Neurobiology of Disease 102 (2017) 70–80

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Epileptiform activity and behavioral arrests in mice overexpressing the calcium channel subunit α2δ-1

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ARTICLE INFO

Article history:
Received 27 November 2013
Revised 17 January 2017
Accepted 25 January 2017
Available online 11 February 2017

Keywords:
Excitation
EEG
Synaptogenesis
Pyramidal cells
Astrocytes
Epilepsy
Epileptogenesis
Nonconvulsive
Neocortex

ABSTRACT

The alpha2delta-1 subunit (α2δ-1) of voltage-gated calcium channels is a receptor for astrocyte-secreted thrombospondins that promote developmental synaptogenesis. Alpha2delta-1 receptors are upregulated in models of injury-induced peripheral pain and epileptogenic neocortical trauma associated with an enhancement of excitatory synaptic connectivity. These results lead to the hypothesis that overexpression of α2δ-1 alone in neocortex of uninjured transgenic (TG) mice might result in increased excitatory connectivity and consequent cortical hyperexcitability and epileptiform activity. Whole cell recordings from layer V pyramidal neurons in somatosensory cortical slices of TG mice showed increased frequency and amplitude of miniature and spontaneous EPSCs and prolonged bursts of polysynaptic EPSCs. Epileptiform field potentials were evoked in layers II/III and V of brain slices from TG mice, but not controls. Dual immunoreactivity for Vglut-2 and PSD95 showed increased density of close appositions in TG mice compared to controls, suggesting an increased number of excitatory synapses. Video-EEG monitoring showed that 13/13 implanted TG mice aged >P21, but not controls, had frequent abnormal spontaneous epileptiform events, consisting of variable duration, high amplitude bi-hemispheric irregular bursts of delta activity, spikes and sharp waves lasting many seconds, with a variable peak frequency of ~1–3 Hz, associated with behavioral arrest. The epileptiform EEG abnormalities and behavioral arrests were reversibly eliminated by treatment with i.p. ethosuximide. Behavioral seizures, consisting of ~15–30 s duration episodes of rigid arched tail and head and body extension, followed by loss of balance and falling, frequently occurred in adult TG mice during recovery from isoflurane-induced anesthesia, but were rare in WT mice. Results show that over-expression of α2δ-1 subunits increases cortical excitatory connectivity and leads to neocortical hyperexcitability and epileptiform activity associated with behavioral arrests in adult TG mice. Similar increases in expression of α2δ-1 in models of cortical injury may play an important role in epileptogenesis.

Significance: Binding of astrocytic-secreted thrombospondins to their α2δ-1 receptor facilitates excitatory synapse formation and excitatory transmission during cortical development and after injury. Upregulation of α2δ-1 is present in models of injury-induced pain and epileptogenic cortical trauma, along with many other molecular alterations. Here we show that overexpression of α2δ-1 alone in TG mice can enhance excitatory connectivity in neocortex and lead to neural circuit hyperexcitability and episodes of electrographic epileptiform activity, associated with behavioral arrests in transgenic mice. α2δ-1 is the high-affinity receptor for gabapentinoids and a potential target for prophylactic treatment of posttraumatic epilepsy and other disorders in which excessive aberrant excitatory connectivity is a pathophysiological feature.

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1. Introduction

Thrombospondins are extracellular matrix proteins that have multiple functions including involvement in inflammation, angiogenesis and platelet aggregation (Adams and Lawler, 2004). They also promote excitatory synapse formation in the central nervous system (Christopherson et al., 2005) by interacting with the voltage gated calcium channel subunit α2δ-1, which also binds gabapentin, a widely used anticonvulsant drug (Eroglu et al., 2009). Initially identified as a non-essential calcium channel subunit (Arikkath and Campbell, 2003), the α2δ-1 protein is the product of a single gene (De Jongh et al., 1990) that is post-translationally cleaved into α2 and δ peptides. The α2 subunit is entirely extracellular, whereas δ is mainly a transmembrane protein with a small intracellular portion (Gurnett et al., 1996). The α2δ-1

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

http://dx.doi.org/10.1016/j.nbd.2017.01.009
0969-9961/Published by Elsevier Inc.

subunit is expressed in multiple cortical areas (Cole et al., 2005) where it is mainly located in presynaptic terminals (Taylor and Garrido, 2008), as well as in tissues outside the nervous system such as heart and muscle (Gong et al., 2001).

Recent results have shown that α2δ-1 is upregulated after nerve and brain injury (Luo et al., 2001), (Luo et al., 2002), (Li et al., 2012). In the neocortical partial isolation (undercut) model of posttraumatic epileptogenesis, gliosis, increases in thrombospondins (TSPs) and α2δ-1, and increased density of excitatory synapses occur in the injured cortex, along with abnormal epileptiform burst discharges (Li et al., 2012). Brief gabapentin treatment reduces the incidence of epileptiform bursts, the density of excitatory synapses and the frequency of excitatory synaptic currents (EPSCs) in this model, suggesting that thrombospondin-induced excitatory synapse formation via interactions with α2δ-1 might contribute to these abnormalities. However cortical injury induces a large number of pathophysiological processes (reviewed in (Prince et al., 2009)) so that it is difficult to determine whether the increases in α2δ-1 play a significant role in development of the epileptogenic activity. Transgenic (TG) mice, overexpressing α2δ-1 subunit proteins, have increased number of excitatory synapses in the visual cortex (Eroglu et al., 2009), and show tactile allodynia without injury (Li et al., 2006) and increased frequency of glutamatergic synaptic currents in spinal cord dorsal horn (Nguyen et al., 2009). Such enhanced excitatory connectivity is one mechanism underlying epileptogenesis and seizures in models of posttraumatic (Jin et al., 2006; Li et al., 2005; Li and Prince, 2002), genetic (Chu et al., 2010) and status epilepticus-induced epilepsy (Esclapez et al., 1999), even in the absence of concurrent decreases in inhibition (Buckmaster and Dudek, 1997a; Buckmaster and Dudek, 1997b; Chu et al., 2010). We hypothesized that increased excitatory synapse formation in uninjured TG mice overexpressing α2δ-1 protein in brain would induce hyperexcitability and epileptiform activity similar to that seen in animal models of epilepsy.

We obtained electrophysiological recordings, immunocytochemical and behavioral data from adult male TG mice that overexpress α2δ1 receptors and from control littermates that have normal complements of α2δ1 ("controls" below). Whole cell recordings were obtained from layer V pyramidal neurons of the somatosensory cortex, an area known for its potential to generate epileptiform activity (Connors, 1984; Graber and Prince, 2004; Hoffman et al., 1994), and video/EEGs were recorded from implanted animals. Results show that an increased density of Vglut2/PSD95 close appositions (putative excitatory synapses), enhanced functional excitatory cortical connectivity and bilaterally synchronous spontaneous paroxysmal electrographic epileptiform activity associated with behavioral arrests that are blocked by ethosuximide, occur in α2δ-1 TG mice.

2. Materials and methods

All experiments were performed according to the National Institutes of Health guide for the care and use of Laboratory animals and all protocols were approved by the Stanford Institutional Animal Care and Use Committee. TG mice, overexpressing α2δ-1 subunit protein, and control littermates were kindly provided by Z. David Luo (Li et al., 2006) and Ben A. Barres. Mice were housed and bred in the research animal facilities at Stanford University. We used only adult ($P > 21$; P0 = date of birth) male mice in our experiments. Transgenic mice overexpressing Cacna2-1 that encodes the voltage-dependent calcium channel subunit α2δ-1, were identified by sending tail samples to Transnetyx (Tennessee, USA) for genotyping.

2.1. Video-EEG recordings

Animals were anesthetized with isoflurane (induction 5%, maintenance 3%), the scalp incised and retracted and small drill holes placed in the skull, leaving the dura intact. Electrodes consisting of 0.003-inch diameter silver wires (Medwire), insulated to within ~0.5 mm of their cut ends, were pre-soldered to a microminiaturize connector and ends placed epidurally through the drill holes over both hemispheres, between bregma and lambda, 2 mm from the midline. A reference electrode was implanted in the midline over the cerebellum. Small pieces of gelfoam were placed over the skull openings, sites of electrode attachment to the connector and skull were insulated and the connector secured to the skull with dental cement. Mice were given carprofen 5 mg/kg s.c. postoperatively and a heat lamp used to prevent hypothermia during recovery from surgery. All mice resumed normal behavior within a few hours after surgery and were allowed to rest for at least 72 h before recordings. To acquire EEG data, the implanted plug was connected to a XLTEK 32 channel box (Natus Medical Incorporated, CA, USA) with a flexible cable. EEG signals were recorded, digitized and stored for later analysis. Video-EEG recording sessions, lasting approximately 3 h, were performed in TG mice ($n = 13$) and controls ($n = 8$), for 3–5 days/week during 2–3 weeks. EEG signals were filtered through a 1-Hz high-pass filter and a 15-Hz low-pass filter, and stored for later analysis. Epileptiform activities were identified as burst-like events of high amplitude containing spikes and sharp waves lasting from 5–90 s, that were clearly distinct from baseline. Generalized seizures were recorded in some additional mice during recovery from isoflurane-induced anesthesia (Results below) and the incidence of these events in control and TG mice quantified.

2.2. In vitro recordings

Mice were anesthetized at $P > 21$ and, using previously described techniques (Faria and Prince, 2010), brains removed and coronal neocortical slices cut and maintained for in vitro recordings. Slices (350 μm) were cut with a vibratome in cold (4 ± 1 °C) "slicing" artificial cerebrospinal fluid (ACSF) containing (in mM): 234 mM sucrose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$ and 11 mM glucose; pH 7.4 when saturated with 95% O_2–5% CO_2. After 1 h of incubation in standard ACSF containing (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose; (33 ± 1 °C), single slices were transferred to a recording chamber where they were minimally submerged and perfused at the rate of 2.5–3 ml/min with ACSF at 33 ± 1 °C.

Simultaneous extracellular field potentials in layers II/III and V of the somatosensory cortex were evoked using a tungsten stimulating electrode positioned in layer V and recorded with ASCF-filled borosilicate glass electrodes. We used the "coastline bursting index" (Korn et al., 1987) to quantify the degree of hyperexcitability in field potentials recorded in TG and control mice. The period analyzed extended from the point immediately following the stimulus artifact to the time point when responses returned to baseline. The total length of line in this region of interest was measured and a baseline event-free segment prior to the stimulus was subtracted. The resulting value of the coastline index correlates with circuit excitability (Korn et al., 1987).

Whole cell voltage-clamp recordings were made from visually-identified layer V pyramidal (Pyr) cells using infrared video microscopy and differential interference contrast optics (Zeiss Axioskop2) and a Multiclamp 700A amplifier (Axon Instruments). Pharmacologically isolated monosynaptic (mono) IPSCs were recorded with patch electrodes pulled from borosilicate glass tubing (1.5 mm OD) that had impedances of 2–3 MΩ when filled with intracellular solution containing (in mM): 70 K-gluconate, 70 KCl, 2 NaCl, 10 HEPES, 4 EGTA, 4 Mg ATP, 0.3 GTP. The IPSCs were inward at the estimated chloride equilibrium potential (E_{Cl}) of −16 mV based on the Nernst equation. EPSCs were blocked with continuous bath perfusion of ACSF containing 50 μM 2-amino-5-phosphonovaleric acid (D-AP5) and 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX; Ascent Scientific). The time to wash in drug was typically <5 min as judged by alterations in peak amplitude of evoked excitatory postsynaptic currents (eEPSCs).