

CALCIUM AND AUTOSOMAL DOMINANT NOCTURNAL
FRONTAL LOBE EPILEPSY (ADNFLE)Five ADNFLE Mutations Reduce the Ca^{2+} Dependence of the Mammalian $\alpha 4\beta 2$ Acetylcholine Response

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Five nicotinic acetylcholine receptor (nAChR) mutations are currently linked to autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). The similarity of their clinical symptoms suggests that a common functional anomaly of the mutations underlies ADNFLE seizures. To identify this anomaly, we constructed rat orthologues (S252F, +L264, S256L, V262L, V262M) of the human ADNFLE mutations, expressed them in *Xenopus* oocytes with the appropriate wild-type (WT) subunit ($\alpha 4$ or $\beta 2$), and studied the Ca^{2+} dependence of their ACh responses. All the mutations significantly reduced 2 mM Ca^{2+} -induced increases in the 30 μM ACh response ($P < 0.05$). Consistent with a dominant mode of inheritance, this reduction persisted in oocytes injected with a 1:1 mixture of mutant and WT cRNA. BAPTA injections showed that the reduction was not due to a decrease in the secondary activation of Ca^{2+} -activated Cl^- currents. The S256L mutation also abolished 2 mM Ba^{2+} potentiation of the ACh response. The S256L, V262L, and V262M mutations had complex effects on the ACh concentration–response relation, but all three mutations shifted the concentration–response relation to the left at $[\text{ACh}] \geq 30 \mu\text{M}$. Co-expression of the V262M mutation with a mutation (E180Q) that abolished Ca^{2+} potentiation resulted in 2 mM Ca^{2+} block, rather than potentiation, of the 30 μM ACh response, suggesting that the ADNFLE mutations reduce Ca^{2+} potentiation by enhancing Ca^{2+} block of the $\alpha 4\beta 2$ nAChR. Ca^{2+} modulation may prevent presynaptic $\alpha 4\beta 2$ nAChRs from overstimulating glutamate release at central excitatory synapses during bouts of synchronous, repetitive activity. Reducing the Ca^{2+} de-

pendence of the ACh response could trigger seizures by increasing $\alpha 4\beta 2$ -mediated glutamate release during such bouts.

COMMENTARY

How can several different point mutations in a ligand-gated ion channel cause a largely homogeneous epileptic phenotype, as found in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)? To understand this issue better, consider the following exercise. Symbolize the rigid amino acid structure of a small part of a large protein by choosing a reasonably long word, such as “convention.” Swapping a single letter in this word (e.g., “v” to “t”) will entirely change its meaning. This action is analogous to a single amino acid being substituted in the protein. The whole protein could then be represented by a sentence of many words, in this case totaling about 600 letters, to correspond roughly to the total number of amino acids in a given nicotinic acetylcholine receptor (nAChR) subunit. Now generate two different 600-letter sentences, keeping everything the same except the two swapped-letter words. Perform this exercise 5 times over, with the two words occupying different positions in the sentences. Difficult? Yet this is pretty much what the nAChR subunits manage to accomplish in ADNFLE. Changing a single amino acid at five different positions among the 600 or so of a nAChR subunit changes the properties of the receptor and, most interestingly, produces the same clinical phenotype.

The study by Rodrigues-Pinguet et al. takes a novel approach to addressing the role of mutated receptor subunits in neurologic disorders. The authors searched for a common denominator in the function of nAChRs assembled from mutant subunits found in ADNFLE patients and were rewarded with a most interesting finding concerning the basic mechanism of the disease. Previous studies focused on the effect of a single mutated subunit and described numerous, and often contradictory, effects on channel function. In contrast, Rodrigues-Pinguet et al. discovered a common functional outcome in five ADNFLE mutations of the nAChRs. The mutations decrease the potentiation of nAChR-mediated responses by Ca^{2+} (elicited by ACh concentrations $\geq 30 \mu\text{M}$).

How does this alteration lead to seizures? Presynaptic nAChRs are known to facilitate both glutamate and γ -aminobutyric acid (GABA) release. Meanwhile, some glutamate receptors (e.g., NMDA and GluR2-deficient AMPA receptors, in particular) are profoundly Ca^{2+} permeable. When activated, these receptors can create a “black hole” for Ca^{2+} , thus depleting extracellular Ca^{2+} levels around excitatory terminals (1,2). After the depletion of Ca^{2+} around these terminals, nAChR activation will be less effective in enhancing glutamate release. Based on this idea, Rodrigues-Pinguet et al. put forward an interesting hypothesis for a preferential modulation of glutamate release by nAChR. In control subjects and, presumably, during sleep spindles, activation of presynaptic nAChRs will be impaired in the vicinity of extracellular Ca^{2+} -depleting glutamate receptors. The diminished extracellular Ca^{2+} will no longer potentiate the effect of nAChRs on excitatory terminals; thus ACh will fail to enhance glutamate release. Meanwhile, GABA release from inhibitory terminals will still be enhanced by nAChR activation because GABA receptors do not deplete extracellular Ca^{2+} levels. The reduced stimulatory effect of ACh on glutamate release will result in a relatively larger inhibition than excitation, thus confining sleep spindles to limited areas of the cortex. In ADNFLE, the diminished Ca^{2+} sensitivity of the mutated receptors would no longer reduce the stimulating effect of ACh on glutamate release when Ca^{2+} disappears from

the extracellular space. In other words, the mutation, having eliminated the marked Ca^{2+} -dependent potentiation of glutamate release by ACh, also has eliminated the decrease in release when Ca^{2+} is absent. This event will equalize the facilitatory action of ACh on glutamate and GABA release, and the resulting larger excitation relative to inhibition will allow the spindles to spread far beyond their normal boundaries. The discovery of a common functional alteration in five ADNFLE mutant receptors represents a new and significant insight into a possible mechanism for the disease, but many puzzles, including its frontal lobe origin and childhood onset, remain unresolved (3). By the way, how are those 600-character sentences doing?

by Istvan Mody, Ph.D.

References

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