2005; Kumar and Buckmaster, 2006; Faria and Prince, 2010; Faria et al., 2012; Ma and Prince, 2012) and in human epileptogenic brain (De Lanerolle et al., 1989; Marco et al., 1996; DeFelipe, 1999). Disinhibition can result from actual loss of interneurons of various subtypes (De Lanerolle et al., 1989; Sloviter, 1987; Houser and Esclapez, 1996; Rosen et al., 1998; Wyeth et al., 2010; Buckmaster and Dudek, 1997) and/or functional alterations in surviving interneurons. In the partial neocortical isolation, an injury/deafferentation model of posttraumatic epileptogenesis ("undercut "or "UC" below), we previously found impaired inhibitory synaptic transmission from fast-spiking (FS) parvalbumin (PV)-containing interneurons onto excitatory neurons, as well as to other FS cells (Ma and Prince, 2012). The decreased GABAergic inhibition appeared to be due to functional abnormalities in presynaptic terminals, as evidenced by increased failures of evoked monosynaptic and unitary IPSCs, decreased release probability (P_r) (Faria and Prince, 2010), increase in the coefficient of variation (CV) of the amplitude of unitary IPSCs (Ma and Prince, 2012) and decreases in presynaptic N-current calcium channels (Faria et al., 2012). Reductions in mIPSC frequency in layer V Pyr cells in UC cortex (Li and Prince, 2002), and topographic restriction of inhibitory connectivity (Jin et al., 2011) were also compatible with presynaptic interneuronal abnormalities. In the current experiments, we assessed potential structural alterations in FS interneurons that might underlie terminal dysfunction. Results show that the density of GABAergic neurons is not reduced in the UC cortex. However, there are significant reductions in vesicular GABA transporter (VGAT), and glutamic acid decarboxylase (GAD) -65 and -67 in their perisomatic GABAergic terminals. In addition, GABAergic boutons were decreased in size and fewer were closely associated with postsynaptic gephyrin, suggesting a reduction in GABAergic synapses, confirmed with electron microscopy that showed decreased density of symmetrical synapses on layer V Pyr cell somata. Similar abnormalities of GABAergic neurons are reported following cortical deafferentation and as a consequence of decreased activity-dependent BDNF gene activation. Reductions in BDNF expression in layer V Pyr cells of the UC and the well-established dependence of interneuronal growth and development on activation of TrkB receptors, suggest that loss of trophic support contributes to functional and structural abnormalities of FS interneurons in the epileptogenic UC cortex.

Partial results of these experiments were presented in a review (Prince et al., 2012).

2. Methods

All procedures were conducted according to National Institutes of Health Guide for the Care and Use of Laboratory Animals and protocols approved by the Stanford Institutional Animal Care and Use Committee. We analyzed FS interneurons of neocortical layer V in the UC model to determine whether there were abnormalities in their axons and presynaptic terminals that could result in defects in GABAergic transmission. Interictal discharges originate in layer V of the UC (Prince and Tseng, 1993; Hoffman et al., 1994) and alterations in FS interneurons in this lamina could contribute to hyperexcitability and epileptiform bursts in in vitro cortical slices (Prince and Tseng, 1993; Hoffman et al., 1994) and seizures in vivo (Chauvette et al., 2016; Ping and Jin, 2016a). We identified FS cells in whole cell recordings from their electrophysiological phenotype (Xiang et al., 1998) and, retrospectively, appearance following biocytin labeling. FS interneurons included predominately PV-positive cells (Uematsu et al., 2008).

2.1. Animals

A total of 21 naïve control and 22 lesioned Sprague-Dawley rats were used in these experiments, as detailed below.

2.2. Partial cortical isolations

These were prepared as previously described (Hoffman et al., 1994); see Supplemental Fig. S5 from Graber and Prince (2006) for details. Male Sprague-Dawley rats aged postnatal day (P) 21–30 were anesthetized with ketamine/xylazine (80/8 mg/kg, i.p.), the skull exposed and a bone window opened over sensory-motor cortex. A 30 g needle, bent at a 90° angle ~3 mm from its tip, was inserted parasagittally, beneath the dura and pia, parallel to the cortical surface and 1 mm from the midline, with care to avoid large vessels. The needle was lowered ~2 mm to make a transcortical cut and rotated through ~135° to make an undercutting lesion, raised to just beneath the pia to make a second transcortical cut, and removed. A third transcortical cut was then made parallel and ~3 mm lateral to the initial one. The bone window was covered with sterile Saran Wrap® and the skin sutured. Animals were allowed to recover for 2–4 weeks prior to the terminal experiment. In previous experiments, >80% of animals with such lesions have had epileptogenic activity in at least one in vitro slice after 2 weeks (Hoffman et al., 1994; Graber and Prince, 1999). Most animals underwent UC surgeries at P28–30, except those used for electron microscopy where UCs were performed at P21.

2.3. Biocytin labeling

Methods for preparing in vitro neocortical slices and identifying FS interneurons for biocytin filling were as previously described (Bacci et al., 2003; Xiang et al., 2002). Eighteen well-filled FS interneurons from sensory-motor cortical layer V of 5 naïve control (7 cells) and 5 UC rats (11 cells) were used for confocal analysis of dendritic and axonal structures. Of these, 5 UC and 5 control FS cells were processed for PV immunoreactivity (IR) and all were positive for PV. Basic techniques were similar to those previously described (Xiang et al., 1998). Postnatal (P49–56) naïve rats and animals that had been subjected to a unilateral partial cortical isolation 3–4 weeks earlier ("undercut" or "UC" animals) were anesthetized with pentobarbital (55 mg/kg), decapitated, and brains removed and immersed in cold "cutting" solution (4 °C) containing (in mM): 234 sucrose, 11 glucose, 24 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$ and 0.5 $CaCl_2$, gassed with 95% O_2/5% CO_2. The undercut cortical area was clearly visible at the pial surface. Coronal slices (300 μm) from naïve and undercut animals were cut with a vibratome from a block of brain containing the undercut, incubated for 1 h at 32 °C and subsequently at room temperature in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$ and 10 glucose; pH 7.4. Slices were transferred one at a time to the recording chamber on the stage of an upright microscope, minimally submerged and perfused (~3 ml/min) with the above ACSF at 32 °C. Recordings and cell labeling were begun after 1 h of incubation. Landmarks, including cortical laminae and sites of transcortical or undercutting lesions, were easily delineated in slices under low power with the upright microscope. Sites for recording and biocytin labeling in lesioned animals were from layer V inside the isolation, and within ~1–2 mm of the transcortical cut, as this is the most epileptogenic territory within the injured cortex (Hoffman et al., 1994). Comparable sites were selected in contralateral homotopic and naïve cortex. Pyramidal (Pyr) cells and interneurons were identified using a 40× or 63× water immersion lens, DIC optics, infrared microscopy and a CCD camera. Cells with a multipolar morphology, a round cell body and absence of a single emerging apical dendrite oriented toward the pial surface were selected for recordings and biocytin labeling (Bacci et al., 2003). (Xiang et al., 1998). Patch clamp techniques were used to obtain current clamp recordings with