Neuronal Nicotinic Acetylcholine Receptors and Epilepsy

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The identification of a genetically transmissible form of epilepsy that is associated with a mutation in CHRNA4, the gene that encodes the α4 subunit of the high-affinity nicotinic acetylcholine receptor, was the first demonstration that an alteration in a ligand-gated ion channel can cause seizures. Since then, nine mutations have been found, and analysis of their physiologic properties has revealed that all of them enhance receptor function.

Introduction

Although genetically determined epilepsies are rare, they offer a unique opportunity to study the mechanisms causing seizures. Recent progress in sequencing the human genome has made it easier to map the precise location of affected genes in genetically based epilepsies and to determine the corresponding protein defects. The identification of the mutations responsible for autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a milestone in epilepsy research (1,2). The observation that ADNFLE is associated with a mutation in the CHRNA4 gene that encodes a subunit of the high-affinity nicotinic acetylcholine receptor (nAChR) was the first demonstration that alteration of a ligand-gated ion channel could cause epilepsy.

Ligand-gated ion channels are proteins of the neuronal postsynaptic membrane that mediate fast neurotransmission. They encompass a ligand-binding site and an aqueous pore through which ions can permeate. Activation of cation-permeable channels causes depolarization (excitation) of the postsynaptic cell, whereas activation of anion-permeable channels causes hyperpolarization (inhibition). Excitatory ligand-gated channels include glutamate, serotonin, and acetylcholine receptors, whereas inhibitory ligand-gated ion channels comprise glycine and γ-aminobutyric acid (GABA) receptors (3,4). Neuronal nAChRs belong to the family of excitatory ligand-gated ion channels.

Sixteen genes encoding for the nAChRs have been identified in humans. Five nAChR genes encode neuromuscular junction receptors, and the remaining 11 encode neuronal nAChRs that are widely expressed in both the peripheral and the central nervous systems (4,5). The structures of nAChRs are highly conserved, with each receptor resulting from the assembly of five subunits arranged around an axis formed by the ionic pore. Each subunit consists of polypeptide of roughly 600 amino acids that is organized in such a way as to span the cell membrane 4 times with both the N- and C-termini lying in the synaptic cleft. The second transmembrane domain of each subunit contributes to the formation of the wall of the ionic pore. The neurotransmitter-binding site is at the interface of two adjacent subunits, and evidence suggests that two acetylcholine (ACh) molecules must bind to the receptor to activate it (6). The structures of serotonin, glycine, and $GABA_A$ receptors are similar to those of nAChRs, and it has been proposed that receptor diversity arose from gene duplication and mutations (7). Conservation among this family of receptors is such that results deduced from one receptor subtype often apply to the other members of the family.

The identification of the first mutation in CHRNA4, which encodes the α4 subunit, raised the question about the possible functional alterations caused by such a mutation and its effects on neuronal networks. We know that α4 assembles with the β2 subunit and constitutes the major high-affinity nicotinic receptor in the brain. Such receptors are widely expressed both in thalamus and cortex. ADNFLE seizures arise mainly during stage II of sleep in the frontal cortex and can, in some patients, propagate and cause tonic–clonic seizures (8). The age at onset of ADNFLE is variable but it begins mostly during the first or second decade of life and persists throughout life. Penetrance of ADNFLE is incomplete, with only ∼70% of the persons carrying a given mutation in CHRNA4 displaying the typical sleep syndrome (2,9). The delayed onset suggests that network changes that occur during adolescence are necessary to reveal the dysfunction. Recordings from thalamic and cortical neurons of the cat have shown the importance of thalamocortical and corticothalamoreticular loops in the generation of sleep spindles (10,11). At present, the most probable hypothesis to account for the triggering of epileptic

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seizures during sleep is that alteration of the properties of α4-containing nAChRs causes an imbalance in the thalamocortical networks with an excess of excitation and synchrony. Seizures could be triggered on these abnormally synchronized spindles.

To understand the functional consequence of the α4 mutation, normal or mutant α4-containing nAChRs were expressed in *Xenopus* oocytes, and the properties of the receptors determined. Results obtained by different laboratories have all shown that when the α4 mutant is expressed with normal β2 subunits, the resulting receptor has marked alteration in response to ACh or nicotine (12–14). It is known that ADNFLE patients are heterozygous, and unless there is abnormal regulation of allele expression, both the nonmutated and mutated alleles must be equally expressed in neurons (2). To mimic the situation in affected individuals, experiments were carried out in which normal and mutated α4 subunits were coexpressed with the normal β2 subunit. These experiments revealed the dominant effects of the α4-S248F and α4-776ins3 mutations and showed that mutant receptors displayed a higher sensitivity to ACh (15).

Since these initial observations, a further five mutations associated with ADNFLE were found in the CHRNA4 genes (16; Leninger T, 2002, personal communication). As predicted from the knowledge that high-affinity nAChRs result from the assembly of α4 and β2 subunits (17), it was expected that mutations in this latter subunit might also result in malfunctioning of the receptor that would cause increased susceptibility to epilepsy. It was recently reported that mutations in CHRNB2, the gene that encodes the β2 subunit, also are associated with ADNFLE (18,19). Moreover, two additional mutations have been identified in other ADNFLE families (Favre I, 2002, personal communication). Electrophysiologic experiments were carried out with the nine different mutant subunits by using the "heterozygous" mode of expression. At present, the only common feature between mutant-containing receptors and controls is a higher affinity of the mutant receptors for ACh that results in enhanced function (16). The initial localization of all CHRNA4 mutations in the second transmembrane domain leads to the hypothesis that only mutations in this critical segment result in appropriate alterations of receptor function to cause ADNFLE. More recent results indicate, however, that mutations can occur in different segments of the proteins, and these also lead to ADNFLE (Favre, personal communication). Indeed, any alteration of a subunit that reduces the energy barrier between the closed and open conformation could enhance receptor function. As more ADNFLE-carrying families are screened, additional mutations may be found.

In situ hybridization with probes specific for the α4 or β2 mRNA revealed that whereas these two subunits are both expressed in some brain areas, such as the thalamus, their expression differs markedly in other brain areas (20–22). This suggests that although either CHRNA4 and CHRNB2 mutations may cause epilepsy, there may be neurologic differences that have not yet been identified.

Because ADNFLE seizures are prevented by the use of the antiepileptic drug (AED) carbamazepine (CBZ), it was postulated that this compound might interact with nAChRs or the affected neuronal network. Determination of the effects of CBZ on α4β2 nAChRs revealed that this compound acts an open channel blocker at concentration that are compatible with those found in the CSF of patients (23). Moreover, it was found that both the α4-S248F and α4-776ins3 mutations increase the receptor sensitivity to this drug (23). Of five of the mutants tested so far, four of them displayed a higher CBZ sensitivity, but the α4-S252L was less sensitive (16). Although no definitive conclusions concerning the mode of action of CBZ in patients can be drawn from these interesting observations, the results clearly show that mutation of a single amino acid can alter the pharmacologic sensitivity of nAChRs.

Bridging the gap between the data obtained in studies on mutated nAChRs and the clinical manifestations of ADNFLE will require a great deal of additional information. First, there must be better localization of the α4β2 nAChRs in the neuronal network. Second, it must be determined whether the mutant receptors cause developmental changes leading to network reorganization. Third, it must be demonstrated that thalamic and cortical network organization and function are conserved between lower mammals and humans. Although data regarding the precise subcellular distribution of the α4 and β2 subunit in the human brain are not yet available, a first estimation of their pattern of distribution can be deduced from in situ hybridization with specific mRNA probes (20–22). These studies provide evidence for the expression in embryonic life and adulthood of both the α4 and β2 subunits in the cortex and the thalamus. However, care must be taken in the interpretation of results. We know that mRNA levels do not necessarily correlate with protein levels. Identification of significant expression of nAChR subunits in earlier stages of development could suggest that circuit remodeling might occur in individuals with ADNFLE. This possibility cannot be fully ruled out, even though, as far as we know, affected individuals do not have impaired cognitive functions.

If it is assumed that gene expression and neuronal network organization are not modified in ADNFLE patients, the question that remains to be answered is how enhanced function of α4β2 nAChRs can trigger epileptic seizures during sleep spindle wave activity. In addressing this problem, we must appreciate the full complexity of how neuronal nAChRs might function in neurons. For instance, although it has been documented that nAChRs mediate conventional synaptic transmission, it was surprising to discover that nAChRs are

present not only in the dendritic and somatic postsynaptic membrane but also in axons and presynaptic boutons. Electrophysiologic studies from brain slices or isolated cells have shed some light on the functions of these extradendritic nAChRs (24). These presynaptic receptors can enhance the release of neurotransmitter in two ways (25). There can be sustained depolarization of the bouton and increased activation of voltage-dependent calcium channels. Alternatively, there can be direct permeation of calcium ions through the activated nAChRs. In contrast, nAChRs expressed in axons may prevent propagation of action potentials. Thus nAChRs not only mediate synaptic transmission but may also modulate neuronal activity in other ways. The finding of mutations in the nAChRs genes in ADNFLE has begun to reveal many new features of the role of these ligand-gated channels in brain function.

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