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Gabapentin attenuates hyperexcitability in the freeze-lesion model of developmental cortical malformation

Lauren Andresen [a,b], David Hampton [a], Amaro Taylor-Weiner [c,1], Lydie Morel [a], Yongjie Yang [a], Jamie Maguire [a], Chris G. Dulla [a,*]

[a] Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Avenue, SC201, Boston, MA, USA
[b] Neuroscience Program, Sackler School of Graduate Biomedical Sciences, Tufts University, 136 Harrison Avenue, SC201, Boston, MA, USA
[c] Broad Institute, 301 Binney Street, 5066, Cambridge, MA 02142, USA

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ABSTRACT

Developmental cortical malformations are associated with a high incidence of drug-resistant epilepsy. The underlying epileptogenic mechanisms, however, are poorly understood. In rodents, cortical malformations can be modeled using neonatal freeze-lesion (FL), which has been shown to cause in vitro cortical hyperexcitability. Here, we investigated the therapeutic potential of gabapentin, a clinically used anticonvulsant and analgesic, in preventing FL-induced in vitro and in vivo hyperexcitability. Gabapentin has been shown to disrupt the interaction of thrombospondin (TSP) with α2δ-1, an auxiliary calcium channel subunit. TSP/α2δ-1 signaling has been shown to drive the formation of excitatory synapses during cortical development and following injury. Gabapentin has been reported to have neuroprotective and anti-epileptogenic effects in other models associated with increased TSP expression and reactive astrocytosis. We found that both TSP and α2δ-1 were transiently upregulated following neonatal FL. We therefore designed a one-week GBP treatment paradigm to block TSP/α2δ-1 signaling during the period of their upregulation. GBP treatment prevented epileptiform activity following FL, as assessed by both glutamate biosensor imaging and field potential recording. GBP also attenuated FL-induced increases in mEPSC frequency at both P7 and 28. Additionally, GBP treated animals had decreased in vivo kainic acid (KA)-induced seizure activity. Taken together these results suggest gabapentin treatment immediately after FL can prevent the formation of a hyperexcitable network and may have therapeutic potential to minimize epileptogenic processes associated with developmental cortical malformations.

Introduction

Developmental cortical malformations, such as polymicrogyria and cortical dysplasia, are a group of neurological disorders with a high incidence of intractable epilepsy (Takano et al., 2006). They are characterized by areas of anatomically disorganized cortex that often evolve into a seizure focus (Leventer et al., 2010). The mechanisms by which these lesions contribute to the onset of seizure activity, however, are poorly understood. To investigate the pathophysiology of cortical malformation, we have utilized the neonatal freeze-lesion (FL) model, which reproduces key features of polymicrogyria including structural abnormalities and cortical hyperexcitability (Jacobs et al., 1996; Luhmann et al., 1998a,b). Briefly, on the day of birth (P0), mice are anesthetized and a freezing probe is applied to the surface of the skull. This results in the formation of a microgyrus at the site of lesion. Epileptiform activity can be evoked in the region adjacent to the lesion (paramicrogyral zone; PMZ) in acute brain slices after a 10- to 11-day latent period (Jacobs et al., 1999). Spontaneous seizures have not been reported in the FL model (but see Kamada et al., 2013) but numerous changes in network connectivity and excitability have been reported (Jacobs and Prince, 2005; Scantlebury et al., 2004).

A number of lines of evidence specifically implicate increased glutamatergic signaling in the pathophysiology of FL-induced hyperexcitability. Increased excitatory input onto both interneurons and pyramidal cells in the PMZ were first reported in the FL model after P14, when acute cortical brain slices generate epileptiform responses (Jacobs and Prince, 2005). Importantly, later studies went on to show that increased excitatory input onto layer V pyramidal cells is present before the onset of epileptiform activity. This supports the hypothesis that network level changes occur during the latent period, before the onset of hyperexcitability at P14 (Zsombok and Jacobs, 2007). Changes in glutamate receptor expression and localization have also been reported in the FL model (Hagemann et al., 2003; Defazio and Hablitz, 2000; Zilles et al., 1998). Excitatory connectivity between cortical layers II and V is specifically increased as assayed by laser-scanning photostimulation (Brill and

* Corresponding author at: Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Avenue, SC203, Boston, MA 02111, USA. Fax: +1 617 636 2413.
Available online on ScienceDirect (www.sciencedirect.com).
[1] Current address.

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Huguenard, 2010). This implicates local intra-cortical circuitry in the hyperexcitable phenotype. Furthermore, glutamate biosensor imaging studies showed that stimulus-evoked extracellular glutamate levels were increased in the FL cortex (Dulla et al., 2012). Taken together, these reports clearly implicate increased excitatory signaling in the FL model. The specific molecular and cellular changes which occur during the latent period and which drive increased excitatory signaling remain largely unknown. Identifying these mechanisms is important as it will provide novel targets for therapeutic interventions.

To this end, we have begun to investigate the role of neuron/astrocyte signaling following FL. It has been shown that neurons in co-culture with astrocytes form more synapses than when cultured alone (Ullian et al., 2001). This effect can be replicated by the addition of astrocyte-conditioned media (Ullian et al., 2004a,b), implicating an astrocyte-secreted protein in driving synaptogenesis. Thrombospondins (TSPs) were identified as the astrocyte-secreted factors responsible for excitatory synapse formation (Christopherson et al., 2005). TSPs have been shown to promote synaptogenesis in the developing CNS, but their expression decreases dramatically in the adult brain (Christopherson et al., 2005). Following injury in the mature cortex, however, reactive astrocytes re-express TSP (Lin et al., 2003; Zhou et al., 2010). Whether neonatal injury induces similar increases in TSP is unknown. The receptor for TSP-mediated synaptogenesis is the calcium channel subunit α2δ-1 (Eroglu et al., 2009; Christopherson et al., 2005). α2δ proteins are accessory subunits of voltage gated calcium channels, which enhance trafficking to the plasma membrane and can influence biophysical properties of the channels. Interestingly, α2δ-1's role in synaptogenesis appears to be independent from calcium channel association (Hoppa et al., 2012).

Recently, the anticonvulsant and antiallodynic drug gabapentin (GBP) was shown to block the interaction between TSP and α2δ-1 (Eroglu et al., 2009). GBP was originally designed as a GABA-mimetic that could freely cross the blood–brain barrier; however, it was subsequently found to have no actions on GABAergic neurotransmission (Sills, 2006). Rather, its therapeutic actions appear to be mediated by antagonizing the interaction of TSP and α2δ-1 (Eroglu et al., 2009; Eroglu, 2009). Recent studies have shown that GBP treatment prevented injury-induced increases in reactive astrocytosis and excitatory synaptogenesis (Li et al., 2012; Lo et al., 2011). Furthermore, GBP treatment attenuated cortical hyperexcitability in a model of posttraumatic epilepsy associated with upregulation of α2δ-1 (Li et al., 2012). Given these findings, along with reports of increased reactive astrocytes in the FL model (Bordey et al., 2001; Campbell and Hablitz, 2008; Dulla et al., 2012), we hypothesized that treating FL animals with GBP during the latent period may attenuate later hyperexcitability in the FL model. We found that both TSP and α2δ-1 immunoreactivity were increased focally 3 and 7 days following FL. We therefore treated FL animals with GBP for one week (P1–P7) and then assessed epileptiform activity and glutamate signaling in acute brain slices (P14–P28) and seizure susceptibility in vivo (>P60). We found that GBP treatment after FL caused long-term decreases in both in vitro and in vivo hyperexcitability. GBP treatment also prevented FL-induced increases in mEPSC frequency. Taken together these results suggest that GBP treatment protects against the onset of hyperexcitability associated with developmental cortical malformation, potentially through the inhibition of TSP/α2δ-1 signaling.

Materials and methods

Production of glutamate FRET biosensor

BL21(DE3) bacteria were transformed with pRSET-FLII^{81}E-1 μ plasmids and streaked on an LB plate with ampicillin (100 μg/ml) (Dulla et al., 2008). After overnight incubation at 37 °C, a single colony was picked and grown in 1 l LB with ampicillin (100 μg/ml) for 3 days at 25 °C in the dark with rapid shaking (300 rpm). Cells were harvested by centrifugation, resuspended in extraction buffer (50 mM Sodium Phosphate, 300 mM NaCl, pH 7.2), and lysed with CelLytic B reagent (Sigma). The FRET sensor was purified by Talon His-affinity chromatography (Clontech). Binding to the resin was performed in batch at 4 °C, washed in a column with extraction buffer, and then eluted with extraction buffer containing 150 mM imidazole.

Animals and freeze lesion surgery

Experimental microgyri in primary somatosensory cortex (right hemisphere) were induced in P0 C57BL/6 mouse pups by freeze lesioning as described previously in rats (Dulla et al., 2012) and other mice FL models (Wang et al., 2012), but with some modifications. Briefly, animals were anesthetized by hypothermia, an incision into the scalp was made, and a copper probe (1 mm X 1.5 mm) cooled to −50 to −60 °C was placed onto the exposed skull for 5 s. Sham operated littermates were generated by leaving the probe at room temperature. After freeze-lesioning, the incision was closed using surgical glue, and pups were warmed and returned to the dam. Mice were treated with once daily i.p. injections of either gabapentin (200 mg/kg) or vehicle from P1 to P7. All guidelines of Tufts University's Institutional Animal Care and Use Committee were followed.

Preparation of brain slices

Cortical brain slices containing sensorimotor cortex (400 μM) were prepared from C57BL/6 mice (P3–P30) of either sex. Briefly, mice were anesthetized with isoflurane, decapitated, and the brains were rapidly removed and placed in chilled (4 °C) low-Ca, low-Na slicing solution consisting of (in mM): 234 sucrose, 11 glucose, 24 $NaHC0_2$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$ and 0.5 $CaCl_2$, equilibrated with a mixture of 95% O_2:5% CO_2. The brain was glued to the slicing stage of a Vibratome 3000 sectioning system and slices were cut in a coronal orientation. The slices were then incubated in 32 °C oxygenated aCSF (in mM: NaCl, 126, KCl, 2.5, NaH_2PO_4, 1.25, $MgSO_4$, 1, $CaCl_2$, 2, glucose, 10, $NaHCO_2$, 26) for 1 h, and then allowed to cool to room temperature and subsequently used for sensor loading and recording.

Loading of FRET-based glutamate sensor protein

Loading of glutamate biosensor was done as previously described (Dulla et al., 2008). A 35 mm tissue culture dish was filled with ≈2 ml aCSF and a 0.4 μm Millicell (Millipore) culture plate was inserted. Care was taken to ensure that no bubbles were present under the plate insert and that no aCSF spilled onto its top surface. A single brain slice was transferred from the incubation chamber onto the plate insert and excess aCSF was removed. The dish containing the slice was then placed in a humidified and warmed (32 °C) chamber equilibrated with 95% O_2:5% CO_2. 50 μl of concentrated glutamate FRET sensor protein (≈50 ng/μl) was then carefully applied to the top surface of the slice. After 5–10 min of incubation, slices were removed from the loading chamber and placed into the recording chamber.

Field recordings

Slices were placed in an interface chamber maintained at 34 °C, superfused with oxygenated aCSF at 2 ml/min and cortical projections were stimulated with a tungsten concentric bipolar electrode at the layer VI–white matter boundary. Electrical stimulation consisted of 10–50 μA, 100 μs pulses at 30 s intervals delivered by a stimulus isolator (World Precision Instruments). Glass micropipettes (resistance ≅ 1 MΩ) were filled with aCSF and placed in layer V of the cortex directly above the stimulation electrode. Electrophysiological data were recorded with an Axon Multiclamp 700A amplifier and Digidata 1322A digitizer (sampling rate = 20 kHz) with pClamp software (Molecular Devices). Threshold