Published in final edited form as:

Epilepsia. 2012 August ; 53(8): e161–e165. doi:10.1111/j.1528-1167.2012.03596.x.

Decreased Viability and Absence-Like Epilepsy In Mice Lacking or Deficient In The GABA_A Receptor Alpha 1 Subunit

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Summary

Autosomal dominant mutations (S326fs328X, A322D) in the GABA_A receptor α 1 subunit are associated with human absence epilepsy and juvenile myoclonic epilepsy, respectively. Because these mutations substantially reduce α 1 subunit protein expression *in vitro*, it was hypothesized that they produce epilepsy by causing α 1 subunit haploinsufficiency. However, in a mixed background strain of mice, α 1 subunit deletion does not reduce viability or cause visually-apparent seizures; the effects of α 1 subunit deletion on EEG waveforms were not investigated. Here, we determined the effects of α 1 subunit loss on viability, EEG spike-wave discharges and seizures in congenic C57BL/6J and DBA/2J mice. Deletion of α 1 subunit caused strain- and sex-dependent reductions in viability. Heterozygous mice experienced EEG discharges and absence-like seizures within both background strains, and exhibited a sex-dependent effect on the discharges and viability in the C57BL/6J strain. These findings suggest that α 1 subunit haploinsufficiency can produce epilepsy and may be a major mechanism by which the S326fs328X and A322D mutations cause these epilepsy syndromes.

Keywords

generalized; inhibition; electroencephalogram; electromyography

Introduction

GABA_A receptors (GABA_ARs), the major inhibitory neurotransmitter receptors in mammalian brains, are pentamers typically composed of two α and β subunits and either a γ or δ subunit. The S326fs328X and A322D mutations in the GABA_AR α 1 subunit are associated with, respectively, absence epilepsy (Maljevic et al., 2006) and juvenile myoclonic epilepsy (Cossette et al., 2002). In heterologous cells, the S326fs328X mutation caused complete α 1 subunit elimination (Kang et al., 2009), and A322D reduced α 1 subunit by 88% (Ding et al., 2010), results suggesting that these mutations caused epilepsy via α 1 subunit haploinsufficiency. However, heterozygous and homozygous α 1 subunit knockout (*Gabra1* KO) mice produced in a mixed genetic background were viable and did not exhibit spontaneous, visually-apparent seizures (Vicini et al., 2001). These surprising *in vivo* findings could result if 1) α 1 subunit haploinsufficiency alone does not cause epilepsy and

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Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

that dominant negative effects are necessary, 2) mouse models of $\alpha 1$ haploinsufficiency do not reproduce the human phenotype, or specific, seizure-prone mouse background strains are needed, or 3) *Gabra1* KO produces only nonconvulsive epileptiform abnormalities that cannot be identified by visual inspection. Therefore, we generated *Gabra1* mutant mice in two congenic strains and performed continuous video/EEG monitoring to determine if they expressed epileptiform activity and/or altered viability.

Methods

Animals

We performed all procedures in accordance with protocols approved by the Vanderbilt University Institutional Animal Care and Use Committee. Mice were housed in a controlled facility with a twelve hour light/dark schedule, a temperature and humidity controlled environment, and ad libitum water and food.

Mice with loxP sequences surrounding *Gabra1* exon nine, B6.129(FVB)Gabra1tm1Geh/J, were previously produced in a mixed background (Vicini et al., 2001) and were backcrossed at least six times into a C57BL/6J background by Jackson Laboratories (stock 004318). We mated them with C57BL/6J mice unconditionally expressing cre-recombinase, B6.CTg(CMVcre)1Cgn/J (Jackson Laboratories stock 006054), to produce heterozygous $\alpha 1$ knockout ($\alpha 1^{+/-}$) mice. We maintained $\alpha 1^{+/-}$ mice on the C57BL/6J strain by continued backcrossing, and thus the mice used in these studies were backcrossed at least ten times into the C57BL/6J strain. We also generated a separate $\alpha 1^{+/-}$ line congenic on the DBA/2J background by backcrossing for at least nine generations with wild type DBA/2J mice (Jackson Laboratories). We genotyped the mice using polymerase chain reaction of mouse tail genomic DNA between P17 and P21 or on the date of death.

Viability and pathological analyses

We mated $\alpha 1^{+/-}$ mice and recorded the number of pups produced and the date of death. We measured the body mass of the mice every one to three days from P7–P30 before they were used for EEG experiments. We performed pathological analyses on six P25 $\alpha 1^{+/+}$, $\alpha 1^{+/-}$, and $\alpha 1^{-/-}$ C57BL/6J mice. The gross morphology and histopathology of all major organs was evaluated by an expert veterinary pathologist.

EEG monitoring

The EEG/electromyography (EMG) methodology has been described (Chung et al., 2009). Briefly, under isoflurane anesthesia, we implanted P30–33 mice with prefabricated skull headmounts (Pinnacle Technology) that acquire two bipolar EEG channels and one subcutaneous nuchal EMG channel. Furthermore, to determine if the discharges occurred synchronously in both cerebral hemispheres, we performed additional recordings with modified headmounts that recorded from electrode screws placed in the left and right frontal regions that were referenced to an inactive electrode on the skull surface. After at least 48 hours of recovery, we obtained 24 hour synchronized video/EEG/EMG recordings. A blinded reviewer analyzed the EEG offline and identified spike-wave discharges (SWD) using criteria established for the analysis of rat models of absence epilepsy (Akman et al., 2010). Briefly SWD were defined as trains (>1s) of rhythmic biphasic spikes, with a voltage at least two-fold higher than baseline and that were associated with aftergoing slow waves. The reviewer quantified the SWD incidence and duration in uniform five minute samples each hour. To determine if SWDs were associated with behavioral arrest, manifestations of absence seizures, we determined whether the longer SWDs (>2s) were associated with attenuation of the EMG signal and behavioral changes on video. Because mouse movements produce slow (1–4 Hz) EMG waveforms, we also objectively quantified the effects of SWD

on movement by measuring the relative EMG spectral power (1–4 Hz delta power, Carefusion) in two seconds segments before, during, and after the SWD. Finally, we determined the effects of the anti-absence seizure drug, ethosuximide, on SWD in DBA/2J mice. Mice were given 200 μ l intraperitoneal (i.p.) saline and a two hour baseline EEG was obtained. The mice then received either ethosuximide (200 mg/kg i.p.) or another dose of 200 μ l saline (placebo) and the incidence of SWD after the drug/placebo treatment was compared with that during the baseline.

Statistical analyses

Statistical analyses were performed using the R 2.12.2 Statistical Package for Windows (R Foundation for Statistical Computing). We first determined the effects of *Gabra1* KO separately in male and female mice; if there was no sex-dependent effect, we grouped males and females together. Parametric data (body mass, SWD incidence) are presented as the mean $\pm$ standard error of the mean and are compared using the Student's t-test or analysis of variance with Tukey posttest, as appropriate. Nonparametric data (Mendelian ratios, mortality) were analyzed by chi-square.

Results

First, we determined if loss of $\alpha 1$ subunit decreased viability, and if such an effect depended upon mouse sex or background strain. C57BL/6J mice produced 211 offspring (199 genotyped), and DBA/2J mice produced 115 offspring (112 genotyped). There was no significant deviation from the expected Mendelian ratios (C57BL/6J: 27% $\alpha 1^{+/+}$, 50% $\alpha 1^{+/-}$, 24% $\alpha 1^{-/-}$, $P = 0.780$; DBA/2J: 34% $\alpha 1^{+/+}$, 47% $\alpha 1^{+/-}$, 19% $\alpha 1^{-/-}$, $P = 0.273$). However, after approximately P19, homozygous knockout increased mortality in both strains ($P < 0.001$, Figure 1). The viability decrease was strain-dependent with a higher mortality rate in the C57BL/6J than DBA/2J strain ($P = 0.028$). After P19, *Gabra1* loss also caused strain-dependent declines in body mass with reductions in both $\alpha 1^{+/-}$ and $\alpha 1^{-/-}$ C57BL/6J mice, but only in $\alpha 1^{-/-}$ in DBA/2J mice (Figure 1). In C57BL/6J mice, body mass was reduced in female, but not male $\alpha 1^{+/-}$ mice. Pathological analysis revealed that the reduced viability did not result from altered systemic or brain development, histogenesis, or disrupted cortical layering (not shown).

We next performed continuous EEG recordings on $\alpha 1^{+/+}$ and $\alpha 1^{+/-}$ mice and identified SWD in both bipolar (Figure 2A1, 2B1) and left and right referential (Figure 2A2, 2B2) recordings. Inspection of the waveform morphology (Figure 2C1 and 2C2) demonstrated spikes, positive transients and waves, components observed in rat models of absence seizures (Robinson and Gilmore, 1980). Consistent with previous reports (Reid et al., 2011), wild type mice possessed rare spontaneous SWDs (DBA/2J: 2 ± 2 SWD/hr, $N = 11$; C57BL/6J: 1 ± 0.4 SWD/hr, $N = 9$). Heterozygous *Gabra1* loss increased the incidence of SWD in both DBA/2J (19 ± 2 SWD/hr; $N = 19$, $P < 0.001$, Figure 2D) and C57BL/6J mice (15 ± 3 SWD/hr, $N = 21$, $P = 0.001$). In the DBA/2J strain, there was no difference in SWD incidence between female and male (17 ± 3 SWD/hr) $\alpha 1^{+/-}$ mice. Interestingly, in the C57BL/6J strain, heterozygous *Gabra1* KO caused a significantly greater increase in SWD incidence in females (23 ± 5 SWD/hr) than males (11 ± 2 SWD/hr; $P = 0.024$, Figure 2D). There was no significant change in the mean SWD duration in either strain.

Because the mean SWD duration was longer in DBA/2J (2.7 ± 0.2 s) than C57BL/6J (1.8 ± 0.1 s) mice, we determined whether SWD were associated with movement arrest in the DBA/2J strain. Visual inspection demonstrated that SWDs were sometimes associated with behavioral arrest on EMG (Figure 2E1) and video (Supplemental Data). We quantified the effect of the SWD on these slow, movement-associated EMG waves by measuring the EMG relative spectral power (1–4 Hz) of 83 sequential SWDs from 14 mice and found a

significant reduction of mean EMG amplitude during SWD relative to times immediately before or after the SWD (Figure 2E2, $P < 0.001$). Finally, we determined that ethosuximide, but not placebo, reduced SWD incidence by 83% (Figure 2F). These data suggest that *Gabra1* deficient mice experience absence-like seizures and interictal SWDs.

Discussion

Our data demonstrate that heterozygous *Gabra1* KO caused SWDs and absence-like seizures in both C57BL/6J and DBA/2J mice. Heterozygous and homozygous $\alpha 1$ loss reduced viability in C57BL/6J mice, but had a milder effect in the DBA/2J background that was limited to the null genotype. Because previous studies reported that $\alpha 1^{+/-}$ and $\alpha 1^{-/-}$ mice were viable, our finding that *Gabra1* mutation reduced viability was somewhat unexpected. However, the previous studies were performed in a mixed C57BL/6J, 129X1/SvJ $\times$ 129S1/Sv, and FVB/N background, whereas ours was performed using mice in congenic C57BL/6J or DBA/2J strains. Our findings could thus be explained by a background strain effect on viability. Our observation that *Gabra1* KO produced a significantly higher mortality rate in C57BL/6J mice than DBA/2J mice supports this conclusion, as does the well-studied effects of mouse background strain on seizures and other aversive phenotypes (Frankel, 2009).

Our pathological examinations demonstrated that the decreased viability did not result from a gross or histological systemic or neurological abnormality. Although we did not observe a definite neurobehavioral phenotype on visual examination, it is possible that formal neurobehavioral testing of *Gabra1* KO mice will reveal strain-dependent abnormalities that will contribute to their reduced viability and whether such an abnormality is associated with their epilepsy phenotype, a question relevant to the increased mortality of human epilepsy patients (Neligen et al., 2010).

In addition to background strain, developmental stage and sex also affected the phenotypes. Viability began to decrease at a specific developmental time point, P19, a finding that could result from the emergence of $\alpha 1$ subunit expression in postnatal life (Yu et al., 2006), or from a developmental change in the chloride reversal potential (Ben-Ari et al., 2011). In C57BL/6J mice, SWDs were significantly more frequent in females than males. This result is consistent with sex-dependence in GABA_AR subunit expression in another rodent absence epilepsy model (Li et al., 2007).

Loss of *Gabra1* expression produced absence-like seizures in two strains of mice and thus recapitulated the absence epilepsy phenotype of the GABRA1(S326fs328X) mutation as well as the absence seizures observed in several of the juvenile myoclonic epilepsy patients with the GABRA1(A322D) mutation (Cossette et al., 2002). These results demonstrate the importance of $\alpha 1$ subunit haploinsufficiency (as opposed to dominant negative effects) in producing the epilepsy phenotypes, and will thereby guide investigations to determine the precise mechanisms by which $\alpha 1$ subunit loss, in particular, and disinhibition, in general, produce generalized seizures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by National Institutes of Health Grants NS055979 and NS064286 to MJG. We thank Dr. Jennifer Kearney, Dr. Gregg Stanwood, and Ms. Nicole Hawkins (Vanderbilt University) for their helpful comments concerning this manuscript.

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Figure 1.

Gabra1 KO reduces post-P19 survival and body mass. Kaplan-Meier curves illustrate that in both the C57BL/6J (A) and DBA/2J (B) strains, homozygous $\alpha 1^{-/-}$ mice (red) had reduced 30 day survival relative to wild type $\alpha 1^{+/+}$ (blue) and heterozygous $\alpha 1^{+/-}$ (green) mice. Note that $\alpha 1^{-/-}$ mice in the C57BL/6J strain had a higher mortality than those in the DBA/2J strain. The reduced 30 day survival of $\alpha 1^{+/-}$ C57BL/6J mice relative to $\alpha 1^{+/+}$ was not statistically significant ($P = 0.066$). Daily gains in body mass from P7–P30 are presented in panels C–E and the effects of genotype on the final P30 mass are statistically analyzed in panel F. Heterozygous C57BL/6J mice had a sex-dependent reduction in body mass with female (C, F), but not male (E, F) $\alpha 1^{+/-}$ having reduced P30 mass; the high mortality of $\alpha 1^{-/-}$ C57BL/6J mice precluded quantifying their P30 body mass. Homozygous, but not heterozygous DBA/2J mice had sex-independent reductions in body mass (E, F). * $P < 0.05$; ns = not significant

Figure 2.

Heterozygous KO causes SWD and absence-like seizures. Sample EEG tracings from C57BL/6J (A1, A2) and DBA/2J (B1, B2) $\alpha 1^{+/-}$ mice with bipolar (A1, B1) and referential (A2, B2) recordings showing typical SWDs. Referential EEG tracings on an expanded time scale from C7BL/6J (C1) and DBA/2J (C2) mice depict the waveform morphology demonstrating spikes (s), positive transients (pt), and waves (w) similar to those seen in rat models of absence epilepsy. D) Heterozygous C57BL/6J mice had a sex-dependent increase in SWDs with females having two-fold more SWD than males. Heterozygous DBA/2J mice had a sex-independent increase in SWD incidence. Sample EEG (black) and EMG (red) tracing showing attenuation of the EMG signal at the beginning of the SWD and its resumption at its conclusion corresponding to ictal behavioral arrest (E1). The mean normalized EMG spectral power (1–4 Hz) during 83 successive SWD (>2s) was significantly lower than the spectral power for the two seconds preceding and following the SWD(E2). A video EEG demonstrating the behavior arrest and EMG attenuation in three successive SWD is provided in Supplemental Data. F) Ethosuximide (ETX), but not placebo, reduced SWD incidence by 83% compared to baseline EEG. * $P < 0.05$; ns = not significant