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Inhibitory synapse deficits caused by familial α1 $GABA_A$ receptor mutations in epilepsy

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ABSTRACT

Epilepsy is a spectrum of neurological disorders with many causal factors. The GABA type-A receptor ($GABA_AR$) is a major genetic target for heritable human epilepsies. Here we examine the functional effects of three epilepsy-causing mutations to the α1 subunit ($\alpha1^{T10'I}$, $\alpha1^{D192N}$ and $\alpha1^{A295D}$) on inhibitory postsynaptic currents (IPSCs) mediated by the major synaptic $GABA_AR$ isoform, α1β2γ2L. We employed a neuron - HEK293 cell heterosynapse preparation to record IPSCs mediated by mutant-containing $GABA_ARs$ in isolation from other $GABA_AR$ isoforms. IPSCs were recorded in the presence of the anticonvulsant drugs, carbamazepine and midazolam, and at elevated temperatures (22, 37 and 40 °C) to gain insight into mechanisms of febrile seizures. The mutant subunits were also transfected into cultured cortical neurons to investigate changes in synapse formation and neuronal morphology using fluorescence microscopy. We found that IPSCs mediated by $\alpha1^{T10'I}$β2γ2L, $\alpha1^{D192N}$β2γ2L $GABA_ARs$ decayed faster than those mediated by α1β2γ2L receptors. IPSCs mediated by $\alpha1^{D192N}$β2γ2L and $\alpha1^{A295D}$β2γ2L receptors also exhibited a heightened temperature sensitivity. In addition, the $\alpha1^{T10'I}$β2γ2L $GABA_ARs$ were refractory to modulation by carbamazepine or midazolam. In agreement with previous studies, we found that $\alpha1^{A295D}$β2γ2L $GABA_ARs$ were retained intracellularly in HEK293 cells and neurons. However, pre-incubation with 100 nM suberanilohydroxamic acid (SAHA) induced $\alpha1^{A295D}$β2γ2L $GABA_ARs$ to mediate IPSCs that were indistinguishable in magnitude and waveform from those mediated by α1β2γ2L receptors. Finally, mutation-specific changes to synaptic bouton size, synapse number and neurite branching were also observed. These results provide new insights into the mechanisms of epileptogenesis of α1 epilepsy mutations and suggest possible leads for improving treatments for patients harbouring these mutations.

1. Introduction

Epilepsy is a serious neurological condition, affecting 2–3% of the world's population (Wallace et al., 2001). It is not a single neurological disorder, but a diverse set of disorders arising from multiple causes, many of which have a genetic component (Helbig et al., 2016; Thomas and Berkovic, 2014). The disorders have in common the recurrence of episodic seizures (Fisher et al., 2005). Brain regions that have been identified as sites of epileptic activity include the neocortex, thalamus, hypothalamus and amygdala (Rogawski and Loscher, 2004). The γ-aminobutyric acid (type A) receptor ($GABA_AR$) is emerging as a major causal agent in some forms of genetic epilepsy. Heritable mutations to the $GABA_AR$ α, β, γ and δ subunits have been identified in family pedigrees of epilepsy (Macdonald et al., 2010) and new mutations continue to be identified (Janve et al., 2016; Kodera et al., 2016). Studies of thalamo-cortical connections of the brain have demonstrated that epileptic states are preceded by the synchronisation of excitatory activity in neuronal populations. This hypersynchronous firing can be a result of impaired GABAergic inhibitory input (Beenhakker and Huguenard, 2009), and is consistent with the presence of epilepsy-like symptoms in animals in which thalamic $GABA_ARs$ have been selectively knocked-out by genetic manipulation (DeLorey et al., 1998). The prevailing hypothesis is that GABAergic inhibitory input is critical in restraining the inherent tendency of recurrently connected excitatory neurons to transition, via positive feedback, into synchronous epileptiform firing (Rogawski and Loscher, 2004).

The $GABA_AR$ belongs to the pentameric ligand-gated ion channel (pLGIC) family (Olsen and Sieghart, 2009). Each subunit comprises a large extracellular N-terminal domain that incorporates the neurotransmitter binding site and four α-helical transmembrane domains termed M1-M4 (Fig. 1). Synaptic $GABA_ARs$ mainly consist of α (α1–6), β (β1–3) and γ (γ1–3) subunits arranged in a pentameric ring as, β-α-β-

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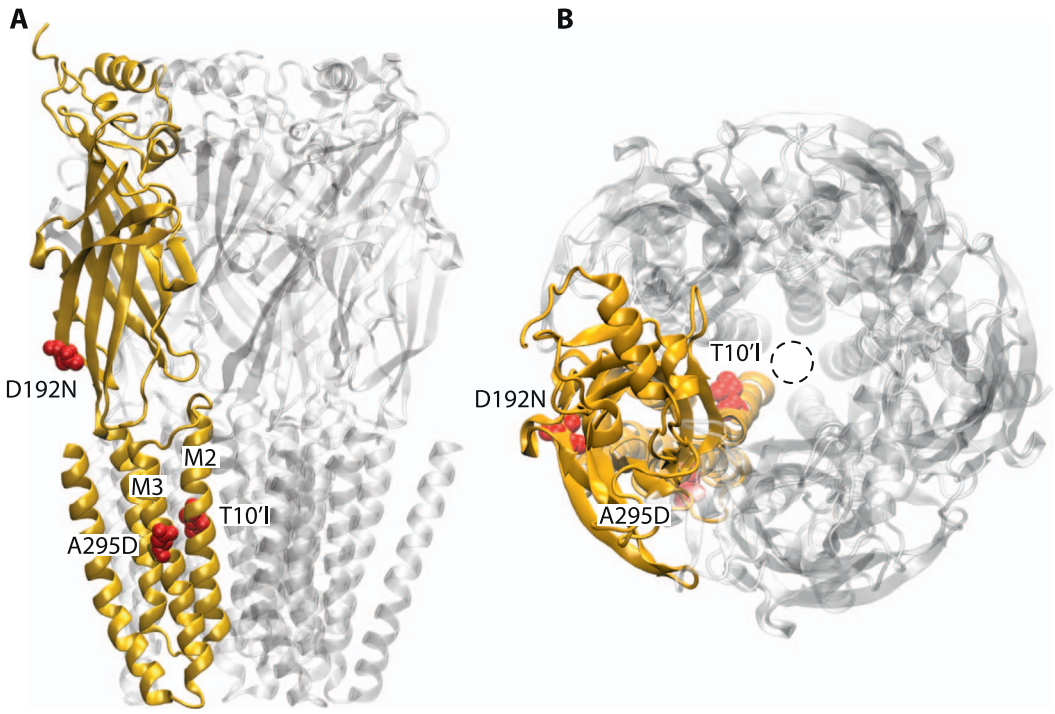

Fig. 1. Structural model of a pentameric $GABA_AR$ showing the positions of the three epilepsy mutant residues (red) in a single highlighted (yellow) subunit. The left panel shows a view of the complex from within the plane of the membrane. The right panel shows a view from the extracellular space along the central pore axis perpendicular to the membrane. The model was generated from the 3 Å crystal structure of the homomeric β3 $GABA_AR$ (Miller and Aricescu, 2014) (PDB access code: 4COF) and rendered in Visual Molecular Dynamics software.

α-γ (Farrar et al., 1999). The most abundant synaptic subtype comprises α1, β2 and γ2 subunits (Pirker et al., 2000). The $GABA_AR$ is a major therapeutic target for the treatment of epilepsy and drugs that block $GABA_AR$ currents, such as bicuculline and picrotoxin, can give rise to seizures (Rogawski and Loscher, 2004). GABA binds at the two interfaces between β (+) and α (−) subunits in the extracellular domain of the receptor and benzodiazepines bind at the homologous, single α-γ subunit interface (Olsen and Sieghart, 2009). Benzodiazepines, such as midazolam, have been used extensively in the treatment of epilepsy (Loscher and Rogawski, 2012; Riss et al., 2008). Carbamazepine is another commonly prescribed anti-epileptic drug that inhibits voltage-gated sodium channels (Kuo et al., 1997) and affects other ion channels that modulate neuronal firing, such as *N*-methyl-D-aspartate receptors (Lancaster and Davies, 1992). It also enhances whole-cell currents mediated by α1β2γ2 $GABA_ARs$ expressed in HEK293 cells or neurons (Granger et al., 1995).

In this study we sought to resolve the molecular mechanisms of epileptogenesis caused by three epilepsy-causing α1 mutations: $\alpha1^{T10'I}$ (or $\alpha1^{T265I}$), $\alpha1^{D192N}$ and $\alpha1^{A295D}$ (Fig. 1), with the aim of identifying novel therapeutic strategies for these disorders. The $\alpha1^{A295D}$ mutation, located in M3, gives rise to autosomal dominant juvenile myoclonic epilepsy. Its clinical presentation is not associated with febrile seizures. The alanine residue at position 295 is highly conserved amongst α subunits. Previous studies of this mutation in recombinantly expressed $\alpha1^{A295D}$–containing $GABA_ARs$ in HEK293 cells demonstrated reduced current amplitude (Cossette et al., 2002; Fisher, 2004) and reduced cell surface expression (21). It is reported to result in subunit misfolding, internalisation and subsequent degradation via the endoplasmic reticulum degradation pathway (Gallagher et al., 2007). The FDA-approved drug, suberanilohydroxamic acid (SAHA), was shown to modestly enhance the functional expression of $\alpha1^{A295D}$–containing $GABA_ARs$ at the cell surface (Di et al., 2013). However, the extent to which synaptic signalling is recovered by SAHA treatment remains to be determined. The $\alpha1^{D192N}$ mutation, which gives rise to idiopathic generalised epilepsy with febrile seizures (Lachance-Touchette et al., 2011), is located in the extracellular domain in a region that lines the base of the GABA binding pocket. This region has been shown to be critical for triggering activation in $GABA_ARs$ (Zhang et al., 2009) and other pLGICs (Miller and Smart, 2010). Whole-cell current recordings revealed accelerated whole-cell deactivation and desensitisation (Lachance-Touchette et al., 2011), although it is unclear whether these changes alter the activation or deactivation time courses of GABAergic inhibitory postsynaptic currents (IPSCs). Little is known about the functional consequences of the $\alpha1^{T10'I}$ mutation. This mutation is located in the middle of the pore-lining M2 domain, near the permeation 'gate' of the receptor (Miller and Smart, 2010) and other residues that are critical for channel conductance and gating (Miko et al., 2004; Shan et al., 2002; Bianchi and Macdonald, 2001; Keramidas et al., 2004; Moorhouse et al., 2002). It was recently discovered as a de novo mutation in a child with epileptic encephalopathy (Epi, 2013).

The effects of epilepsy mutations on the magnitudes, rise and decay times of IPSCs were investigated in α1β2γ2L $GABA_ARs$. Because it is not possible to control $GABA_AR$ subunit composition in neurons, we employed a heterosynapse preparation in which the postsynaptic $GABA_ARs$ comprised only the subunits of interest (Dixon et al., 2014). These recordings were also used to determine the effects of temperature (22, 37 and 40 °C) and anti-epileptic drugs (midazolam and carbamazepine) and on IPSC rise and decay kinetics. We also investigated the effects of SAHA on $GABA_ARs$ incorporating the $\alpha1^{A295D}$ mutation (Di et al., 2013). Finally, fluorescence microscopy was employed to monitor the effects of mutations and SAHA on neuronal morphology and on $GABA_AR$ surface expression efficiency and synapse number.

2. Materials and methods

2.1. HEK293 cell culture and transfection

Methods for preparing neurons and HEK293 cells for heterosynapse recordings have previously been detailed (Dixon et al., 2014). Briefly, euthanasia of timed-pregnant rats was performed via CO_2 inhalation as approved by the University of Queensland Animal Ethics Committee (approval number: QBI/142/16/NHMRC/ARC). The cortices of e18 rat