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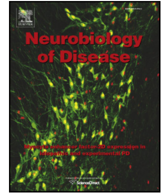

Altered intrathalamic $GABA_A$ neurotransmission in a mouse model of a human genetic absence epilepsy syndrome

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ABSTRACT

We previously demonstrated that heterozygous deletion of Gabra1, the mouse homolog of the human absence epilepsy gene that encodes the $GABA_A$ receptor ($GABA_AR$) α1 subunit, causes absence seizures. We showed that cortex partially compensates for this deletion by increasing the cell surface expression of residual α1 subunit and by increasing α3 subunit expression. Absence seizures also involve two thalamic nuclei: the ventrobasal (VB) nucleus, which expresses only the α1 and α4 subtypes of $GABA_AR$ α subunits, and the reticular (nRT) nucleus, which expresses only the α3 subunit subtype. Here, we found that, unlike cortex, VB exhibited significantly reduced total and synaptic α1 subunit expression. In addition, heterozygous α1 subunit deletion substantially reduced miniature inhibitory postsynaptic current (mIPSC) peak amplitudes and frequency in VB. However, there was no change in the expression of the extrasynaptic α4 or δ subunits in VB and, unlike other models of absence epilepsy, no change in tonic $GABA_AR$ currents. Although heterozygous α1 subunit knockout increased α3 subunit expression in medial thalamic nuclei, it did not alter α3 subunit expression in nRT. However, it did enlarge the presynaptic vesicular inhibitory amino acid transporter puncta and lengthen the time constant of mIPSC decay in nRT. We conclude that increased tonic $GABA_A$ currents are not necessary for absence seizures. In addition, heterozygous loss of α1 subunit disinhibits VB by substantially reducing phasic GABAergic currents and surprisingly, it also increases nRT inhibition by prolonging phasic currents. The increased inhibition in nRT likely represents a partial compensation that helps reduce absence seizures.

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Introduction

Epilepsy is a disorder in which the brain exhibits an enduring predisposition to generate seizures. It is a common disease that affects approximately 1% of the population and resists optimal medical therapy in approximately one third of cases. Typical absence seizures are a common seizure type that cause brief interruptions of consciousness and are associated with rhythmic 3 Hz bi-hemispheric spike-and-wave discharges on EEG. They occur in several different genetic generalized epilepsy (GGE) syndromes including childhood absence epilepsy (CAE), juvenile absence epilepsy, and juvenile myoclonic epilepsy. Although anticonvulsant medications are often effective in reducing absence seizures, approximately 50% of CAE patients fail optimal medical management (Glauser et al., 2013) and thus are at risk for injury as well as memory and behavioral deficits (Kernan et al., 2012; Lin et al., 2013).

Studies of rodent models of absence seizures suggested that typical absence seizures start in layer VI of the somatosensory cortex and quickly spread to the remainder of the cortex and two thalamic nuclei, the ventrobasal (VB) and reticular (nRT) nuclei (Meeren et al., 2002; Polack et al., 2007). Although the VB and nRT nuclei do not initiate the epileptic discharges in these models, unilateral VB/nRT lesions abolish absence seizures in rodents, a result that emphasizes the importance of these nuclei in seizure generation (Meeren et al., 2009). The cortex, VB, nRT, and interconnections among these regions comprise the thalamocortical network that is thought to be the core network involved in absence seizures.

Recent studies revealed that different pharmacological and genetic absence epilepsy models possess defects in different components of the thalamocortical network (Cope et al., 2009; Tan et al., 2007; Paz et al., 2011; Errington et al., 2011). Understanding the aberrant neurophysiology in different models of absence epilepsy will lead to the development of new pharmacological and, possibly, neurostimulation therapies for medically intractable absence seizures.

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We have studied the pathophysiology of a human absence epilepsy gene, GABRA1, which encodes the α1 subunit of the $GABA_A$ receptor ($GABA_AR$). The $GABA_AR$, the major inhibitory ligand-gated ion channel in the brain, is a pentamer composed of five subunits which arise from eight gene families; four of the gene families contain multiple isoforms (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–3). $GABA_ARs$ composed of different subunit isoforms are expressed in different brain regions at different times in development and exhibit different physiological properties. Heterozygous loss-of-function mutations in the α1 subunit have been associated with three GGE syndromes that confer absence seizures (Cossette et al., 2002; Lachance-Touchette et al., 2011; Maljevic et al., 2006), and heterozygous Gabra1 knockout (Het-KO) mice exhibit absence seizures (Arain et al., 2012). Previously, we found that neocortical neurons reduce endocytosis of $GABA_ARs$ from the cell surface which compensates for the heterozygous α1 subunit deletion by: 1) increasing the surface expression of the residual α1 subunit protein driven by the wild type allele and 2) increasing both the total and surface expression of the α3 subunit (Zhou et al., 2013).

Here, we determined the effects of heterozygous α1 subunit deletion on $GABA_AR$ expression and GABAergic physiology in the thalamus with a particular focus on the VB and nRT nuclei. Unlike adult cortex which expresses α1–5, β1–3, γ, and δ $GABA_AR$ subunits, $GABA_AR$ subunit expression in adult VB and nRT is much more limited. Adult VB expresses only α1, α4, β1–2, γ and δ subunits (Hortnagl et al., 2013). Synaptic receptors in VB neurons predominantly consist of α1βγ receptors and mediate phasic GABAergic currents (Peden et al., 2008). Extrasynaptic receptors in VB neurons consist of α4βδ (Chandra et al., 2006; Porcello et al., 2003) and α1βγ (Peden et al., 2008) receptors, but it is predominantly the α4βδ receptors that mediate tonic $GABA_A$ currents (Chandra et al., 2006; Porcello et al., 2003; Cope et al., 2005). Adult nRT neurons express predominantly α3, β1–2, and γ subunits (Hortnagl et al., 2013) and conduct phasic, but not tonic $GABA_A$ currents (Cope et al., 2005).

We show that, unlike cortex, neither VB nor nRT compensates for the heterozygous loss of α1 subunit by increasing total or synaptic expression of residual α1 or α3 subunits. Tonic currents in VB are unaltered and mIPSC peak amplitudes are reduced. Het-KO nRT neurons increase the size of GABAergic vesicular inhibitory amino acid transporter (VIAAT) puncta and prolong the decay of phasic synaptic $GABA_A$ currents. These findings demonstrate that heterozygous α1 subunit deletion produces a unique alteration of intrathalamic $GABA_A$ neurotransmission with disinhibition in VB and increased $GABA_A$ inhibition in nRT.

Materials and methods

Animals

All procedures were performed using protocols approved by the Vanderbilt University Institutional Animal Care and Use Committee. The mice were housed in a facility with a temperature and humidity controlled environment, a twelve hour light/dark schedule, and ad libitum food and water. We previously described the generation of mice with an unconditional deletion of the α1 subunit in a congenic C57BL/6J background (Arain et al., 2012). In addition, in some electrophysiology experiments, we used wild type and Het-KO mice that also expressed enhanced yellow fluorescent protein (EYFP) in parvalbumin-containing neurons (Jackson Laboratories, B6;SJL-Tg(Pvalb-COP4*H134R/EYFP)15Gfng/J, stock # 012355) in order to visualize the parvalbumin neurons in nRT in live brain slices. We mated Het-KO and wild type mice and used female pups at postnatal ages 33–37 because our previous EEG studies demonstrated frequent absence seizures in female Het-KO mice at this age (Arain et al., 2012). There is no difference in survival between wild type and Het-KO mice (Arain et al., 2012). On the day of the experiment, the mice were anesthetized with isoflurane and sacrificed. Brains were rapidly dissected and placed for sectioning in cutting solution kept at 0 °C.

Antibodies

We used the primary antibodies listed in Table 1 and the secondary antibodies listed in Table 2. Previously, we verified the specificity of the anti α1 subunit and anti α3 subunit antibodies in immunohistochemistry studies using complete α1 subunit and α3 subunit deletion mice (Zhou et al., 2013). The specificity of the anti β2/3 and anti VIAAT antibodies in immunohistochemistry studies was demonstrated in other publications (Gutierrez et al., 1994; Zander et al., 2010).

Brain slice biotinylation assay and Western blots

We used a vibratome (Leica VT1200S) to make three to four coronal brain slices (300 μm) encompassing the thalamus. We then biotinylated the cell surface proteins using the procedures and solutions described previously (Zhou et al., 2013). After biotinylation, we dissected the thalami and, in some experiments, microdissected thalamic regions containing either the VB/nRT nuclei or the medial thalamic nuclei. The region we designated as "VB/nRT" was microdissected from the coronal sections as is shown in Fig. 2A. We cut along the internal capsule from the hippocampus (dorsal point) to the hypothalamus (ventral point). We then located the midpoint, "M," between the thalamic midline and the internal capsule and made two diagonal cuts from the dorsal point and the ventral point to "M" to isolate the VB/nRT region. The region we designated the "medial thalamic nuclei" was obtained by making vertical cuts from the hippocampus, through "M" to the hypothalamus bilaterally. We made protein lysates from the dissected brain tissue and purified the cell-surface and total proteins as previously described (Zhou et al., 2013).

The total and surface proteins were analyzed on 10% SDS-PAGE gels followed by electrotransfer to nitrocellulose membranes. To ensure linearity of detection, 10 and 20 μg of total protein and 10, and 20 μl of surface protein were applied to the gel, and we verified that the signal from each protein increased in proportion to the amount loaded on the gel. The nitrocellulose membranes were blocked for 1 h with 5% nonfat dry milk in Tris buffered saline containing 0.1% Tween pH 7.4. The membranes were then incubated with primary antibody at 4 °C overnight and then with secondary antibody at room temperature for 1 h. We imaged the blots using an infrared fluorescent imaging system (Licor).

Quantification of RNA editing of the α3 subunit

Because VB does not express the α3 subunit, we could quantify α3 RNA editing in VB/nRT to determine the fraction of edited α3 subunit RNA in nRT. We dissected VB/nRT regions from brain slices as described above and quantified Gabra3 RNA editing using a high-throughput multiplexed transcript analysis as described previously (Hood et al., 2014).

Immunohistochemistry and confocal microscopy

We used the immunohistochemistry protocol described by Schneider Gasser et al. that allows for light fixation of cytoplasmic proteins but avoids over-fixation that prevents detection of clustered proteins in GABAergic synapses (Schneider Gasser et al., 2007). Briefly, we cut 2 mm coronal block slices (Zivic Instruments) in freshly-dissected brain tissue that encompassed the thalamus. We fixed the block slices in 4% paraformaldehyde dissolved in 100 mM sodium phosphate buffer for 30 min at 0 °C and then cryoprotected them in 30% sucrose in phosphate buffered saline (PBS) at 4 °C overnight. We generated 15 μm coronal sections using a cryostat (Leica) onto Colorfrost Plus glass slides (Thermo Scientific).

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