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$GABA_A$ receptor epilepsy mutations

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Abstract

Idiopathic generalized epilepsy (IGE) syndromes are diseases that are characterized by absence, myoclonic, and/or primary generalized tonic-clonic seizures in the absence of structural brain abnormalities. Although it was long hypothesized that IGE had a genetic basis, only recently have causative genes been identified. Here we review mutations in the $GABA_A$ receptor α1, γ2, and δ subunits that have been associated with different IGE syndromes. These mutations affect $GABA_A$ receptor gating, expression, and/or trafficking of the receptor to the cell surface, all pathophysiological mechanisms that result in neuronal disinhibition and thus predispose affected patients to seizures.

Keywords: $GABA_A$ receptor; Mutations; Epilepsy; Myoclonus; Review; Electrophysiology; Trafficking

1. Epilepsy genes

Epilepsy affects more than 0.5% of the population in the world [1], and genetic factors play an important role in many of the idiopathic generalized epilepsies (IGEs) and in some partial epilepsies [1–5]. Recently, monogenic mutations of ion channel genes have been found in patients with inherited epilepsies. These epilepsy mutations have been identified in multiple voltage- and ligand-gated ion channels (LGICs) including the sodium, calcium and potassium voltage-gated ion channels and nicotinic cholinergic and $GABA_A$ receptor ligand-gated ion channel [2,4].

$GABA_A$ receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system and have been repeatedly documented to play a critical role in animal models of seizures [6–13], and recently epilepsy mutations have also been identified in human $GABA_A$ receptor genes (Fig. 1) [2,4]. This review will focus on the recently described human $GABA_A$ receptor channel epilepsy mutations.

2. $GABA_A$ receptors

$GABA_A$ receptors are formed by the assembly of multiple subunit subtypes (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ, and ρ1–ρ3) into a pentamer, although the most common and likely subunit composition has been determined to contain two α subunits, two β subunits and a γ subunit [14,15]. Once assembled, $GABA_A$ receptors form chloride ion channels, and $GABA_A$ receptor currents can be modulated by a number of positive and negative allosteric regulators, including barbiturates, benzodiazepines, and neurosteroids, as well as bicuculline, picrotoxin, and zinc. $GABA_A$ receptors have been shown to be involved in both phasic, inhibitory synaptic transmission and tonic, perisynaptic inhibition.

2.1. Phasic inhibition

Inhibitory postsynaptic currents (IPSCs) are triggered by release of presynaptic GABA that binds to postsynaptic $GABA_A$ receptors. IPSCs have a rapidly activating current (rise time of ~1 ms or less) that decays to baseline over tens to hundreds of ms. Initial studies of factors that shape IPSCs used the "concentration jump" technique to mimic the transient application of GABA during IPSCs. Brief

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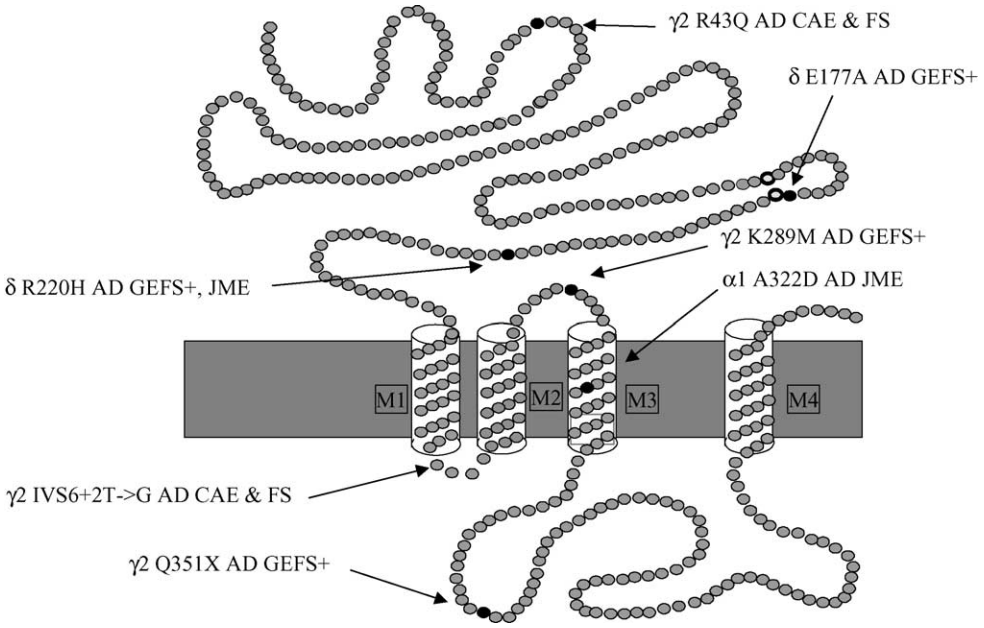

Fig. 1. Diagram of the putative membrane topology of the $GABA_A$ receptor, showing the location of recently identified mutations associated with generalized epilepsies in humans.

pulses of GABA applied to excised patches evoked currents that activated rapidly (<1 ms) and decayed slowly and biphasically (10–200 ms time constants) [16,17], suggesting that post-synaptic factors primarily determined IPSC shape. Studies with recombinant αβγ $GABA_A$ receptors showed that similar long duration IPSC-like currents were evoked by brief GABA applications, also suggesting that IPSC shape depended on intrinsic $GABA_A$ receptor properties [18,19]. Brief GABA pulses applied to recombinant αβγ receptors, therefore, provide a good model of synaptic currents.

2.2. Tonic inhibition

While phasic, inhibitory synaptic transmission is well characterized, there is compelling evidence for tonic, non-synaptic communication. Various neurotransmitters are known to circulate in the extracellular space [20], and $GABA_A$ receptors are also found in extra-synaptic locations. Extracellular neurotransmitter concentrations may be affected by spillover from synaptic transmission or by membrane transporters [21–23]. Currents that are consistent with the presence of both synaptic and extra-synaptic $GABA_A$ receptors have been recorded from hippocampal neurons in slices [24–26]. Properties conferred by the δ subunit would be consistent with a role for αβδ receptors in tonic inhibition. αβδ receptors desensitize more slowly and less extensively than αβγ receptors and have a lower GABA EC_{50} [19,27–29], ideal properties for a receptor sensing low GABA concentration for sustained time periods. Additionally, α6β3δ receptors have a low GABA EC_{50} (~200 nM) and may also be the extracellular target for low concentrations of GABA [30]). It should be noted that although Nusser et al. [31] suggested that α6β3δ receptors mediated tonic inhibition, α1/6β2/3γ2 receptors were also observed in non-synaptic membranes and the tonic inhibition in cultured cerebellar neurons was benzodiazepine sensitive, consistent with α1βγ2 receptors [32], suggesting that both δ and γ subunit-containing receptors may mediate tonic inhibition.

3. $GABA_A$ receptor epilepsy genes

The initial $GABA_A$ receptor mutations associated with IGEs were found in the γ2 and α1 subunits, consistent with a genetic defect in phasic, inhibitory GABAergic synaptic inhibition, and recently we have reported that GABRD, the gene encoding the δ subunit, is a susceptibility locus for IGEs (Fig. 1) [33]. These findings suggest that compromise of both phasic αβγ and tonic αβδ GABAergic inhibition may lead to generalized epilepsy. We used expression of αβγ and αβδ $GABA_A$ receptors in human embryonic kidney 293T (HEK293T) cells and application of GABA with the rapid application technique to excised macropatches and lifted cells to determine the effect of α1 [34], γ2 [35] and δ [33] $GABA_A$ receptor subunit gene mutations associated with IGEs on phasic, αβγ ''IPSC-like'' and tonic αβδ ''perisynaptic-like'' currents.

3.1. $GABA_A$ receptor γ2 subunit mutation (K289M)

A family with an autosomal dominant generalized epilepsy similar to generalized epilepsy with febrile seizures plus (GEFS+) was shown to have a K289M mutation in the $GABA_A$ receptor γ2 subunit [36]. This residue is located in

the short extracellular loop between transmembrane domains M2 and M3 (M2–M3 loop) (Fig. 1), a region that has been implicated in the gating of ligand-gated ion channels [37–40]. Recordings from oocytes expressing homozygous α1β2γ2(K289M) $GABA_A$ receptors revealed smaller amplitude currents relative to wild type receptor current amplitudes [36].

We reinvestigated the effects of this mutation [28]. Wild type α1β3γ2L and mutant α1β3γ2L(K289M) $GABA_A$ receptors were transiently expressed in a mammalian expression system (HEK293T cells). We used a rapid application concentration jump technique (open tip application rise time ∼400 μs) to apply GABA [41] for long (400 or 6 s) or brief (2–5 ms) durations. We used the excised outside-out patch clamp recording technique to determine the effects of these mutations on the pharmacological and biophysical properties of transient macropatch and single channel wild type α1β3γ2L and mutant α1β3γ2L(K289M) $GABA_A$ receptor currents (Fig. 2). Wild type α1β3γ2L $GABA_A$ receptor macropatch currents evoked by 400 ms applications of 1 mM GABA were large (∼500 pA) (Fig. 2A and E), desensitized with two time constants (∼7.5 and 130 ms) (Fig. 2B and C) and deactivated with two time constants with a weighted time constant of ∼200 ms (Fig. 2F). In our study, homozygous α1β3γ2L(K289M) currents evoked by 400 ms applications of 1 mM GABA did not have reduced current amplitude as reported by [36]. The mutation also did not alter the rate of activation (Fig. 2D) or desensitization (Fig. 2B and C) but did result in faster deactivation (∼100 ms) (Fig. 2F). When currents were evoked by brief applications of 1 mM GABA, the weighted current deactivation rate was reduced from ∼70 to ∼35 ms (Fig. 3A1, A2 and B). Analyses of single channel currents from $GABA_A$ receptors containing the γ2K289M mutation revealed that mean open times of γ2K289M mutations were four-fold shorter than wild type α/β/γ receptors, consistent with its faster whole-cell current deactivation time. Brief, rapid GABA applications to excised macropatches have been shown evoke currents that are very similar to inhibitory post-synaptic currents (IPSCs) [17,19]. Reduction of the duration of the GABA-evoked rapid application current by the γ2L(K289M) mutation suggests that the mutation results in reduced inhibitory post-synaptic current duration, thus producing disinhibition that may lead to epilepsy.

The basis for the mutation-induced acceleration of deactivation that we reported is unclear. However, insights into the coupling of binding of GABA to subsequent gating of the channel have been obtained recently. The extracellular N-terminal domain is directly connected to M1; however, evidence from several sources has suggested that the N-terminal domain also interacts with M2 and the M2–M3 loop. Detailed structural information is available for the extracellular domain of ligand-gated ion channels based on homology with the acetylcholine binding protein (AChBP) [42], which has been crystallized [43]. The ACh

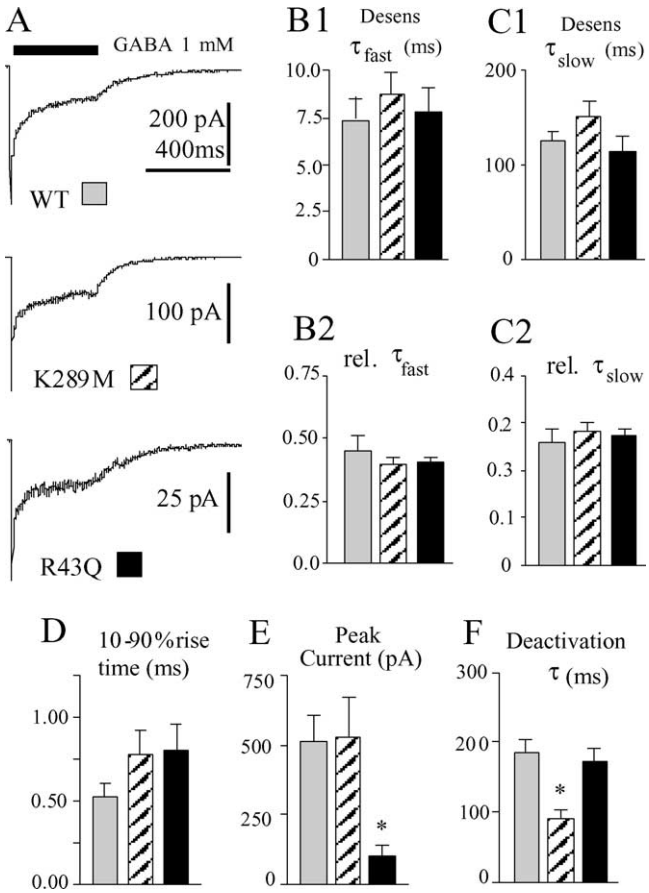

Fig. 2. Macroscopic kinetic properties of wild type α1β3γ2L and mutant α1β3γ2L(K289M) and α1β3γ2L(R43Q) $GABA_A$ receptor currents. (A) Representative currents from wild type α1β3γ2L or mutated α1β3γ2L(K289M) and α1β3γ2L(R43Q) receptors evoked by 400 ms jumps into 1 mM GABA. The time scale of top trace applies to all three traces. (B1 and C2) Neither fast (B1) nor slow (C1) time constants of desensitization, nor their relative contributions (B2 and C2) were significantly altered by the γ2L(K289M) mutations. (D) The 10–90% rise time of the current was not significantly altered by the mutations. (E) The peak current amplitudes were significantly smaller for α1β3γ2L(R43Q), but not α1β3γ2L(K289M), $GABA_A$ receptors (asterisk, $p < 0.01$). (F) The current deactivation rate after removal of GABA was significantly faster for α1β3γ2L(K289M), but not α1β3γ2L(R43Q), $GABA_A$ receptors (asterisk, $p < 0.001$). (Fig. 2 from [35] with permission).

binding pocket is formed at subunit interfaces by three loops (A–C) from the positive side and three β strands (D–F) from the negative side of the protomer. Using a mutagenesis approach, critical residues in the $GABA_A$ receptor β2 subunit in two binding domains equivalent to loops B (Y157, T160) and C (T202, Y205) were identified [44]. Using the substituted cysteine accessibility method (SCAM), residues in $GABA_A$ receptor β2 subunit in domains equivalent to loop A (β strand 4; Y97, L99) [45] and loop C (β strand 10; S204, Y205, R207, S209) [46] and in $GABA_A$ receptor α1 subunit residues in domains equivalent to loop D (β strand 2; F64, R66) [47] and loop F (V178, V180, D183) [48] were identified. These data suggest that the $GABA_A$ receptor binding pocket is similar to that of the AChBP binding pocket,

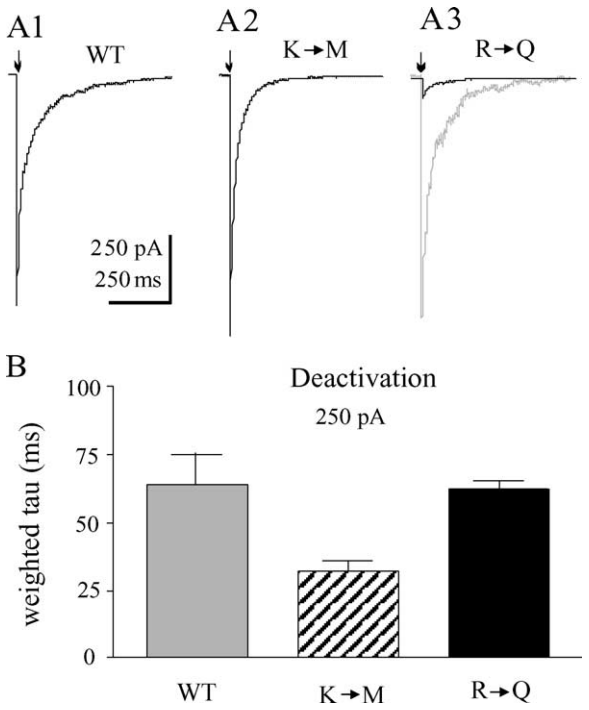

Fig. 3. Brief wild type α1β3γ2L and mutant α1β3γ2L(K289M) and α1β3γ2L(R43Q) $GABA_A$ receptor currents. (A) Representative currents illustrate deactivation rates of α1β3γ2L (A1), α1β3γ2L(K289M) (A2), and α1β3γ2L(R43Q) (A3) $GABA_A$ receptor currents in response to brief (<5 ms) pulses of GABA (1 mM). Scale bars apply to all three solid traces. The solid trace in A3 is expanded 10-fold (gray trace) for comparison of deactivation current time course. (B) The weighted deactivation time constants are shown for wild type and mutated channels. Deactivation was significantly faster for α1β3γ2L(K289M) $GABA_A$ receptor currents (hatched bar; asterisk, $p < 0.05$). α1β3γ2L (R43Q) $GABA_A$ receptor deactivation (solid bar) was not different than that of wild type receptors (gray bar). (Fig. 3 [35] with permission).

consistent with a high degree of homology between the AChBP and $GABA_A$ receptors.

Disease mutations in the M2–M3 loop of other ligand-gated ion channels have been identified including in the nAChR (congenital myasthenia; [49]) and the glycine receptor, (hyperekplexia; [50]). These mutations also decreased ligand-gated current, suggesting disruption of coupling. Based on the AChBP it was suggested that $GABA_A$ receptor α subunit loops 2 and 7 interact with a K residue in the M2–M3 loop to couple GABA binding to gating [39]. Using SCAM, gating was shown to induce a conformational change in and/or around the N-terminal half of the M2–M3 loop [51]. Thus, coupling of binding to channel gating may involve both direct (M1) and indirect (loops 2, 7, M2–M3 loop) pathways. The gating process has been further clarified using electron microscopy of nAChRs [40,52]. Loop 2 of both α subunits is positioned such that it contacts the distal M2, just before the beginning of the M2–M3 loop. ACh binding induces both loop 2s to rotate 15° about an axis passing through the disulphide bridge, normal to the membrane. The loop 2 rotations are associated with M2 rotations, which are translated to the gate, presumably causing the gate to open. Thus the major transduction of binding to gating appears to pass in the α subunits from loop 2 to the distal M2 and the K in the middle of the M2–M3 loop and then to the M2 gate.

These data suggest an important role for the M2–M3 loop and suggest that the γ2L(K289) mutation may be in a position to disrupt biophysical function of the receptor. However, since the γ2L subunit does not appear to be directly within the α subunit transduction pathway, it may be in a position to modify other properties of the receptor channel such as deactivation.

3.2. $GABA_A$ receptor γ2 subunit mutation (R43Q)

Wallace et al. [53] reported a missense mutation, R43Q (in the N-terminal extracellular domain of the γ2 subunit) in affected individuals of a large family having both childhood absence epilepsy (CAE) and febrile seizures (Fig. 1). Recordings from *Xenopus* oocytes injected with α1β2γ2(R43Q) $GABA_A$ receptor subunits suggested no differences in GABA EC_{50}, current amplitude or apparent desensitization. However, the receptors were insensitive to functional modulation by the benzodiazepine diazepam. Although this raised the interesting possibility of an endogenous ligand at the benzodiazepine recognition site, that interpretation depends upon the exclusion of any other functional consequences of the mutation.

We reinvestigated the effect of this mutation [35]. We reported that the γ2(R43Q) mutation did not alter benzodiazepine sensitivity but did reduce peak current amplitude. We used the rapid application concentration jump technique (open tip application rise time ∼400 ms) to apply GABA [41] for long (400 or 6 s) or brief (2–5 ms) durations. We used the excised outside-out patch clamp recording technique to determine the effects of these mutations on the pharmacological and biophysical properties of transient macropatch and single channel wild type α1β3γ2L and mutant α1β3γ2L(R43Q) $GABA_A$ receptor currents (Fig. 2). Wild type α1β3γ2L $GABA_A$ receptor macropatch currents evoked by 400 ms applications of 1 mM GABA were large (∼500 pA) (Fig. 2A and E), desensitized with two time constants (∼7.5 and 130 ms) (Fig. 2B and C) and deactivated with two time constants with a weighted time constant of ∼200 ms (Fig. 2F). In our study, homozygous α1β3γ2L(R43Q) currents evoked by 400 ms applications of 1 mM GABA had reduced current amplitude (Fig. 2E). The mutation also did not alter the rate of activation (Fig. 2D), desensitization (Fig. 2B and C) or deactivation (Fig. 2F). When currents were evoked by brief applications of 1 mM GABA, the weighted current deactivation rate was unchanged but current amplitude was reduced (Fig. 3A1, A3 and B).

A different effect of the γ2(R43Q) mutation was reported by Bowser et al. [54]. Using similar techniques this group reported that the mutation increased the rate of desensitization and slowed deactivation. In addition, the kinetic properties of the wild type currents were altered

with minimal desensitization. Furthermore, they reported a small decrease in benzodiazepine sensitivity. The basis for the altered wild type current and these different effects of the γ2(R43Q) mutation are unclear.

We have explored the basis for the effect of the γ2(R43Q) mutation on peak current amplitude (Kang and Macdonald, submitted). We expressed wild type and mutant α1β2γ2S receptors with free assembly and with assembly fixed by using γβα and βα tethered constructs to exclude any contribution of α1β2 receptors to the reduced current. We demonstrated that the current reduction was due to reduced surface expression of receptor protein (Fig. 4). By expressing γ2S subunit coupled to EYFP (enhanced yellow fluorescent protein), we determined that the majority of the mutant α1β2γ2S(R43Q) receptors were retained intracellularly in a mesh-like pattern and that only very weak fluorescence appeared on the membrane surface. Co-expression of α1β2γ2S receptors with cell membrane- or endoplasmic reticulum (ER)-specific markers revealed that the γ2S(R43Q)-containing receptors colocalized extensively with the fluorescence of the ER marker but only slightly with that of the cell membrane marker. Thus, the reduced surface expression of mutant hα1β2γ2S(R43Q) was due to receptor retention in the ER. This increased ER retention was probably due to the impaired protein assembly, folding and trafficking.

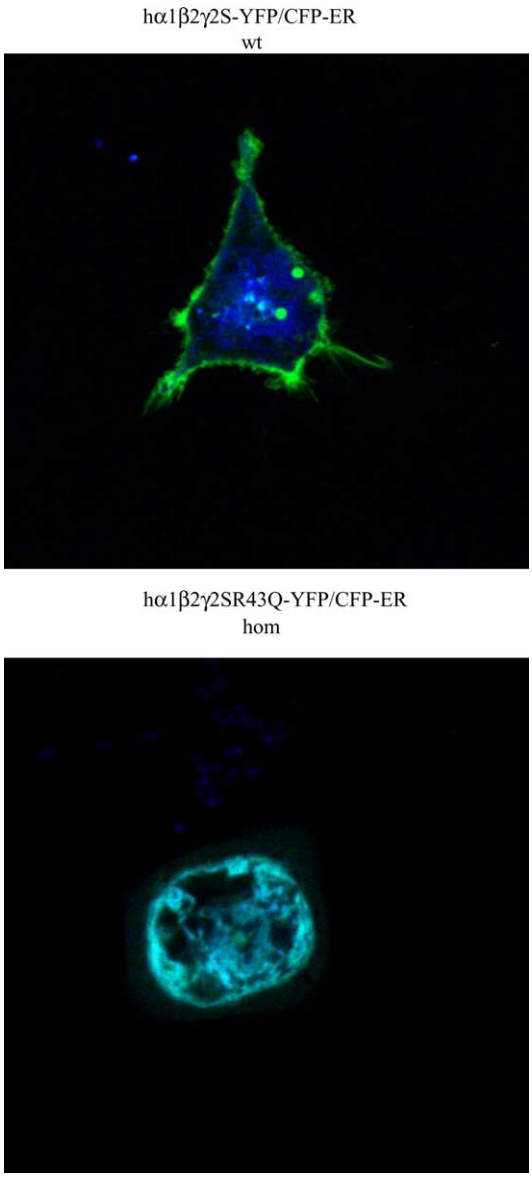

Fig. 4. Representative confocal fluorescence images of Cos-7 cells transfected with hα1β2γ2S-EYFP or hα1β2γ2S(R43Q)-EYFP receptors. Wild type (WT) α1β2γ2S-EYFP receptors were primarily in the cell membrane and had weak colocalization with pECFP-ER. Homozygous α1β2γ2S(R43Q)-EYFP receptors were found primarily in intracellular compartments and co localized with the pECFP-ER marker. (Kang and Macdonald, unpublished).

3.3. $GABA_A$ receptor γ2 subunit mutation (Q351X)

A γ2 subunit mutation (Q351X) localized in the intracellular loop between M3 and M4 (Fig. 1) was identified in a family with GEFS+, which introduced a premature stop codon at Q351 [55]. With homozygous expression in oocytes, GABA sensitivity was abolished, suggesting a nonfunctional $GABA_A$ receptor. When this mutation was further analyzed by Harkin et al. [55], using a green fluorescent protein (GFP)-tagged γ subunit, they found that the receptor, although assembled, did not exhibit surface expression but remained trapped in the endoplasmic reticulum. Thus, this mutation would be expected to reduce surface expression of functional $GABA_A$ receptor complexes, which would lead to decreased GABAergic inhibition and presumably increased excitatory activity.

3.4. $GABA_A$ receptor γ2 subunit truncation mutation (IVS6 + 2T → G)

Interestingly, a splice-site mutation in γ2 subunit has been identified in a family with childhood absence epilepsy and febrile convulsions (FC) [56] (Fig. 1). The effect of this mutation on $GABA_A$ receptor function is unknown, but was predicted to lead to nonfunctional protein. A point mutation in the gene was found that leads to a splice-donor site mutation in intron 6 (IVS6 + 2T → G), resulting in production of a truncated γ2 subunit protein. To date, electrophysiological experiments to study the effects of this mutation have not been reported. Due to the site of the truncation (just upstream of M1), it is questionable whether a $GABA_A$ receptor subunit that contains this mutation would be expressed and incorporated into a functional $GABA_A$ receptor.

3.5. $GABA_A$ receptor α1 subunit mutation (A322D)

A mutation of a highly-conserved alanine residue in the third transmembrane segment (M3) in the $GABA_A$ receptor α1 subunit (A322D) is associated with an autosomal

dominant juvenile myoclonic epilepsy (ADJME, [57]). Unlike γ- and δ-subunits, which are only present in one copy within each $GABA_A$ receptor pentamer, $GABA_A$ receptor α subunits are typically present in two copies in each pentamer with one α-subunit positioned between a β and γ subunit ($\alpha_{\beta\alpha\gamma}$) and the other α-subunit positioned between two β-subunits ($\alpha_{\beta\alpha\beta}$) [15]. Thus, if wild type and mutant subunits are all efficiently assembled into $GABA_A$ receptors, the presence of the autosomal dominant mutation, α1A322D would be expected to produce four different $GABA_A$ receptor pentamer assemblies: α1β2α1β2γ2 (wild type), α1(A322D)β2α1β2γ2 (Het-$_{\beta}$αγ), α1β2α1(A322D)β2γ2 ($Het_{\beta\alpha\beta}$), α1(A322D)β2α1(A322D)β2γ2 (homozygous).

Homozygous currents differed substantially from wild type currents: their peak current amplitudes were reduced by 90%, their GABA EC_{50} was increased by 100-fold, and they lacked GABA-evoked desensitization ([57–59]. In addition, single channel recordings of homozygous α1(A322D) receptors demonstrated that their mean open times were reduced from 2.23 to 0.54 ms and that the contribution of the longest open state was reduced from 14.8 to 0.8% [58].

Because patients with ADJME were heterozygous, not homozygous, for the α1(A322D) mutation, we transfected HEK293T cells with wild type β2 and γ2 subunits and either wild type α1 subunit, α1(A322D), or a 50:50 mixture of wild type α1 subunit and α1(A322D) [59]. This transfection strategy was expected to recapitulate the heterozygous phenotype by generating a binomial mixture of wild type, $Het_{\beta\alpha\beta}$, $Het_{\beta\alpha\gamma}$, and homozygous receptors. Mean peak current amplitudes from heterozygously transfected cells were substantially smaller than those of wild type currents but larger than those of homozygous mutant currents (Fig. 5A); the heterozygous currents had similar activation, desensitization, and deactivation current

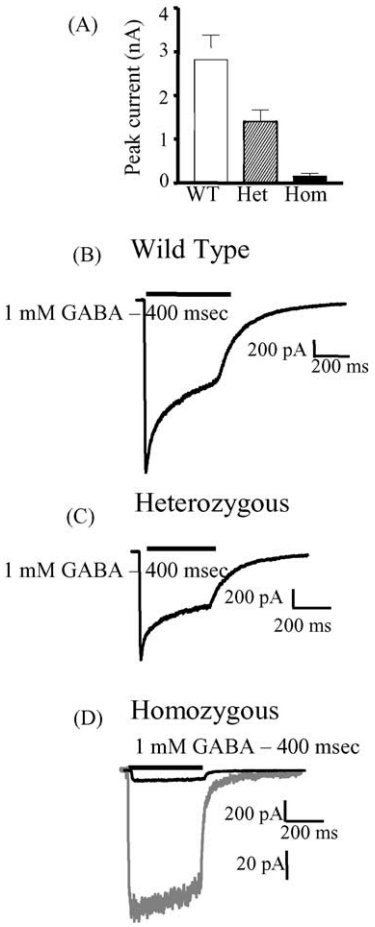

Fig. 5. The ADJME α1A322D mutation alters mean peak currents and current kinetics. (A) Mean peak currents (±S.E.M.) from homozygous mutant receptors ($N = 5$) were smaller than heterozygous receptors ($n = 16$, $p < 0.001$) which were smaller than wild type peak current amplitudes ($N = 17$, $p < 0.05$). Specimen current traces obtained from (B) wild type, (C) heterozygous or (D) homozygous mutant transfections after application of 1 mM GABA. The gray trace in (D) is plotted in an expanded scale (lower scale bar) in order to display the current kinetics timecourse. (modified from [59]).

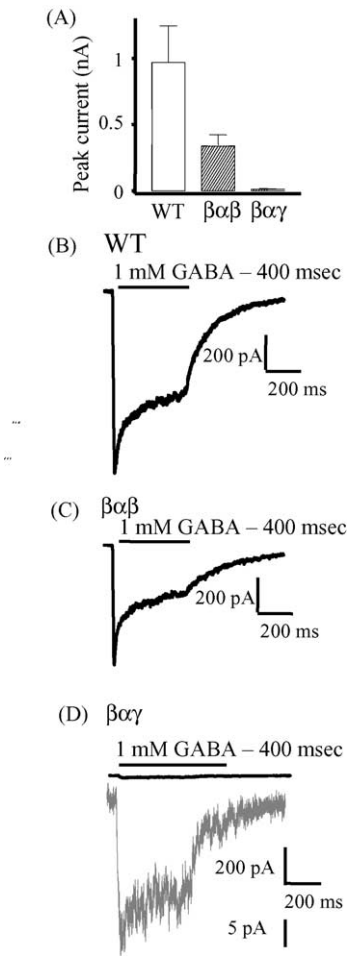

Fig. 6. The α1A322D ADJME mutation is asymmetric. (A) Mean peak currents (±S.E.M.) of wild type tethered receptors ($N = 20$) were larger than those of $Het_{\beta\alpha\beta}$ currents ($N = 17$, $p < 0.05$) which were substantially larger than $Het_{\beta\alpha\gamma}$ currents ($n = 8$, $p < 0.01$). Specimen current traces from (B) wild type (WT), (C) $Het_{\beta\alpha\beta}$ or (D) $Het_{\beta\alpha\gamma}$ receptors. The gray trace in (D) is depicted in an expanded scale (lower scale bar) in order to display current kinetic time course. (modified from [59]).

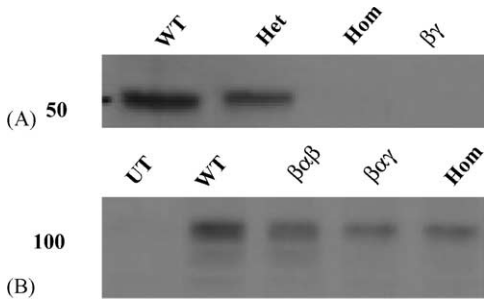

Fig. 7. The ADJME α1A322D mutation reduces α1 subunit expression. (A) Western blots of whole-cell lysates (20 μg protein) from HEK293T cells transfected with, β2 and γ2 subunits and either wild type α1 (WT), a 50:50 mixture of wild type and α1(A322D) (Het), α1(A322D) subunit (Hom), or only β2 and γ2 subunits without α1 subunit (βγ) were probed with a monoclonal antibody directed against the α1 subunit N-terminus. Heterozygous α1 subunit expression was smaller than wild type (56 ± 13% wild type expression, $N = 4$) but larger than homozygous mutant (6 ± 4% wild type expression, $N = 5$). (B) Western blots of whole-cell lysates (30 μg protein) from HEK293T cells transfected with tethered wild type (WT), $Het_{\beta\alpha\beta}$ (βαβ), $Het_{\beta\alpha\gamma}$ (βαγ), or homozygous mutant (Hom) were probed with a monoclonal antibody targeted against the β2 subunit. β–α expression was greatest in wild type tethered receptors compared with $Het_{\beta\alpha\beta}$ (71 ± 9% relative to wild type, $N = 5$), $Het_{\beta\alpha\gamma}$ (51 ± 16% relative to wild type, $N = 5$) or homozygous mutant (36 ± 14% relative to wild type, $N = 4$). (modified from [59]).

kinetics as those from wild type, but differed from those of homozygous mutant (Fig. 5B and C) [59]. Next, we used tethered concatamers to selectively target the α1(A322D) mutation to either the $\alpha_{\beta\alpha\beta}$ or $\alpha_{\beta\alpha\gamma}$ subunit and thus selectively generate either $Het_{\beta\alpha\beta}$ or $Het_{\beta\alpha\gamma}$ receptors without generating a binomial mixture of receptors. With this method, we demonstrated that the α1(A322D) mutation had markedly different consequences depending on which α subunit was mutated; $Het_{\beta\alpha\beta}$ receptor mean peak current amplitude was less than wild type (35%) but substantially greater than that of $Het_{\beta\alpha\gamma}$ receptors (Fig. 6).

We showed that the primary reason for the reduced peak current amplitudes for both tethered and untethered mutant receptors was a reduced level of whole-cell and surface receptor expression (Fig. 7). It is possible that α1(A322D) reduced α1 subunit expression by inhibiting correct $GABA_A$ receptor folding and assembly. $GABA_A$ receptors are folded and assembled in the endoplasmic reticulum and incorrectly assembled receptors are degraded. A similar disruption of expression has been reported from nonconservative mutations of M3 residues in the AChR [60]. Although reduction of receptor expression is likely the most important factor in the diminution of whole-cell peak currents, the α1(A322D) mutation also increased the GABA EC_{50} value and altered whole-cell current kinetics, thus demonstrating that it directly altered channel function. This conclusion is consistent with prior structure–function studies. In the $GABA_A$ receptor, water-soluble probes react with α1 subunit M3 residues near α1A322 only in the presence GABA, suggesting that in the presence of agonist, M3 undergoes an agonist-induced conformational change and thus is important in signal transduction [61]. Furthermore, site directed mutagenesis and SCAM studies demonstrated that M3 residues are important for $GABA_A$ receptor modulation by benzodiazepines, ethanol, propofol, and volatile anesthetics [62–65].

3.6. $GABA_A$ receptor δ subunit mutation (E177A)

We have completed an initial characterization of new $GABA_A$ receptor δ subunit gene (GABRD) epilepsy mutations/polymorphisms [33]. Two putative missense mutations in GABRD were identified: E177A was detected in a small GEFS+ family (Fig. 1), and R220C was detected in a second small GEFS+ family. Both changes were heterozygous and neither was detected in controls derived from anonymous blood donors primarily of Caucasian origin. Compared to wild type receptors, homozygous and heterozygous α1β2Sδ(E177A) $GABA_A$ receptors had decreased $GABA_A$ receptor current amplitudes (Fig. 8).

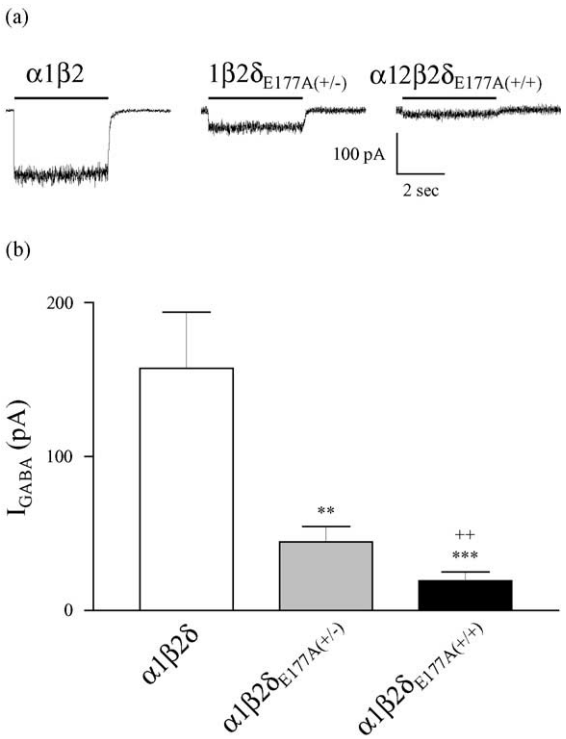

Fig. 8. The GEFS+ δE177A variant reduces α1β2δ current amplitudes. (a) Typical examples of the whole-cell currents evoked by 1 mM GABA from wild type (WT) δ subunit-, heterozygous (HET) or homozygous (HOM) δE177A variant subunit-containing $GABA_A$ receptors. (b) Incorporation of either HET or HOM δE177A variant into $GABA_A$ receptors resulted in reduction of maximal GABA currents. Compared to WT receptors ($n = 39$), the maximal currents were significantly decreased for HET ($n = 18$) or HOM ($n = 25$) δE177A variant subunit-containing receptors. The maximal currents were also significantly different between HET and HOM δE177A variant subunit-containing receptors. (modified from [33]). $^{**}p < 0.01$ compared with WT receptors; $^{***}p < 0.001$; $^{++}p < 0.01$ compared with HET receptors.

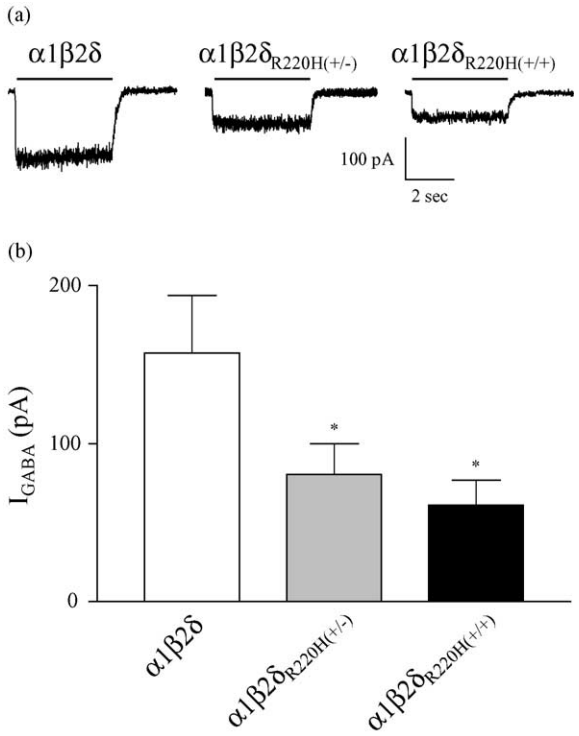

Fig. 9. The JME δR220H polymorphism reduces α1β2δ current amplitudes. (a) Typical examples of the whole-cell currents evoked by 1 mM GABA from wild type (WT) δ subunit-, heterozygous (HET) or homozygous (HOM) δR220H polymorphic subunit-containing $GABA_A$ receptors. (b) Incorporation of either HET or HOM δR220H variant into $GABA_A$ receptors resulted in reduction of maximal GABA currents. Compared to WT receptors ($n = 39$), the maximal currents were significantly decreased for HET ($n = 33$) or HOM ($n = 21$) δR220H variant subunit-containing receptors. No significant difference in maximal currents was observed between HET and HOM δR220H variant subunit-containing receptors. (modified from [33]). $^*p < 0.05$ compared with WT receptors.

3.7. $GABA_A$ receptor δ subunit mutation (R220H)

A base change was also identified, which resulted in a missense mutation (R220H) [33]. GABRD R220H (Fig. 1) was found to be carried by IGE, GEFS+ and FS patients as well as control blood bank individuals. When present homozygously, the δR220H mutation was associated with a patient with JME. To determine the effects of the δ(R220H) polymorphism, we recorded currents evoked by GABA (1 mM). Heterozygous α1β2Sδ(R220H) receptors had significantly decreased peak currents compared to wild type controls (Fig. 9). We proposed that GABRD R220H combines additively as a susceptibility allele with other yet to be identified susceptibility alleles responsible for the complex epilepsies, and proposed that the reduced current associated with the variant was likely to be associated with disinhibition [33]. Since δ subunit-containing receptors are involved in tonic inhibition, we suggested that alteration of tonic, perisynaptic inhibition may contribute to the common IGEs. The basis for the mutation-induced reduction in current is unknown.

4. Conclusions

The studies reviewed here demonstrate that missense and truncation mutations of $GABA_A$ receptor subunit proteins that are associated with different IGE syndromes alter $GABA_A$ function and/or expression when expressed in immortalized mammalian cell lines. However, these studies represent just the beginning of the characterization of the pathophysiology of the $GABA_A$ receptor related IGEs. The biophysical basis for the increased rate of whole-cell current the deactivation of $GABA_A$ receptors containing the γ2K289M mutation was explained by reduced mean single channel open times, but mechanistic explanations for the altered current kinetic properties of the α1A322D, δE177A, and δR220H mutations have not yet been elucidated. Such an understanding is necessary to predict and tailor the actions of antiepileptic drugs in these epilepsy syndromes.

The studies described in this review also demonstrated that the α1A322D, γ2R43Q, and γ2Q351X mutations decreased $GABA_A$ receptor subunit cell surface expression. However, the mechanisms by which their expression is reduced is unknown. Given their position in the interior of the subunit proteins, it is likely that the α1A322D and γ2Q351X mutations inhibit proper protein folding. Misfolded $GABA_A$ receptor subunit proteins do not assemble properly in the ER and are then degraded (for a review see [66]; the reduction of expression of total α1A322D and γ2Q351X is consistent with such an entrapment and degradation in the endoplasmic reticulum ER. In contrast to the α1A322D and γ2Q322X mutations, the γ2(R43Q) mutation is located at the subunit's N-terminus, and thus based on the above discussion, it is not surprising that γ2(R43Q) does not reduce total γ2 subunit expression; γ2 subunit folding in the ER is not disrupted. It is of considerable interest to determine how γ2(R43Q) reduces expression on the cell surface. Does γ2(R43Q) disrupt a cell surface targeting sequence and is such a sequence responsive to pharmacological manipulation?

One of the most intriguing questions surrounding the IGE mutations is how can their dysfunction cause paroxysmal symptoms (seizures) but not affect interictal neurological function. One possible explanation is that endogenous $GABA_A$ receptor modulators such as partial agonists, pH, neurosteroids, and phosphorylation affect wild type and mutant receptor current kinetics and trafficking differently and that only under specific modulation conditions are the patients prone to seizures. Therefore, the effects of these modulators on the aforementioned pathophysiological mechanisms need to be determined.

Finally, it should be emphasized that the studies reviewed here were all performed in either *Xenopus* oocytes or immortalized mammalian fibroblast cell lines. The alterations of the mutations' expression and biophysical properties in neurons are unknown. Not only could neural cellular processes produce $GABA_A$ receptors with

different current kinetics and expression profiles than are seen in immortalized fibroblasts, but neurons could also target the wild type and mutant receptors differently to synaptic, perisynaptic and extra-synaptic locations. This differential trafficking could cause changes in phasic and tonic currents that could not be predicted from studies in nonpolarized immortalized cell lines. Unlike immortalized fibroblasts, neurons express many subtypes of endogenous $GABA_A$ receptor subunits. It is possible that, unlike an immortalized fibroblast, a neuron may compensate for a $GABA_A$ receptor subunit mutation by upregulating other $GABA_A$ receptor subunit subtypes. This possibility should be evaluated to determine the ultimate effect of a single $GABA_A$ receptor subunit mutations on GABA-evoked currents.

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