Insights into channel function via channel dysfunction

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The nicotinic synapse has been a touchstone for advances in neuroscience ever since Jean Nicot, the French ambassador to Portugal, sent some tobacco seeds home to Paris in 1550 with a note that the New World plant had interesting effects when smoked. Now the muscle nicotinic acetylcholine receptor (nAChR) is a well-studied example of ligand-gated ion channels. After a motor neuron is stimulated, the nerve impulse reaches the presynaptic terminal, where it evokes release of acetylcholine (ACh) into the synapse. The nAChR depolarizes the postsynaptic muscle and triggers muscle action potentials; muscle contraction follows. To date, several nAChR subtypes have been successfully isolated, purified, imaged, and expressed, and unitary currents have been recorded from these channels (1). Researchers continue to unravel the molecular mechanisms of these macromolecules that are embedded in membranes at vertebrate nerve-muscle synapses, at invertebrate nicotinic synapses (which explains why nicotine-producing tobacco plants have a select advantage against invertebrate pests), and in the vertebrate central

system (which explains Jean Nicot's fascination with those leaves). However, the precise structural events that trigger channel opening or "gating" remain mostly unknown.

Site-directed mutagenesis reveals some information about nAChR function but fails to give us an appreciation for the physiological significance of the receptor's biophysical properties. During the last decade, the venerable pharmacological approaches have been aided by newer insights from molecular genetics. We now appreciate that a number of naturally occurring mutations within nAChRs lead to severe

disorders. These ion-channel–associated disorders are collectively known as channelopathies. The nAChR is a heteropentamer assembled from α, β, γ, (or ε) and δ subunits (Figure 1). Each subunit is thought to contain a large N-terminal domain, followed by four transmembrane regions (M1–M4) with a large cytoplasmic loop between M3 and M4. In the central nervous system, at least five mutations linked to autosomal dominant nocturnal frontal lobe epilepsy lie within, or immediately adjacent to, M2 — the putative pore-forming region of neuronal nAChR subunits (2, 3). At the nerve-muscle synapse, at least 26 mutations in the extracellular, transmembrane, and cytoplasmic do-mains of the nAChR lead to different forms of congenital myasthenic syndrome (CMS) (4, 5). Thus these clinical cases aid in assigning physiological functions to the biophysical properties of nicotinic receptors.

In this issue of the *JCI*, Shen and colleagues report the channel kinetics from a valine to leucine mutation (V132L) located in the α subunit of the muscle nAChR within the signature cystine loop (cys-loop) (6). The cys-loop is a highly conserved structure found in
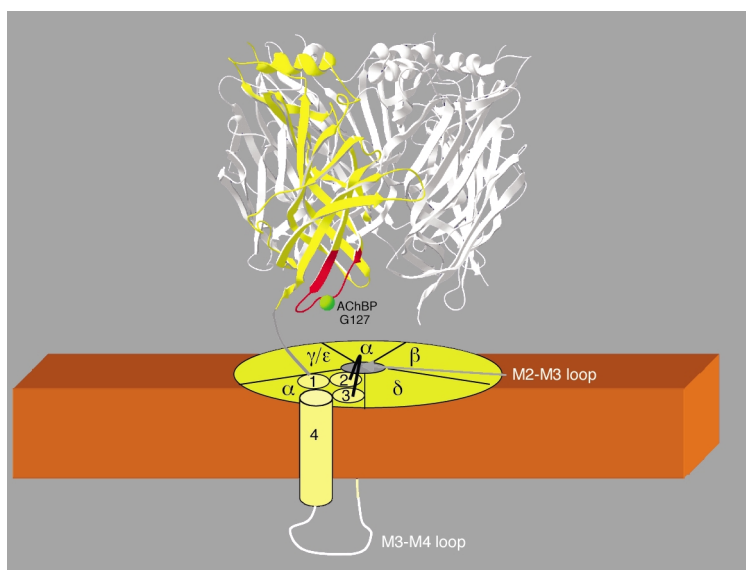
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Nonstandard abbreviations used: nicotinic acetylcholine receptor (nAChR); acetylcholine (ACh); congenital myasthenic syndrome (CMS); cystine loop (cys-loop); acetylcholine binding protein (AChBP); slow-channel CMS (SCCMS); fast-channel CMS (FCCMS).

Figure 1
The structure of AChBP is illustrated over a representation of the transmembrane domain of nAChR. Each subunit (α, β, γ or ε, δ) is labeled by its Greek symbol. The α subunit is shown in yellow, except for most of the signature cys-loop, which is shown in red, and AChBP G127 is shown in green (this residue aligns with nAChRα V132). The membrane spanning regions M1–M3 are represented on the bilayer surface, and M4 is also shown in cross section.

every subunit of the ligand-gated ion channel superfamily. In a high-resolution structural model of the nAChR N-terminus, based on the crystal structure of a snail (*Lymnae stagnalis*) acetylcholine-binding protein (AChBP), the cys-loop is located near the membrane surface (highlighted in red in Figure 1). Sequence analysis reveals that the AChR cys-loop contains a higher proportion of hydrophobic residues than the AChBP loop (7, 8). Furthermore, cryoelectron microscopy imaging of *Torpedo* electroplax membranes enriched with nAChR suggests that the cys-loop resides near the second (M2) and third (M3) transmembrane domains, where it may couple agonist binding to channel activation (8, 9).

The αV132L mutation leads to a severe form of postsynaptic CMS. Postsynaptic CMS mutations are classified as either slow- (SCCMS) or fast-channel congenital myasthenic syndromes (FCCMS), depending on their effects on the amplitude and waveform of postsynaptic currents at the muscle membrane. SCCMS mutations enhance agonist affinity or gating efficiency. FCCMS mutations reduce agonist affinity for the open channel or impair gating (4). In the present manuscript, the authors detail the detrimental effects of a FCCMS mutation on ACh binding and channel gating (6). Similar to other FCCMS mutations, αV132L attenuates the postsynaptic response to ACh. Specifically, αV132L reduces ACh binding affinity for the closed channel state by 30-fold. Consequently, ACh released into the synaptic space is predicted to activate only 10% of the available binding sites, much less than normal. Moreover, there is a fivefold decrease in the total ACh current due to an accelerated decay of the synaptic response. In short, receptors containing the αV132L mutation have pathologically little activation, and that for pathologically brief periods. This would be consistent with weak or nonexistent muscle stimulation in response to motor neuron activity.

Do subunits with homologous sequences look alike?

Interestingly, amino acid substitutions at positions corresponding to αV132L in the β, ε, and δ subunits yielded significantly different phenotypes (6). Substitution of the aligning V residue in the β and ε subunits produces virtually no effect, while substitution in the δ subunit produces a receptor with nearly the opposite phenotype of αV132L. Specifically, δV134L has slight effects on ACh binding affinity, but gating efficiency is reduced nearly fourfold. We suspect that each subunit's signature cys-loop folds and functions in a generally similar manner, but there is clearly some functional and struc-tural asymmetry at a more detailed level. Indeed, based on cryoelectron microscopy studies of the *Torpedo* nAChR in the open state (9), individual subunits do assume asymmetrical conformations and presumably have unique local environments. Apparently, even a small structural perturbation may lead to asymmetric changes in function.

Implications from CMS studies

Progress continues into the 21st century. Shen and colleagues, including the Sine and Auerbach labs, are contributing to the study of the molecular pathophysiology of CMS. Furthermore, their biophysical analyses of the CMS mutations have provided us with some basic insights into the relationship between the structure and function of nAChRs. However, we still await refined structural information on the nAChR ion channel in order to further exploit present gating predictions based on similar kinetic studies.

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