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ALTERATIONS IN AMPA RECEPTOR SUBUNIT EXPRESSION IN CORTICAL INHIBITORY INTERNEURONS IN THE EPILEPTIC STARGAZER MUTANT MOUSE

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Abstract—Absence seizures arise from disturbances within the corticothalamocortical network, however the precise cellular and molecular mechanisms underlying seizure generation arising from different genetic backgrounds are not fully understood. While recent experimental evidence suggests that changes in inhibitory microcircuits in the cortex may contribute to generation of the hallmark spike-wave discharges, it is still unclear if altered cortical inhibition is a result of interneuron dysfunction due to compromised glutamatergic excitation and/or changes in cortical interneuron number. The stargazer mouse model of absence epilepsy presents with a genetic deficit in stargazin, which is predominantly expressed in cortical parvalbumin-positive (PV^+) interneurons, and involved in the trafficking of glutamatergic AMPA receptors. Hence, in this study we examine changes in (1) the subunit-specific expression of AMPA receptors which could potentially result in a loss of excitation onto cortical PV^+ interneurons, and (2) PV^+ neuron density that could additionally impair cortical inhibition. Using Western blot analysis we found subunit-specific alterations in AMPA receptor expression in the stargazer somatosensory cortex. Further analysis using confocal fluorescence microscopy revealed that although there are no changes in cortical PV^+ interneuron number, there is a predominant loss of GluA1 and 4 containing AMPA receptors in PV^+ neurons in stargazers compared to non-epileptic controls. Taken together, these data suggest that the loss of AMPA receptors in PV^+ neurons could impair their feed-forward inhibitory output, ultimately altering cortical network oscillations, and contribute to seizure generation in stargazers. As such the feed-forward inhibitory interneurons could be potential targets for future therapeutic intervention for some absence epilepsy patients.

Key words: absence epilepsy, somatosensory cortex, AMPA receptor, stargazer, parvalbumin, feed-forward inhibition.

INTRODUCTION

Absence epilepsy is a generalized non-convulsive form of epilepsy characterized by a sudden brief loss of consciousness and concomitant spike wave discharges (SWDs) measuring 2.5–4 Hz on an electroencephalogram (EEG) (Crunelli and Leresche, 2002). Absence seizures, which account for approximately 10% of pediatric epilepsies, can occur hundreds of times a day (Berg et al., 2010) and are associated with cognitive deficits, learning difficulties and behavioral disorders in some affected children (Pavone et al., 2001; Caplan et al., 2008; Killory et al., 2011; Tosun et al., 2011). In addition to the adverse effects on a child's learning and social adjustment, there is an increased risk of physical injury if they occur during activities such as swimming or riding a bicycle on the road (Wirrel et al., 1996). Although absence seizures are known to arise from disturbances within the corticothalamocortical (CTC) circuitry (McCormick and Contreras, 2001), the precise underlying mechanisms are not fully understood and appear to be multifactorial as evidenced by the variability in patients' responses to anti-epileptic drugs (AEDs). Currently available AEDs fail to control seizures or induce intolerable side effects in approximately a third of patients, and even exacerbate seizures in some cases (Regesta and Tanganelli, 1999; Glauser et al., 2010, 2013). This variability in patient response to drug treatment suggests different cellular and molecular mechanisms within specific microcircuits are potentially capable of switching the normal oscillatory firing pattern within the CTC network into pathological SWDs. Hence, there is a critical need to understand the various mechanisms that underlie generation of absence seizures, in order to identify novel therapeutic targets for treatment of absence seizures, which arise from different genetic backgrounds.

Recent studies, in some rodent models of absence epilepsy, have implicated region and synapse specific changes in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression (Menuz and Nicoll, 2008; Kennard et al., 2011; Paz et al., 2011; Barad et al., 2012; Maheshwari et al., 2013) in seizure generation. AMPA receptors (AMPARs), formed from tetrameric combinations of GluA1–4 subunits, mediate most of the fast excitatory glutamatergic synaptic

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Abbreviations: AEDs, anti-epileptic drugs; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CT, corticothalamic; CTC, corticothalamocortical; E, epileptic; EEG, electroencephalogram; GABA, γ-aminobutyric acid; NE, non-epileptic; NMDA, N-methyl-d-aspartate; PBS, phosphate buffered saline; PV^+, parvalbumin-positive; RTN, reticular thalamic nucleus; SWD, spike-wave discharge; TARP, transmembrane AMPA receptor regulatory protein; TC, thalamocortical; VGlut2, vesicular glutamate transporter 2; VP, ventral posterior thalamus.

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transmission in the brain (Opazo and Choquet, 2011). They are trafficked to synapses and modulated by a family of transmembrane AMPA receptor regulatory proteins (TARPs), including the prototypical TARPγ2 stargazin (Chen et al., 2000; Tomita et al., 2005; Nicoll et al., 2006). Their presence at excitatory inputs to fast spiking inhibitory interneurons within the CTC network provides feed-forward inhibition that prevents runaway excitation within the network.

GluA4 containing AMPARs are particularly abundant at synapses on parvalbumin containing (PV^+) feed-forward inhibitory neurons within the CTC network; namely the inhibitory interneurons in the reticular thalamic nucleus (RTN) and somatosensory cortex (Kondo et al., 1997; Mineff and Weinberg, 2000). Recent studies have indicated that a selective loss of GluA4-AMPARs in the RTN and thus excitatory input to these PV^+ inhibitory neurons (Menuz and Nicoll, 2008; Paz et al., 2011), contributes to the generation and maintenance of pathological oscillations in the thalamus. For example, the stargazer mouse, an established model of absence epilepsy in which a spontaneous recessive mutation on chromosome 15 causes a stargazin protein deficit (Noebels et al., 1990), shows a significant loss of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSC) in RTN neurons (Menuz and Nicoll, 2008). This aberration results from a selective reduction in GluA4-AMPAR expression at corticothalamic (CT) synapses in inhibitory RTN neurons, but not at excitatory synapses on relay neurons in the ventral posterior (VP) thalamic region (Barad et al., 2012). Similarly, the Gria4-knockout mouse, which lacks GluA4 subunit expression and also presents with absence epilepsy, has been shown to have a selective impairment in CT-RTN synaptic transmission, but not in CT-TC or TC-RTN synaptic function. This ultimately leads to the increased CT excitation of the thalamic relay neurons via CT-TC-RTN-TC pathway. As a consequence, there is a selective loss of feed-forward inhibition but not of feedback inhibition in the thalamus, creating an enhanced oscillatory network that promotes the generation of SWDs (Paz et al., 2011). Collectively, the data from these mouse models of absence epilepsy implicate reduction in feed-forward inhibition but not feedback inhibition in the generation of seizure activity in the thalamus.

While most studies to date, have concentrated on the changes in inhibitory microcircuits within the thalamic component of the CTC network, emerging evidence indicates that SWDs are initiated in the somatosensory cortex (Meeren et al., 2002; Polack et al., 2007). A recent study proposed that seizure exacerbation by some N-methyl-D-aspartate (NMDA) receptor (NMDAR) antagonists in stargazers is due to the reduced excitation of cortical PV^+ inhibitory neurons (Maheshwari et al., 2013). This is because in the absence of AMPAR-mediated excitation, NMDARs, which are unaffected by the stargazin mutation, are thought to drive the excitation of inhibitory neurons (Lacey et al., 2012); as such blocking their activity could cause a further reduction in inhibition and thus enhance seizure activity. Nevertheless, the precise mechanism underlying seizure initiation in the cortex is still unclear and could either be a result of either cortical disinhibition (Maheshwari and Noebels, 2014), enhanced tonic inhibition (Cope et al., 2009) or even enhanced excitation (Kennard et al., 2011). Furthermore, since loss of inhibitory neurons have also been implicated in several neurological diseases including epilepsy (Kann, 2015), a reduction in numbers of cortical PV^+ inhibitory interneurons, in addition to any loss of excitatory input to these neurons, could contribute to a net loss of feed-forward inhibition within the cortex and runaway excitation. Currently, it is unknown whether changes in AMPAR expression in the cortex of the stargazer model are specifically linked to changes in GluA4-AMPARs in inhibitory PV^+ interneurons to which thalamocortical (TC) neurons project and hence is a subunit-specific effect, or whether other subclasses of AMPAR are involved, as seen in other models of absence epilepsy (Hanada, 2014). Moreover, it is unknown whether altered cortical inhibition is also associated with changes in inhibitory interneuron number or primarily a result of their dysfunction. Hence, the aim of the current study was first, to investigate changes in the subunit-specific expression of AMPARs in stargazer cortex that could potentially result in a loss of excitation onto cortical PV^+ interneurons, and second, to identify any changes in PV^+ neuron density that could additionally impair cortical inhibition.

To this end, we quantified the AMPAR subunit levels in whole-tissue lysates of the somatosensory cortex with Western blotting and found subunit-specific losses in the global expression of GluA1–4 in the stargazer somatosensory cortex. In addition we examined the expression levels of all AMPA receptor subunits throughout the stargazer cortical layers 1–6 with confocal fluorescence microscopy and demonstrated that although the density of cortical PV^+ interneurons is unchanged between non-epileptic (NE) and stargazer mice, there is both a reduction in the somatic expression and dendritic trafficking of AMPA receptors in stargazer cortical PV^+ neurons, which could impact on their feed-forward inhibitory function to induce hypersynchronous activity in the stargazer cortex.

EXPERIMENTAL PROCEDURES

Animals

Breeding stock of stargazer mice obtained from the Jackson Laboratory (Bar Harbor, USA) were mated to produce epileptic stargazer (stg/stg) and NE wildtype (+/+) and heterozygote (+/stg) control littermates. Mice were maintained on a 12-h light/dark cycle with access to food and water ad libitum. 9–12 week-old adult male mice were used, with genotypes confirmed by PCR of tail DNA based on recommendations of Jackson Laboratory. All experimental procedures were performed in line with approved protocols by the University of Otago Animal Welfare Office and Ethics Committee.

Antibodies

The antibodies used in this study are listed in Table 1. Preadsorption control tests to confirm primary antibody

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