

IS TOO MUCH INHIBITION TO BLAME IN AUTOSOMAL DOMINANT NOCTURNAL FRONTAL LOBE EPILEPSY?

Seizures and Enhanced Cortical GABAergic Inhibition in Two Mouse Models of Human Autosomal Dominant Nocturnal Frontal Lobe Epilepsy. Klaassen A, Glykys J, Maguire J, Labarca C, Mody I, Boulter J. *Proc Natl Acad Sci U S A* 2006;103(50):19152–19157. Selected mutations in the human $\alpha 4$ or $\beta 2$ neuronal nicotinic acetylcholine receptor subunit genes cosegregate with a partial epilepsy syndrome known as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). To examine possible mechanisms underlying this inherited epilepsy, we engineered two ADNFLE mutations (*Chrna4*^{S252F} and *Chrna4*^{+L264}) in mice. Heterozygous ADNFLE mutant mice show persistent, abnormal cortical electroencephalograms with prominent delta and theta frequencies, exhibit frequent spontaneous seizures, and show an increased sensitivity to the proconvulsant action of nicotine. Relative to WT, electrophysiological recordings from ADNFLE mouse layer II/III cortical pyramidal cells reveal a >20-fold increase in nicotine-evoked inhibitory postsynaptic currents with no effect on excitatory postsynaptic currents. i.p. injection of a subthreshold dose of picrotoxin, a use-dependent γ -aminobutyric acid receptor antagonist, reduces cortical electroencephalogram delta power and transiently inhibits spontaneous seizure activity in ADNFLE mutant mice. Our studies suggest that the mechanism underlying ADNFLE seizures may involve inhibitory synchronization of cortical networks via activation of mutant $\alpha 4$ -containing nicotinic acetylcholine receptors located on the presynaptic terminals and somatodendritic compartments of cortical GABAergic interneurons.

COMMENTARY

Cholinergic projections, originating primarily in the basal forebrain, influence neuronal excitability throughout the cerebral cortex and hippocampus. Although extensive, the projections are sparsely distributed, making detailed physiological studies of the effects of cholinergic inputs difficult, and therefore the precise functions of the cholinergic system are not well understood. In general, activity of cholinergic neurons correlates with cortical activation during wakefulness and REM sleep (1). Acetylcholine acts at both ionotropic nicotinic acetylcholine receptors (nAChRs) and metabotropic muscarinic acetylcholine receptors (mAChRs). mAChRs influence a variety of important brain processes, such as attention, memory, and the sleep/wake cycle. Pilocarpine, a muscarinic agonist, causes seizures in high doses and is used to generate status epilepticus in a widely studied animal model. An important role for nAChRs in seizures and epilepsy was confirmed by the association of mutations in

certain nAChR genes in a hereditary form of epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).

Twelve different nAChR subunits ($\alpha 2$ –10 and $\beta 2$ –4) have been identified that may combine to form pentameric ligand-gated, cation-selective channels. Based on subunit homologies and proposed structural similarities, nAChRs belong to a family that includes the GABA_A receptors. Of the many possible subunit combinations, only $\alpha 4\beta 2$ - and homomeric $\alpha 7$ -containing receptors appear to be expressed at high levels in the brain. These two receptor configurations are characterized by high- and low-affinity binding of agonist, respectively. Like the GABA_A receptor, after opening, the nAChR rapidly enters a closed, desensitized state. Unlike the GABA_A receptor, which contains a chloride channel, nAChR activation results in a brief depolarizing, excitatory potential. The nAChRs also have variable permeability to Ca²⁺ ions, enabling them to influence intracellular signaling pathways in addition to their depolarizing effects.

Because of the relative paucity of nAChR-containing postsynaptic sites identified in anatomical studies, it has been proposed that the majority of signaling mediated by nAChRs occurs via “volume transmission,” that is, via activation of receptors at

nonsynaptic sites (2). Most acetylcholine signaling in the brain appears to be mediated through its action at presynaptic terminals, where it depolarizes and/or increases calcium influx to enhance neurotransmitter release. Although acetylcholine has been shown to increase release of many neurotransmitters, the evidence for nAChR-mediated presynaptic effects is strongest at GABAergic neurons. There is evidence of localization of nAChRs to other subcellular sites, including dendrites and somata, and many other roles in modulating neuronal excitability have been suggested (2).

In 1995, a missense mutation in the $\alpha 4$ subunit gene (*CHRNA4*) was found to underlie ADNFLE, and subsequently five additional mutations both in the $\alpha 4$ and $\beta 2$ subunit (*CHRN2*) genes were associated with the same disease (3). ADNFLE is characterized by hyperkinetic seizures that occur mostly during non-REM sleep. All of the identified disease-causing mutations are located near the proposed pore of the ion channel, and electrophysiological studies of the mutated receptors have revealed a variety of altered properties, including decreased Ca^{2+} permeability in some mutant receptors and increased desensitization in others. One common finding among these mutations is that sensitivity to acetylcholine is increased (4). Extensive studies of the mutant channels have failed to offer a single mechanistic explanation for the clinical manifestations of the mutations. One of the more interesting questions that arose from the investigations is why mutations in a receptor that is widely expressed throughout the brain cause seizures with a focal onset in the frontal lobes. The implication of the results of earlier studies was that altered nAChR function affects local neuronal network behavior in a complex manner that cannot be explained by channel properties alone (3).

The recent work by Klaassen et al. is a major advance in our understanding of the pathophysiology of ADNFLE. These researchers engineered two mouse lines with mutations in the $\alpha 4$ subunit, *Chrna4*^{S252F} (an amino acid exchange) and *Chrna4*^{+L264} (an insertional mutation), which correspond to those in human families. The heterozygous mice were studied in detail because this genotype replicates the human condition in ADNFLE. Both mutant strains of mice had abnormal EEGs, characterized by increased slow activity and repetitive spontaneous seizures associated with sudden onset of rhythmic high-voltage, low-frequency, and asymmetric spike-and-wave discharges. They also demonstrated an increased susceptibility to nicotine-induced seizures.

In an effort to determine the cellular physiological changes underlying the epileptic phenotype, whole cell recordings were performed in cortical pyramidal neurons in brain slices from the mutant mice. No changes in frequency or amplitude of spontaneous EPSCs or IPSCs were observed under baseline conditions when compared with wild-type controls. However, application

of nicotine to the brain slices from ADNFLE mice, but not wild type, revealed a dramatic and selective effect on IPSCs. The amplitude and frequency of spontaneous IPSCs were increased by nicotine in ADNFLE mice, and the net effect was given a quantitative value by calculating the mean inhibitory current as a function of time. As expected for a nAChR-mediated effect, nicotine produced an increase in the mean inhibitory current, which decayed during continued application of the drug, presumably corresponding to activation followed by desensitization of the receptors. Nicotine created approximately a 20-fold increase in the mean inhibitory current in both mutant strains, compared with a 2.5-fold increase in neurons from wild-type mice. Using selective agonists and antagonists, Klaassen et al. argue that the enhanced nicotine response was mediated by $\alpha 4\beta 2$ receptors. To determine the mechanism by which nicotine increased GABAergic output, it was applied to slices after blocking both voltage-gated sodium channels (with TTX) and calcium channels (with cadmium). Under these conditions, there was no difference in the occurrence of spontaneous miniature IPSCs (mIPSCs) between wild-type and ADNFLE mice, neither was there any change in the occurrence of mIPSCs in the presence of nicotine in wild-type mice. However, nicotine increased the frequency and amplitude of mIPSCs in ADNFLE mice. To explain these combined findings, the authors suggest that mutant nAChRs mediate a presynaptic elevation of Ca^{2+} in the terminals of inhibitory neurons, facilitating the release of GABA-containing synaptic vesicles from their release sites.

The findings of Klaassen and colleagues, therefore, suggest that an exaggerated effect of acetylcholine on presynaptic nAChRs enhances the release of GABA in ADNFLE mutants. To confirm the seemingly paradoxical finding that increased inhibitory output in the cortex could underlie the generation of seizures in ADNFLE mice, a GABA_A receptor antagonist, picrotoxin, was administered in doses low enough to have no effect on wild-type mice. ADNFLE mice, in contrast, showed a normalization of their EEGs and a cessation of spontaneous seizures.

The authors propose a model in which GABAergic interneurons innervate a network of cortical pyramidal neurons. Acetylcholine, acting through presynaptic nAChRs, transiently enhances the release of GABA and causes a strong inhibition that, when relieved, results in a synchronization of the pyramidal network output. The effect of acetylcholine is greatly enhanced in the ADNFLE mutants, resulting in hypersynchronization and seizures. The adjunct experiments showing that picrotoxin, which normally has convulsant properties as a result of its effect on GABA_A receptors, actually normalized the EEG and stopped seizures is strong evidence that this model is correct. Currently, however, there is no direct evidence that



acetylcholine released from cholinergic projections can synchronize populations of pyramidal neurons. Moreover, this model will have to be reconciled with the models of cholinergic activity corresponding to arousal, because nocturnal frontal lobe seizures are most common in stage 2 sleep. The findings in this study provide new insights into a type of partial epilepsy that, although caused by a mutation in a widely distributed receptor, may arise from a complex interaction involving cholinergic modulation of specific interneuron populations and excitatory neuronal networks. If true, the findings also may have implications for other forms of epilepsy and their treatment. The idea that some GABAergic neurons have more of a proepileptic than an antiepileptic function is not new. However, the idea that distinct populations of interneurons may respond differently to drugs, such as nicotine, to modulate cortical excitability

raises the possibility that new antiepileptic drug strategies could exploit these mechanisms.

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