

CALCIUM CHANNEL “GAITING” AND ABSENCE EPILEPSY

Dysfunction of the Brain Calcium Channel $Ca_v2.1$ in Absence Epilepsy and Episodic Ataxia

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Brain 2004;127(Pt 12):2682–2692

The molecular basis of idiopathic generalized epilepsy remains poorly understood. Absence epilepsy with 3-Hz spike-wave EEG is one of the most common human epilepsies and is associated with significant morbidity. Several spontaneously occurring genetic mouse models of absence epilepsy are caused by dysfunction of the P/Q-type voltage-gated calcium channel $Ca_v2.1$. Such mice exhibit a primary generalized spike-wave EEG, with frequencies in the range of 5 to 7 Hz, often associated with ataxia, evidence of cerebellar degeneration and abnormal posturing. Previously, we identified a single case of severe primary generalized epilepsy with ataxia associated with $Ca_v2.1$ dysfunction, suggesting a possible link between this channel and human absence epilepsy. We now report a family in which absence epilepsy segregates in an autosomal dominant fashion through three generations. Five

members exhibit a combination of absence epilepsy (with 3-Hz spike-wave) and cerebellar ataxia. In patients with the absence epilepsy/ataxia phenotype, genetic marker analysis was consistent with linkage to the *CACNA1A* gene on chromosome 19, which encodes the main pore-forming α_{1A} subunit of $Ca_v2.1$ channels ($Ca_v2.1\alpha_1$). DNA sequence analysis identified a novel point mutation resulting in a radical amino acid substitution (E147K) in $Ca_v2.1\alpha_1$, which segregated with the epilepsy/ataxia phenotype. Functional expression studies using human *CACNA1A* cDNA demonstrated that the E147K mutation results in impairment of calcium channel function. Impaired function of the brain calcium channel $Ca_v2.1$ may have a central role in the pathogenesis of certain cases of primary generalized epilepsy, particularly when associated with ataxia, which may be wrongly ascribed to anticonvulsant medication.

COMMENTARY

If the reply to a clinician's question of “What brings you here?” is “I have difficulty walking,” you may be dealing with an inherited disorder of P/Q-type calcium channels. Such is the case for spinocerebellar ataxia 6 (SCA6) and episodic ataxia 2 (EA2) patients seen in movement disorder clinics (1,2). Defects in the same gene might explain remarkably dissimilar problems encountered in other settings, such as migraine in a headache clinic, hemiplegia, febrile ataxia, or nystagmus in the emergency department, and coma in the intensive care unit (3,4). Now, at last, the epilepsy clinic can be added to the list, as shown in the article by Imbrici et al., which has identified a mutation of this channel in a three-generation pedigree with three per second absence epilepsy and ataxia (AEA). The finding was long in coming, given that mice with an abnormal gait bearing spontaneous mutations in the same gene were found to show absence epilepsy more than 25 years ago (5). This fortunate overlap in the clinical features of human and mouse validates a new phenotypic pair, prized by those hoping to find solutions to the puzzle of epileptogenesis by using an experimental neurogenetic approach.

Why did the previously reported human P/Q mutations not show epilepsy as well? This question is one part of the curious problem involving apparently unrelated phenotypes arising from defects in a single ion-channel gene. P/Q-type calcium channels control neurotransmitter release at presynaptic terminals throughout the nervous system and share this property with N-type channels, yet deletion of the N-type channel gene in mice has no effect on either gait or epilepsy (no human N-type mutations have yet been described). Thus, the function of the P/Q-type channel alone does not fully explain the mechanism of the spike-wave seizure disorder. A full understanding of the mechanism of this disorder lies somewhere within the developing brain, amidst a tangled path that must be methodically unraveled.

The first step in understanding the mechanism of the synchronization defect is to determine how the mutations in this allelic series alter P/Q-channel properties. In the case of absence epilepsy and ataxia, the authors found a point mutation that resides in the second membrane-spanning segment within the first of four transmembrane domains. Furthermore, functional studies in frog oocytes showed a decrease in evoked calcium current after expression of the mutant channels, with no evidence of a dominant negative interaction when

wild-type channels were coexpressed. Because the clinical disorder showed dominant transmission, it is likely that the mutation arises from haploinsufficiency of the wild-type allele. Imbrici and colleagues also observed that the reduced current could be partially overcome by elevating the expression of two auxiliary subunits, $\alpha 2\delta 2$ and $\beta 4$. Each of these subunits is decreased in two other ataxic mouse mutants with absence epilepsy, *ducky* and *lethargic*, which is consistent with impaired membrane targeting. Indeed, impaired neurotransmitter release and a reduced channel density at nerve terminals have been noted in the P/Q mutant mouse *tottering*. One usual caveat is that kinetic properties of the current observed in oocytes may vary when studied in mammalian cells; however, neurons of the P/Q channel point mutants *tottering*, *leaner*, and the null mutant (despite different degrees of cellular pathology) all share reduced calcium entry, an ataxic gait, and spike-wave seizures.

The opposite effects may occur with mutant P/Q phenotypes lacking epilepsy. The study of mutations leading to spinocerebellar ataxia 6 (an expansion of a polyglutamine sequence encoded by CAG trinucleotide repeats in the C terminus region) and episodic ataxia 2 or familial hemiplegic migraine (scattered missense or nonsense mutations throughout the channel) report increased calcium entry through mutant P/Q channels (6,7). Similarly, the threshold for cortical spreading depression is higher in mice with mutations that decrease calcium entry and lower in mutants with augmented calcium entry (8,9).

Thus, the primary effect of the mutation on the magnitude of calcium entry may have some predictive value in determining whether an inherited calcium channelopathy leads to absence epilepsy, but once again, this hypothesis provides no simple explanation for the spike-wave phenotype. P/Q channels are distributed throughout the entire CNS; why should the abnormal oscillations preferentially arise within the thalamocortical system? One possibility is that downstream cellular plasticity in specific neurons might further define the neurologic phenotype. Indeed, a secondary elevation of thalamic T-type calcium currents in calcium channel mouse mutants with impaired transmitter release favors the spike-wave phenotype (10). In other circuits, downstream plasticity involving L-type calcium channels, enhanced G-protein modulation, and synaptic pathology may lead to cerebellar deficits. The cell-type-specific rescue mechanisms that allow most synapses to escape involvement and the mysterious factors that determine whether the channel mutation leads to an episodic signaling deficit or progressive cellular atrophy are still largely unknown. Clearly a mutant calcium channel may trigger a broad range of biologic cascades that differ according to brain area, time of onset, activity, and of course, the coexpression of other genes. Finally, the appearance of the mutant syndrome must be evaluated against the constantly changing genetic background

of human pedigrees. Interestingly, within some families, both spinocerebellar ataxia and familial hemiplegic migraine phenotypes may result from the same single missense mutation (11,12). Presumably, allelic variation at other genetic loci can influence the phenotypic expression of the mutation. Thanks to continuing discoveries in the genetics of human seizure disorders, P/Q-type calcium channel mutants are no longer orphans in the epilepsy clinic, but where and when will they surface next?

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