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Acetylcholine Receptors and Myasthenia Gravis (40611)

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Myasthenia gravis is a neuromuscular disorder characterized by weakness and fatigability of muscles. The clinical features of myasthenia were first noted by Willis (27) in 1672 and were thoroughly described by the turn of the century (4). The neuromuscular junction was implicated in the disease process more than 40 years ago, but it was not until 1973 that the acetylcholine (ACh) receptors were identified as the precise site of the defect (13). Important advances have been made in understanding the mechanism of the autoimmune attack on ACh receptors (7), but many important questions remain.

In order to understand the pathogenesis of myasthenia gravis, it is necessary to review briefly the physiology of transmission of ACh at the neuromuscular junction (14) (Fig. 1). ACh is synthesized at motor nerve endings and is packaged in membrane-bounded vesicles, or "quanta," containing approximately 10,000 molecules each. ACh is released spontaneously or in response to electrical impulses conducted by the motor nerves. The released ACh crosses a narrow space and combines with ACh receptors which are clustered at the peaks of postsynaptic folds immediately opposite the ACh release sites. The combination of ACh with receptors causes a rapid increase in permeability of the membrane to sodium and potassium ions, resulting in electrical depolarization. The spontaneous release of individual quanta of ACh gives rise to depolarizations of the order of 1 mV called miniature end-plate potentials, or "mepps." Nerve impulses release approximately 150–200 quanta of ACh, giving rise to much larger end-plate potentials (epps), which trigger muscle action potentials and, consequently, muscle contractions.

In the early 1960s Elmqvist and colleagues observed that mepp amplitudes in myasthenic patients' muscles was reduced to about 20% of the normal value (12). They postulated that this was due to a presynaptic defect in the motor nerve, with a decreased amount of ACh contained in each quantum. However, we wondered whether the low mepp amplitudes might be accounted for by reduced numbers of available ACh receptors. We tested this hypothesis with the aid of α-bungarotoxin (α-BuTx) a purified fraction of a snake venom that binds specifically, irreversibly, and quantitatively to ACh receptors at neuromuscular junctions. α-BuTx is readily labeled with ^{125}I and can therefore be used to quantify ACh receptors.

We obtained "motor point biopsies," containing neuromuscular junctions, from 10 patients with myasthenia gravis and from normal and disease controls. The muscles were saturated with ^{125}I-α-BuTx *in vitro*, and the excess was removed by washing. The number of ACh receptors per neuromuscular junction was calculated from the bound radioactivity and the number of neuromuscular junctions present. In the normal muscles, the average number of receptor sites per neuromuscular junction was 3.7×10^7, while the myasthenic junctions had only 0.7×10^7, less than 20% of the normal number (11, 13). This strongly suggested a defect of ACh receptors.

In order to determine whether a reduction of available ACh receptors could account for the physiological and clinical abnormalities in myasthenia, we produced an experimental model in rats by blocking a proportion of the muscle ACh receptors with another highly specific snake toxin, α-cobra toxin (22). The rats were weak, requiring artificial ventilation. They showed the typical physiological features of myasthenia, including decremental responses on repetitive nerve stimulation, curare sensitivity, and improvement with anticholinesterase agents, which are used routinely as clinical diagnostic tests for myasthenia in man. These findings confirmed the theory that a reduction of ACh receptors could account fully for the physiological features of human myasthenia gravis.

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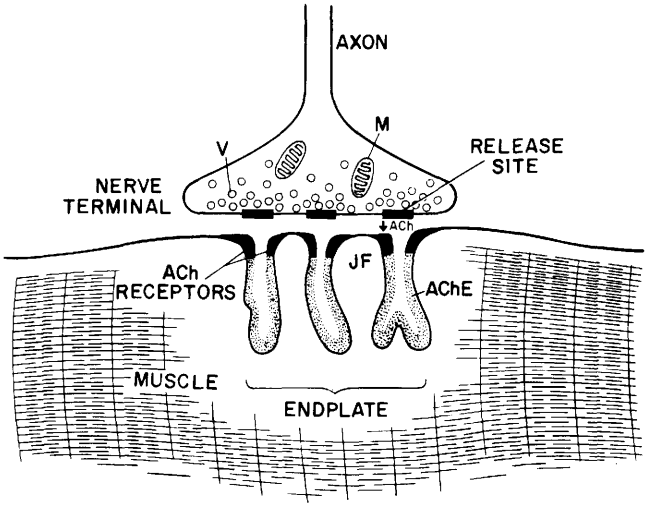

FIG. 1. Diagram of neuromuscular junction. Vesicles (V) release their acetylcholine (ACh) contents at specialized release sites. After crossing the narrow synaptic space (path indicated by arrow) ACh reaches the ACh receptors, which are most densely situated at the peaks of the junctional folds (JF). Acetylcholinesterase (AChE) in the clefts rapidly hydrolyzes the ACh. M, Mitochondria. Reprinted with permission from the N. Engl. J. Med. **298**, 136 (1978).

How is the ACh receptor defect produced in man? Several clues suggested an autoimmune process. The association between myasthenia gravis and other autoimmune disorders (23), as well as the high proportion of thymic abnormalities in patients with myasthenia (5), first suggested an autoimmune disorder. A further important clue that the autoimmune attack might be directed against ACh receptors came from the work of Patrick and Lindstrom who were attempting to raise antibodies to purified ACh receptors (derived from the electric organs of electric eels, one of the richest sources of ACh receptors). They found that rabbits injected with ACh receptor in Freund's adjuvant developed a condition analogous to myasthenia gravis (20). Further, antibodies against ACh receptors were identified in the serum of myasthenic patients (1).

The question of whether the circulating antibodies are instrumental in causing the receptor deficit, or merely represent a secondary response to receptor damage caused by some other agent, is of great importance in understanding the pathogenesis of myasthenia. One well-known clinical observation suggested that circulating antibodies might be pathogenic: Some infants born to myasthenic mothers are affected by myasthenic weakness during the neonatal period, with complete recovery after several weeks (18). However, numerous experimental attempts to transfer a myasthenia-producing factor from humans to animal preparations in the past had failed (19). In most studies of attempted transfer, animals or nerve–muscle preparations were exposed to the myasthenic serum for a brief time, usually minutes or a few hours. Based on the idea that a relatively prolonged period of exposure might be required for the effect to take place, we undertook to transfer immunoglobulins from the serum of human myasthenic patients to experimental mice (26). Daily injections of immunoglobulin (Ig) from myasthenic patients or controls were given to mice in doses sufficient to maintain levels of IgG equivalent to those in the humans, for 1 to 14 days. Some of the experimental mice became clinically weak and showed decremental responses on repetitive nerve stimulation. Passive transfer of Ig from 94% of the patients produced a reduction of ACh receptors, a reduction of mepp amplitudes, or both in the muscles of the recipient mice. Serum from only 1 of 16 patients failed to produce a reduction of either ACh receptors or mepp amplitudes. Injections of Ig from control individuals had no effect on the recipient mice (25).

By the use of this passive transfer model, we have found that only the IgG fraction produced the features of myasthenia, while

other serum immunoglobulins had no effect. Furthermore, the early part of the complement system enhanced the effect of IgG. Depletion of C3 (in the recipient mice) diminished, but did not eliminate, the effect of myasthenic patients' IgG on ACh receptors. However elimination of the latter part of the complement system (C5) did not reduce the effect of myasthenic patients' IgG. Thus, IgG appeared to play an important role in the pathogenesis of myasthenia gravis, enhanced by the early part of the complement cascade.

Theoretically, circulating antireceptor antibody might reduce the number of available ACh receptors by several different mechanisms:

(a) It might *block* the active sites of the receptors by binding at or near them.

(b) It might *alter the turnover* of ACh receptors, either by increasing the rate of degradation, or by decreasing the rate of synthesis, or both.

Evidence from tissue culture studies now suggests that both accelerated degradation of ACh receptors (2, 3, 10, 15) and receptor blockade (10, 15) may be involved. The methods used for measuring receptor degradation in tissue culture have previously been well worked out. Skeletal muscle from rat fetuses is grown in culture, and the ACh receptors are labeled with ^{125}I-α-BuTx. As the labeled receptors undergo degradation, the attached ^{125}I-α-BuTx is broken down to iodotyrosine, which is released into the culture medium. The rate of receptor degradation can be determined from the amount of radioactivity released into the medium per unit time; it is normally approximately 4% per hour for rat skeletal muscle. When Ig from myasthenic patients was added to the cultures, receptor degradation rate increased two- to threefold, as compared with cultures treated with Ig from control patients. The antibody-induced acceleration is triggered by IgG alone, without requiring other humoral or cellular components of the immune system. The antibody alters the receptors so that they are preferentially degraded at the rapid rate. Only those receptors with bound antibody are rapidly degraded, while other receptors in the same cultures are degraded at normal rates (8). This indicates that the effect is not due to an overall acceleration of the cells' ACh receptor-degrading mechanisms, but rather to a specific mechanism involving ACh receptor–antibody complexes.

We wondered whether this effect might be due to the ability of antibodies to crosslink antigens (in this case ACh receptors). Accordingly, we prepared various divalent (Fab'_2) and monovalent (Fab) fragments from myasthenic IgG (9). The divalent fragments produced acceleration of receptor degradation, while the monovalent fragments did not (Fig. 2). The fact that crosslinking alone accounted for the effect was demonstrated in two ways:

(i) In one series of experiments monovalent antibodies were first added to the cultures. The cultures were then incubated with a second, "piggyback" antibody directed against the antibody fragments (not against

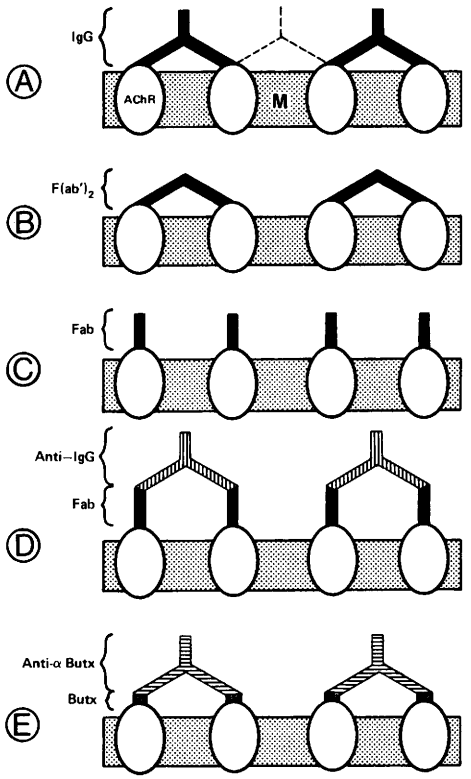

FIG. 2. Diagrammatic representation of crosslinking of receptors by the various antibody fragments. M, Muscle membrane; AChR, acetylcholine receptor; α-BuTx, α-bungarotoxin. Acceleration of degradation was observed in the experimental conditions shown in A, B, D, and E. Fab alone failed to accelerate acetylcholine-receptor degradation. Reprinted with permission from the N. Engl. J. Med. **298**, 1120 (1978).

ACh receptors). The effect of the second antibody was to crosslink the monovalent fragments, which in turn were bound to the receptors. This indirectly crosslinked the ACh receptors and had the same accelerating effect on ACh receptor degradation as the original divalent antibodies.

(ii) We prepared antibodies against α-BuTx by immunizing rabbits with α-BuTx in Freund's adjuvant. When cultures labeled with ^{125}I-BuTx were treated with the anti-BuTx antibodies, the degradation rate was again accelerated two- to threefold. In this case the antibodies were bound to the α-BuTx and thus indirectly produced crosslinking of ACh receptors. Taken together, these findings strongly suggest that the ability of antibodies from myasthenic patients to crosslink ACh receptors accounts for their effects on receptor degradation. It remains to be shown how crosslinking induces accelerated receptor degradation. It is possible that the crosslinked aggregates may be recognized by the muscle membrane, because of altered size or mobility, and selected for preferential degradation. Alternatively, the crosslinked receptors may be degraded by the normal process, but the number of receptors ingested in each "bite" would be increased because they are bound together. We have not yet measured the number of ACh receptors crosslinked together, but this model would predict aggregates of two to four, based on the 2.5- to 3.5-fold increase of degradation rates observed.

In the culture system, the accelerated degradation process accounts for part of the loss of ACh receptor sites observed after exposure to myasthenic immunoglobulin. In addition, blockade of receptor sites by the antibody has been shown to occur in skeletal muscle cultures, as well as in other assay systems (1, 10, 15). There is evidence that the blocking effect is due to attachment of the antibody near, rather than directly at, the active site of the receptor, producing steric hindrance of the active site (17).

Synthesis of ACh receptors does not appear to be affected by immunoglobulin from myasthenic patients. Using the indirect methods now available, we have found no change in synthesis and incorporation of new receptors in the rat muscle culture system after treatment with immunoglobulin from myasthenic patients (8).

These effects of myasthenic immunoglobulin on receptor degradation and blockade apply not only to the culture system in which they were first tested, but also to ACh receptors at intact neuromuscular junctions. Although there are important differences between *extrajuntional* receptors (like those found in cultured myotubes) and ACh receptors at innervated neuromuscular junctions, the same effect of accelerated receptor degradation occurs *in vivo* at intact neuromuscular junctions (24).

Although more is known about the ACh receptor and the humoral autoimmune mechanisms in myasthenia gravis than in any other receptor disorder, many important questions remain. For example, it is not yet known how the autoimmune process originates. The juxtaposition of muscle-like cells that bear ACh receptors (16) and immunocompetent cells in the thymus suggests that the thymus may be the arena in which autoimmunity originates in this disease. The possibility that an autoimmune process may be triggered by an alteration in either the "myoid cells" or the thymic lymphocytes is plausible (7), and it has been suggested that a viral infection may trigger the autoimmune response (6). Another unsettled question is the range of different immune mechanisms that may operate in patients with myasthenia gravis. Although the ACh receptor is probably always the target of the autoimmune process in myasthenia, it is likely that the specific immune mechanisms used differ in individual cases. The role of cell-mediated mechanisms in myasthenia has not yet been well studied. It is known that lymphocytes from myasthenic patients undergo blast transformation in the presence of purified ACh receptors (21). However, it is not yet known whether the sensitized lymphocytes are T cells or B cells, and whether they are capable of participating in cell-mediated effector mechanisms (7).

Conclusions. This review has dealt with recent advances in the understanding of myasthenia gravis. The fundamental abnormality affecting the neuromuscular junctions of myasthenic patients is a reduction of ACh receptors, due to an autoimmune attack directed against the receptors. Antibodies to ACh receptors are present in most patients,

and there is evidence that they have a pathogenetic role in the disease. The mechanism of antibody action involves acceleration of the rate of degradation of ACh receptors, attributable to crosslinking of the receptors by the antibody. In addition, antibodies produce blocking of the ACh receptors. The possibility that cell-mediated mechanisms may participate in the immune responses in some myasthenic patients remains to be explored. Perhaps the most important gap in the present understanding of myasthenia gravis concerns the origin of the autoimmune process, although there is in direct evidence that the thymus gland may be involved. Future research will be directed toward developing therapeutic methods to deal with the specific immunological abnormalities of individual myasthenic patients.

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