Neurobiology of Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Three epilepsy-associated *GABRG2* missense mutations at the γ+/β− interface disrupt $GABA_A$ receptor assembly and trafficking by similar mechanisms but to different extents

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ARTICLE INFO

Article history:
Received 3 March 2014
Revised 22 April 2014
Accepted 25 April 2014
Available online xxxx

Keywords:
$GABA_A$ receptors
Genetic generalized epilepsy
GABRG2(*N79S*) mutation
GABRG2(*R82Q*) mutation
GABRG2(*P83S*) mutation
Loss of function
Dominant negative effects
Subunit interface
Impaired receptor assembly

ABSTRACT

We compared the effects of three missense mutations in the $GABA_A$ receptor γ2 subunit on $GABA_A$ receptor assembly, trafficking and function in HEK293T cells cotransfected with α1, β2, and wildtype or mutant γ2 subunits. The mutations R82Q and P83S were identified in families with genetic epilepsy with febrile seizures plus (GEFS+), and N79S was found in a single patient with generalized tonic–clonic seizures (GTCS). Although all three mutations were located in an N-terminal loop that contributes to the γ+/β− subunit–subunit interface, we found that each mutation impaired $GABA_A$ receptor assembly to a different extent. The γ2(R82Q) and γ2(P83S) subunits had reduced α1β2γ2 receptor surface expression due to impaired assembly into pentamers, endoplasmic reticulum (ER) retention and degradation. In contrast, γ2(N79S) subunits were efficiently assembled into $GABA_A$ receptors with only minimally altered receptor trafficking, suggesting that N79S was a rare or susceptibility variant rather than an epilepsy mutation. Increased structural variability at assembly motifs was predicted by R82Q and P83S, but not N79S, substitution, suggesting that R82Q and P83S substitutions were less tolerated. Membrane proteins with missense mutations that impair folding and assembly often can be "rescued" by decreased temperatures. We coexpressed wildtype or mutant γ2 subunits with α1 and β2 subunits and found increased surface and total levels of both wildtype and mutant γ2 subunits after decreasing the incubation temperature to 30 °C for 24 h, suggesting that lower temperatures increased $GABA_A$ receptor stability. Thus epilepsy-associated mutations N79S, R82Q and P83S disrupted $GABA_A$ receptor assembly to different extents, an effect that could be potentially rescued by facilitating protein folding and assembly.

Introduction

Epilepsy is a common neurological disorder that affects about 1% of the world's population (Sander, 2003), and genetic epilepsy (GE) syndromes comprise ~30% of all cases (Reid et al., 2009; Steinlein, 2004). Many epilepsy mutations in affected individuals in families with GEs have been found in ion channels, including γ-amino butyric acid (GABA) type A ($GABA_A$) receptors, which are heteropentameric chloride ion channels that mediate the majority of inhibitory neurotransmission in the CNS. The receptor is composed of five subunits, and the predominant synaptic receptors are composed of two α subunits, two β subunits and one γ2 subunit. The most common epilepsy-associated $GABA_A$ receptor gene (*GABR*) is *GABRG2*, and epilepsy mutations in γ2 subunits have been shown to decrease receptor function by altering receptor biogenesis or channel function (Macdonald and Kang, 2009). Three *GABRG2* mutations R82Q, P83S and N79S (numbered based on the immature γ2 subunit containing the signal peptide) were reported to be associated with generalized epilepsies and are all located in the same structural loop in the N terminus of γ2 subunits, suggesting that they might impair $GABA_A$ receptor function similarly.

R82Q is one of the best characterized epilepsy-associated *GABRG2* mutations. It was originally found in a large family with genetic epilepsy with febrile seizures plus (GEFS+) (Marini et al., 2003; Wallace et al., 2001), contributing to childhood absence epilepsy and febrile seizures. A single nucleotide substitution caused a highly conserved arginine residue located within a loop between the α-helix and the β1-sheet (the α–β1 loop) in the extracellular N terminus to be replaced by a glutamine (Fig. 1A), resulting in impaired surface expression of γ2 subunits and decreased $GABA_A$ receptor currents (Bianchi et al., 2002; Eugene et al., 2007; Frugier et al., 2007; Hales et al., 2005; Kang and Macdonald, 2004; Sancar and Czajkowski, 2004). Heterozygous knock-in mice carrying this mutation displayed spontaneous spike-wave discharges and thermal-induced seizures (Reid et al., 2013; Tan et al., 2007), consistent with R82Q being an epilepsy-causing mutation.

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http://dx.doi.org/10.1016/j.nbd.2014.04.015
0969-9961/

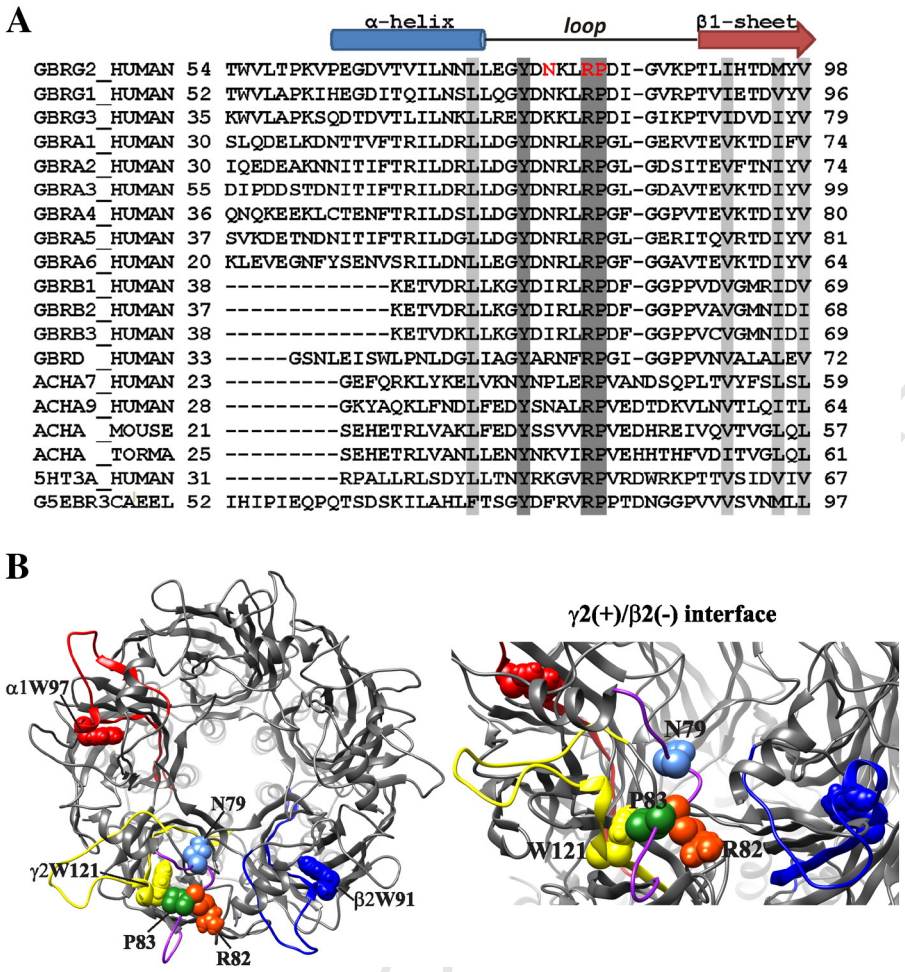

Fig. 1. Mutant residues were located in the α–β1 loop that contributes to the γ+/β− subunit–subunit interface. A. Sequences of N-terminal α-helix, α–β1 loop and β1-sheet domains of human α(1–6), β(1–3), γ(1–3) and δ subunits from the $GABA_A$ receptor family were aligned with sequences of the nicotinic acetylcholine receptor α subunit (ACHA(7,9)), 5-hydroxytryptamine 3_A receptor subunit (5HT3A) and glutamate-gated chloride channel GluCl α subunit (G5EBR3). Sites of missense mutations in the γ2 subunit were highlighted in red. In all sequences, identical residues were highlighted in dark gray and conserved residues were highlighted in light gray. The α-helix, α–β1 loop and β1-sheet domains were also represented across subunits above the alignments. B. On the left, a structural model of the α1β2γ2 $GABA_A$ receptor, as viewed from the synaptic cleft, was shown. Sites of missense mutations in γ2 subunit, located at the γ2(+)/β2(−) interface, were shown in space-filling representation, i.e., N79 in light blue, R82 in orange, and P83 in green, and the α–β1 loop where these three residues were located was shown in purple. Homologous motifs for α1β2γ2 receptor assembly at the respective complementary (−) interfaces (α1: red; β2: dark blue; γ2: yellow) and conserved tryptophan residues located in these motifs (α1W97, red; β2W91, dark blue; γ2W121, yellow) were also represented. On the right, an enlarged 45° side view of the γ+/β− subunit–subunit interface with a close-up of missense mutations in the α–β1 loop was also shown.

However, whether this mutation has dominant negative effects on other $GABA_A$ receptor subunits and how it affects subunit–subunit interactions is still controversial (Frugier et al., 2007; Hales et al., 2005). A recent study showed that while loss of γ2 subunit function could account for the absence of seizure phenotype, the R82Q mutation might be responsible for the febrile seizure phenotype (Reid et al., 2013), further suggesting that the R82Q mutation had effects in addition to haploinsufficiency.

Recently, another epilepsy-associated *GABRG2* mutation, P83S, which is also located within the α–β1 loop of the γ2 subunit, was identified in a three generation GEFS+ family (Lachance-Touchette et al., 2011). Although this mutation was found in all affected individuals in this family and was predicted to have damaging effects, it was reported that $GABA_A$ receptor channel function was not affected by the mutation, and the effects on receptor trafficking were not addressed. How this mutation contributes to epileptogenesis is therefore still uncertain.

Finally, it was reported that a *GABRG2* mutation, N79S, also located in the α–β1 loop of the γ2 subunit, was found in a single patient with generalized tonic–clonic seizures (GTCS) (Shi et al., 2010). The mutation was reported to only modify the steepness of the GABA concentration–response curve (Migita et al., 2013).

All three mutations are located in the N-terminal domain of γ2 subunits that forms part of the γ2+/β2− subunit interface (Fig. 1B), suggesting that they may produce similar impairments of subunit oligomerization and receptor assembly (Hales et al., 2005). In the present study, we compared the effects of these three epilepsy-associated *GABRG2* mutations on surface expression and function of α1β2γ2 receptors in transfected HEK293T cells and rat cortical neurons and found that they impaired assembly and trafficking of $GABA_A$ receptors by similar mechanisms but to different extents.

Materials and methods (1249)

Expression vectors

The coding sequences of human α1, β2 and γ2 $GABA_A$ receptor subunits were cloned into pcDNA3.1 expression vectors (Invitrogen). All subunit residues were numbered based on the immature peptide. Mutant γ2 subunit constructs were generated using the QuikChange site-directed mutagenesis kit (Stratagene). An HA or FLAG epitope was inserted at a functionally silent site (between the 4th and 5th residue of the mature peptide) to facilitate our experiments (Connolly

Please cite this article as: Huang, X., et al., Three epilepsy-associated GABRG2 missense mutations at the γ+/β− interface disrupt $GABA_A$ receptor assembly and trafficking by similar mechanisms but to different extents, Neurobiol. Dis. (2014), http://dx.doi.org/10.1016/j.nbd.2014.04.015