Analysis of a $GABA_A\gamma2$ (R43Q) knock-in mouse model of familial epilepsy

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Epilepsy is a common neurological disorder that causes paroxysmal electrical discharges in the brain. It can be difficult to treat, with up to 30% of all patients unable to achieve satisfactory pharmacological control of their seizures, and many others living with a host of adverse side effects such as sedation and cognitive impairment. Genetic analysis of familial forms of epilepsy led to the discovery of ion channel gene mutations linked to a range of epilepsy syndromes, thus pointing to changes in ion channel function as the primary causative agent of many common forms of epilepsy. Lessons learned from the analysis of these mutations and how they alter neurobiology and neurophysiology are vital to our gaining knowledge of the fundamental mechanisms of seizure genesis, and provide the key to developing better strategies to control epilepsy. We have begun this process with a detailed examination of an autosomal dominant mutation of a $GABA_A\gamma2$ receptor subunit [GABRγ2(R43Q)] found in a large family with GEFS+ (Generalised Epilepsy with Febrile Seizures plus). The study of mouse models harbouring human epilepsy causing mutations is one way in which a more direct link between genes and phenotypes can be made that not only permits the molecular study of mutated genes *in vivo*, but which may also provide a direct link to phenotype. We therefore generated a knock-in mouse model with the GABRγ2(R43Q) mutation, to investigate the *in situ* functional consequences of this genetic lesion on inhibitory synapses, and to assess the potential involvement of this mutation in the development of epilepsy.

Wild type mice ($\gamma2^{R43/R43}$) and mice heterozygous for the GABRγ2(R43Q) mutation ($\gamma2^{R43/Q43}$) were anaesthetised i.p. with ketamine (100 mg/kg) and xylazine (15 mg/kg), and instrumented for EEG recording. Video and EEG analysis of recordings revealed absence seizures, with correlated 3-7 Hz spike and wave discharges (SWD) in the mutant animals. Human patients carrying the GABRγ2(R43Q) mutation display absence seizures with 3 Hz SWD; recapitulation of the human phenotype in our mouse model is a vital validation step, and suggests that underlying pathological mechanisms may be shared between mouse and human. The heterozygous mutant mice also showed an elevated sensitivity to challenge with the pro-convulsant drug, pentylenetetrazol (40-120 mg/kg, s.c.).

Miniature Inhibitory Postsynaptic Currents (mIPSCs) were analysed in Layer 2/3 cortical neurons using the whole cell patch clamp technique, in acute brain slices obtained from P14-16 mice decapitated after anaesthesia by inhalation of isoflurane. Analysis of wild type, heterozygous and homozygous mutant ($\gamma2^{Q43/Q43}$) mice demonstrated a reduction in amplitude ($\gamma2^{R43/R43}$ = 67.5 ± 2.91 pA, $2^{R43/Q43}$ = 58.4 ± 2.22 pA, $\gamma2^{Q43/Q43}$ = 40.0 ± 2.62 pA), and a slower rate of decay ($\gamma2^{R43/R43}$ = 4.7 ± 0.15 ms, $\gamma2^{R43/Q43}$ = 5.9 ± 0.32 ms, $\gamma2^{Q43/Q43}$ = 31.4 ± 2.62 ms). The heterozygous phenotype was much more subtle than would be expected from a simple gene dosing effect. Interestingly, the frequency of detectable synaptic events was also drastically reduced in the homozygous mutant animals ($\gamma2^{R43/R43}$ = 7.17 ± 0.83 Hz, $\gamma2^{Q43/Q43}$ = 0.85 ± 0.15 Hz). We are currently characteristing mIPSCs in the thalamocortical relay circuit, which has been implicated in the generation of the SWD of absence epilepsy.

To determine whether the altered characteristics of the inhibitory currents were due to a pre- or post-synaptic pertubation, immunohistochemical analysis was performed in brain slices obtained from P12-P16 mice killed after pentobarbitone anaesthesia (100 mg/kg, i.p). In homozygous mutant mice, punctate staining of glutamic acid decarboxylase (GAD), the marker of GABAergic synaptic inputs, was unaltered compared to wild type controls. However, in contrast, immunoreactivity for $GABA_A$ α1 and γ2 subunits was virtually absent from most brain regions, including the cortex, thalamus and cerebellum. The distribution of γ2 subunits was investigated further in cortical neurons, maintained in primary cultures for 14-16 days. Pregnant female mice were killed by cervical dislocation, and cortices dissected from decapitated E15-E16 embryos. To determine the location of $GABA_A$ receptor subunits in cultured neurons, extracts of total cellular proteins and cell surface proteins were prepared: the cell surface proteins were biotinylated in living cultures, and subsequently extracted on an immobilised avidin column. In heterozygous and homozygous mutant animals, the γ2 subunit was detected in extracts of total cellular protein, but its expression was reduced in cell surface protein extracts. Our studies suggest that a reduction in cell surface expression of the γ2 subunit, along with a reduction in amplitude and altered deactivation kinetics of mIPSCs, are the key molecular deficits responsible for absence epilepsy in this model.