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Commentary

Mutations causing muscle weakness

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Transmission of signals from nerves to muscles is critical for life, so substantial safety factor mechanisms have evolved to ensure its success. Despite this, diseases can impair even this robust system. Characterizing these disease mechanisms greatly contributes to understanding this fundamental process. Neuromuscular transmission serves as a model for helping to understand neurotransmission throughout the nervous system. Mutations in critical components of the neuromuscular junction or autoimmune responses to them cause muscle weakness and fatigability termed ''myasthenia.''

More than 20 years ago, Andrew Engel and his co-workers began to identify congenital myasthenic syndromes (reviewed in ref. 1). Syndromes were found that reflected deficits in almost every step of neuromuscular transmission: reduced numbers of synaptic vesicles of acetylcholine (ACh), reduced evoked release of vesicles, defective synthesis or packaging of ACh, deficiency of acetylcholinesterase (AChE), deficiency of ACh receptors (AChRs), and altered AChR function. Clinical characterizations of the patients were followed by biochemical, electrophysiological, and electron microscopic characterization of biopsies containing neuromuscular junctions. Now several critical synaptic components have been cloned and their structures have been determined in some detail. This has led to a remarkable series of papers in which Engel and his co-workers have identified patients with mutations in subunits of their AChRs, transfected cells with mutated AChRs, elegantly characterized their properties, then provided a detailed explanation of the complex effects of these mutations on the patient's neuromuscular transmission (1). These AChR mutations will be briefly considered again later.

One of the congenital myasthenic syndromes that Engel identified revealed a virtual absence of AChE at neuromuscular junctions. The catalytic subunit of AChE has been cloned and its structure has been determined by x-ray crystallography (2). However, these patients revealed no mutations in the catalytic subunit (3). This mystery is now solved. In this issue of the *Proceedings* (4), Engel and co-workers report cloning, from human tissue, the collagen tail structure, COLQ, on which the catalytic subunits are arrayed, finding truncation mutations in patients' COLQ, and characterizing cells transfected with combinations of AChE and various COLQ mutations. COLQ was initially cloned from animals by Jean Massoulié and co-workers (5). Understanding the significance of this mutation requires a description of neuromuscular transmission.

The brain's decision to contract a skeletal muscle results in activation of a spinal motor neuron, triggering an action potential that proceeds from the cell body in the spinal cord along the axon to the nerve ending in the muscle (6). Depolarization of the nerve ending activates voltage-sensitive calcium channels in the active zones of the nerve ending. These permit entry of calcium ions that trigger fusion of several vesicles containing ACh with the presynaptic membrane, then each vesicle dumps about 10^4 ACh molecules into the synaptic cleft. The ACh must run the gantlet of AChE located in the basal lamina between the presynaptic nerve membrane and the postsynaptic muscle membrane. Hydrolysis of ACh to choline and acetic acid by AChE quickly terminates transmission so that the muscle can be stimulated again to provide fine motor control. ACh that reaches the muscle postsynaptic membrane binds to AChRs, thereby triggering opening of a cation channel through the center of the protein. Cations flow passively through this channel at 5×10^4 per millisecond, resulting in a depolarization that can trigger an action potential which is propagated down the muscle to trigger contraction. AChE cleaves 5 ACh molecules per millisecond per catalytic site, thereby removing all excess ACh before AChR channels close, about 2 msec after their initial activation. The overall role of neuromuscular transmission is to amplify the small currents of the motor nerve action potential sufficiently to trigger an action potential in the vastly larger muscle fiber. There is a safety factor of at least 3 in terms of excess ACh release and AChRs activated over what is minimally required to ensure neurotransmission. Thus, substantial damage by disease processes can occur while some neuromuscular transmission remains.

The first myasthenia whose molecular basis was determined was myasthenia gravis, which is caused by an antibody-mediated autoimmune response to AChRs that reduces the number of AChRs and disrupts the postsynaptic membrane (7). Then it was discovered that in Lambert–Eaton myasthenic syndrome an antibody-mediated autoimmune response to voltage-gated calcium channels reduces the number of these channels, thereby impairing release of ACh from the presynaptic membrane (8). Despite the safety factor for neuromuscular transmission, it is the target not only of disease processes but also of many toxins because blocking neuromuscular transmission provides a way to rapidly paralyze the victim. Inhibition of AChE is the mode of action of nerve gases such as sarin and insecticides such as malathion, but partial inhibition of AChE is used to treat myasthenia gravis to compensate for the loss of AChRs (9). AChRs are the targets of toxins in the venom of cobras or cone snails, toxins in arrow-poison frog skin, or the plant toxin curare.

Discovering the structure of AChE has involved the efforts of many laboratories, especially those of Jean Massoulié, Terrone Rosenberry, Israel Silman, and Palmer Taylor (reviewed in ref. 9). All AChE associated with synapses derives from a single gene, but alternatively transcribed C-terminal regions allow for the assembly of several forms of AChE. AChE catalytic subunits have about 575 amino acids. X-ray crystallography reveals ellipsoidal subunits with the active site at the bottom of a narrow gorge (2). These subunits can associate through hydrophobic tails with lipid bilayers or with one another in pairs or tetramers. The tetrameric form predominates in human brain. The tetrameric form can be disulfide bonded to a glycolipid tail that anchors it in the membrane. A form of AChE characteristic of skeletal muscle consists of

Abbreviations: AChE, acetylcholinesterase; AChR, acetylcholine receptor; COLQ, collagen-like tail of AChE.

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catalytic tetramers anchored at the ends of each of three branches of the 433-amino acid chains of a collagen-like tail termed COLQ. This produces a tree-shaped form in which the triple helix of the collagen-like tail forms a trunk that at its C-terminal roots is anchored in the basal lamina by heparan sulfate proteoglycans and at its top branches out to display a tetramer of catalytic subunits at the N terminus of each of these branches.

Engel and co-workers report in this issue (4) one patient who lacks a proline-rich attachment domain of COLQ, and this lack prevents any association with catalytic subunits. Five other mutations truncate COLQ after this attachment domain. These mutations bind a tetramer of catalytic subunits but do not associate with other COLQ chains or incorporate into the basal lamina. In patients, this results in greatly reduced AChE at neuromuscular junctions, prolonged synaptic currents, and repeated muscle fiber action potentials. This results in degeneration of the postsynaptic membrane's normal folded structure and loss of AChRs. In response to this excitotoxicity the presynaptic ending is smaller and fewer vesicles of ACh are released per impulse. The net effect is impaired neuromuscular transmission causing muscle weakness and in some cases postural deformity (lordosis).

AChRs at mature neuromuscular junctions are composed of five subunits of about 400–500 amino acids each organized around the central cation channel like barrel staves in the order $\alpha 1 \varepsilon \alpha 1 \delta \beta 1$ (8, 10). In another AChR form, which is present before innervation or after denervation, the ε subunit is replaced by a γ subunit. Congenital myasthenic syndromes have been found due to more than 50 mutations in $\alpha 1$, $\beta 1$, and ε subunits (reviewed in ref. 1). Substitution of γ subunits for ε provides partial compensation for ε subunit mutations. Mutations that truncate subunits can prevent assembly of functional AChRs. Mutations near the ACh binding site on $\alpha 1$ subunits have been found that reduce affinity for ACh and thus inhibit AChR function. Other mutations in this region increase affinity for ACh, resulting in excessive channel opening, which causes excessive entry of calcium that activates proteases and other processes that alter synapse morphology and reduce the number of AChRs. Mutations in the channel lining domains of several subunits prolong channel opening, resulting in similar excitotoxic damage as a result of excessive AChR activation. Some AChR mutations cause only mild myasthenia. For example, in one patient with subclinical weakness, an AChR mutation was not appreciated until an AChR inhibitor given as a surgical muscle relaxant prevented breathing (11).

Few mutations of neurotransmitter receptors are currently known. Neuronal AChRs are formed from combinations of $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, or $\alpha 6$ subunits with $\beta 2$, $\beta 3$, or $\beta 4$ subunits or homomeric combinations of $\alpha 7$, $\alpha 8$, or $\alpha 9$ subunits. Only two mutations of neuronal AChRs have been reported thus far (12). Mutations that impair the function of $\alpha 4$ subunits have been found to cause a rare form of epilepsy. Glycine receptors belong to the same gene superfamily as do AChRs, but they have an anion-specific channel and thus serve an inhibitory role rather than the excitatory role of AChRs. A mutation in the $\alpha 1$ subunit of glycine receptors causes exaggerated startle responses as a result of inhibiting receptor function (13).

The large number of mutations of muscle AChRs that Engel and co-workers have found suggests that there are probably many mutations of various neurotransmitter receptors present in the population. By analogy with the current work on AChE, one may now also expect to find mutations in other enzymes involved in neurotransmitter synthesis, transport, or degradation. Many of these may result in subtle phenotypes not necessarily associated with disease. Characterizing these mutations will be much more difficult to achieve with the elegance that Engel has applied to muscle AChRs and AChE because the normal functional roles of these neural synaptic components are not so well characterized and because neural tissue is not easily biopsied. Studies of these mutations yet to be discovered will need all the help they can get from reference to the elegant models provided by the studies of mutations at neuromuscular junctions.

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