of vesicles and the amount of ACh released varies from transmission to transmission, but activation of the various pools ensures a sufficient amount to depolarize the postsynaptic membrane to threshold.

Calcium has a leading role in the release of ACh and in mobilization of ACh pools. Depolarization of the presynaptic terminal opens voltage-sensitive calcium channels. There is a linear relationship between terminal calcium concentration and the amount of presynaptic ACh released. Under normal circumstances, raising the terminal calcium concentration increases the amount of presynaptic ACh released but does not augment NMJ transmission (Table 1). This is because sufficient amount of ACh is normally released to ensure the generation of a muscle fiber AP. There are maneuvers that increase calcium availability that are important in the diagnosis and treatment of NMJ disorders. The postsynaptic ACh receptors interact with varying amounts of ACh. Under normal conditions there is always a sufficient amount of ACh to ensure depolarization of the end-plate to threshold with the generation of an AP (Fig. 4). The variability in the time to reach threshold in microseconds is of no clinical significance, but it can be measured by SFEMG.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal</td>
<td>Transient symptoms from myasthenic mother</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Localized, usually ocular only</td>
</tr>
<tr>
<td>Adult group I</td>
<td>Generalized, both bulbar and generalized</td>
</tr>
<tr>
<td>Adult group II</td>
<td>Acute fulminating, bulbar and generalized with respiratory failure</td>
</tr>
<tr>
<td>Adult group III</td>
<td>With muscle atrophy, evolving from group II</td>
</tr>
<tr>
<td>Adult group IV</td>
<td>Late severe, evolving from groups I and II</td>
</tr>
</tbody>
</table>

Modified from Ref. 12.

Natural History

Untreated, the natural history of MG is difficult to determine because many current therapies such as prednisone were available even early in the clinical description of the disease. In an early series at the turn of the century, it was found that a third of affected patients progressively worsened. Another one third had relapsing and remitting symptoms, and the remainder improved and led active lives for a period of time (13).

The contemporary natural history includes several important clinical points. Despite a large number of therapeutic regimens to enhance strength and promote remission, the most important improvement in clinical care has been the advent of positive pressure ventilation and respiratory critical care units to reduce mortality and morbidity from myasthenic crisis (14) and to permit safe performance of thymectomy. When the clinical course of a large number of patients are plotted, the maximal state of weakness during an exacerbation is usually experienced within the first 3 years, and the extent and severity will be evident during this time. Therefore, if ocular MG has not progressed to generalized involvement within 3 years, it is unlikely that it will do so (14).

Pathology

The pathologic alterations in MG are at the postsynaptic membrane and include simplification of the postsynaptic end-plate region with fewer and more shallow folds and a reduction in the number of ACh receptors. These changes result from antibody attachment to the receptors and activation of the complement cascade and receptor lysis by membrane attack complex (15,16). There are undoubtedly other mechanisms involved because some antibodies detected in the serum do not directly bind to the ACh receptor. In normal individuals, the half-life of the ACh receptor is approximately 12 days, but 3 days in MG.
The various types of ACh receptor antibodies in MG are operationally defined and based on laboratory testing procedures. Binding antibodies are detected by reaction of the patient serum with solubilized human skeletal muscle ACh receptor. Modulating antibodies are detected by reactivity with ACh receptor on living muscle and may be positive when the binding antibody is negative. Blocking antibodies are detected by binding of sera at or near the neurotransmitter binding site on solubilized human ACh receptors. There is a hierarchy of testing based on the frequency of occurrence (Table 3). Despite testing for three types of antibodies, only 80 to 86% of patients are seropositive. The false-positive rate is extremely low (17), and the specificity for MG is high.

Approximately 15% of patients with MG are seronegative based on the antibody panel described above. Seronegative patients are believed to have an autoimmune pathogenesis similar to the seropositive cases because defects in NMJ transmission can be passively transferred to experimental animals by serum or by the IgG fraction (18), but their diagnosis can be problematic because 50% have ocular symptoms alone and other diagnostic tests may be equivocal (19). Other organ-specific autoantibodies occur at a higher than expected frequency in patients with MG (20), including those to thyroid microsomes, thyroglobulin, and to gastric parietal cells. In seronegative patients with equivocal but suggestive symptoms of MG, these antibodies are supportive of the diagnosis of autoimmune MG (21).

**Autoimmune Features**

MG is a prototypic autoimmune antibody-mediated disease. Although the clinical focus is on the humoral arm of the immune system and autoantibodies to the ACh receptor, the disease starts with the cellular arm of the immune system, perhaps with a breakdown of T-cell tolerance. The normal process of T-cell tolerance is not fully understood, but it takes place in the thymus gland (22), as does the loss of tolerance in MG. While awaiting a full understanding of the physiologic and pathophysiologic processes of autoantibody diseases, several points are pertinent to the clinical diagnosis and management of MG. Because it is a specific autoantibody disease, there must be exposure of T cells to the ACh receptor, either from myoid cells in the thymus or peripherally. The exposure and subsequent breakdown of self-tolerance is a complex process and probably involves genetic susceptibility. The result is activation of B cells and the production of specific autoantibodies. Once this process has begun, there are immune mechanisms that regulate T-cell activity that affect the production of specific autoantibodies (22).

**Pathophysiology**

The pathophysiologic abnormality in MG results from a reduced number of ACh receptors. Fewer sodium channels open and the resultant EPPs have a slower rising phase and reduced amplitude. If the EPP amplitude does not depolarize the end-plate membrane to threshold, a muscle fiber AP will not be produced, NMJ transmission will fail, and the fiber will not contract or generate force (Table 4). A slower rise phase of the EPP, if it has sufficient amplitude to reach threshold, will have no clinical effect, although the slight delay in initiating the muscle fiber AP can be detected by SFEMG.

When NMJ transmission fails in MG, any method to increase the concentration of ACh at the receptor site increases the number of channel openings and improves amplitudes. There are two methods to increase the concentration of ACh. One is to increase the quantal release of ACh, which can be accomplished physiologically by increasing the concentration of calcium in the presynaptic terminal. A high-frequency train of presynaptic APs will overwhelm the calcium buffering capacity and result in greater quantal release. The second method is by reducing the rate of ACh hydrolysis by inhibiting the enzyme AChE. This can be accomplished by several drugs such as edrophonium and pyridostigmine. The effect of increasing the ACh concentration on a model of NMJ transmission can be seen in Table 4.

**Diagnosis**

The diagnosis of a defect in NMJ transmission may be clear when the history indicates and examination findings reveal fluctuating weakness and fatigue, especially of ocular, bulbar, and proximal limb muscles. The physical examination should include prolonged testing of strength.
TABLE 4. Descriptive model of the dynamics at an abnormal NMJ due to myasthenia gravis during low-frequency repetitive stimulation

<table>
<thead>
<tr>
<th>Stimulus number</th>
<th>ACh pool</th>
<th>Available ACh quanta</th>
<th>ACh quanta released</th>
<th>Resultant EPP amplitude a (mV)</th>
<th>Action potential threshold reached</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immediate</td>
<td>1,000</td>
<td>200</td>
<td>36</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Immediate</td>
<td>800</td>
<td>160</td>
<td>28</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Immediate</td>
<td>640</td>
<td>128</td>
<td>24</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Mobilizable</td>
<td>&gt;640</td>
<td>&gt;128</td>
<td>&gt;30</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Mobilizable</td>
<td>&gt;640</td>
<td>&gt;128</td>
<td>&gt;30</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The effects of higher calcium concentration on the release of ACh are normal in MG. However, there are fewer functioning ACh receptors and the resultant EPP is lower than normal. The greater amount of ACh released with mobilization has some effect on the EPP amplitude, resulting in a greater likelihood of an EPP reaching threshold for an action potential. Numbers representing ACh quanta are approximate.

a In this model, the EPP amplitude must be greater than 30 mV to reach threshold for generation of a muscle fiber action potential.

NMJ, neuromuscular junction; ACh, acetylcholine; EPP, end plate potential.

if necessary to bring out fatigue, particularly when the patient is already medicated with prednisone. It is essential to determine whether an NMJ disorder is due to a postsynaptic or a presynaptic defect, such as Lambert-Eaton myasthenic syndrome (LEMS). Elevated titters of ACh receptor antibodies are highly correlated with MG but can also occur in LEMS (22a). It is important therefore to carry out electrophysiological testing to recognize a presynaptic defect by facilitation of the CMAP after exercise. Positive ACh receptor antibodies in patients with LEMS are thought to represent a nonpathologic epiphenomenon and not the coexistence of both diseases.

**Tensilon Test**

Defects in NMJ transmission can be demonstrated by showing a brief improvement in transmission in the form of restoration of strength by giving a short acting AChase inhibitor. The rationale is based on demonstration of a clear difference comparing before, during, and after the intravenous administration of Edrophonium (Tensilon), which blocks AChase for two or three minutes. It is important that there be clearly weak muscle groups to follow because when symptoms and signs are mild and limited to ocular muscles, false positive diagnoses of MG may occur (22b). There are protocols for performing the Tensilon test in a double-blinded, placebo-controlled manner (39). It is emphasized that a positive Tensilon test does not distinguish between the various forms of presynaptic and post synaptic NMJ transmission failure.

**Repetitive Stimulation**

For there to be a demonstrable decrement to repetitive stimulation, a finite number of NMJs must have a failure of transmission. Abnormal NMJs fail during even low levels of repetitive stimulation, which forms the basis for this electrophysiologic test. A practical and effective testing paradigm is to measure the amplitude of the CMAPs to five shocks at 2 to 4 Hz (23,24). The NMJ safety factor ensures sufficient ACh released to produce APs in all muscle fibers, and the CMAP amplitude will not change from shock to shock. However, when there is a reduction in the number of receptors due to a postsynaptic defect or a reduction in ACh release resulting from the presynaptic defect, there may be failure of transmission at some NMJs. This will be evident by a reduction in the CMAP amplitude by the third shock, as the immediate pool of ACh is depleted. This is termed the decremental response (Fig. 5). The extent of the CMAP amplitude decrement depends on the number of NMJs that fail and may not be

![FIG. 5. Response to 3-Hz repetitive stimulation in a patient with myasthenia gravis. Top trace illustrates the decremental response with a maximal decrement of 17.5% with the fourth response. Bottom trace illustrates partial repair after 10 seconds of maximal muscle activation with a maximal decrement of 5.9%.](#)
evident in very mild defects in NMJ transmission. The increase in ACh release by the activation of the mobilizable ACh pool after the third shock will, in turn, result in an increase in the number of NMJs successfully transmitting, and the CMAP amplitude to the fourth shock will show an increase. This results in a U-shaped decremental pattern to the five CMAPs (Fig. 5).

Increasing the presynaptic calcium concentration increases the amount of ACh released and restores NMJ transmission at some failed junctions. This can be accomplished by having the patient voluntarily maximally activate the muscle group being tested for 10 to 15 seconds. If repetitive nerve testing is repeated in 1 to 2 seconds after exercise, there will be partial repair of the initial decrement.

NMJ transmission is most prone to failure 2 to 4 minutes after 10 to 15 seconds of maximal exercise. If repetitive nerve stimulation is performed at this time, a greater degree of decrement will be observed or the subtle defect will be evident (Fig. 5) (24), so-called posttetanic exhaustion.

A number of procedural and technical issues increase the sensitivity of repetitive stimulation. Physiologically, the safety factor suggests that there should be no decrement to repetitive stimulation in a normal muscle (Table 1). However, many texts and reference manuals allow for some decrement in normal muscles and consider up to 8% normal. There are a number of possible reasons for the low percentage decrement in normal muscle. One is a change in stimulation current during the stimulation train due to movement of the limb. This can be reduced by using stimulus intensities of 150% of maximum and by immobilizing the limb (25). Most EMG machines calculate decrement from the negative peak CMAP amplitude. Many also use the isoelectric line before the first CMAP as their baseline for amplitude measurement. If there is movement of the isoelectric line for subsequent shocks, there may be calculation errors. Measurement of peak-to-peak CMAP amplitude is less susceptible to these types of errors (26).

Repetitive stimulation can be incorporated into routine nerve conduction studies that are usually performed on distal limb muscles. Decrement may not be observed in these muscles if the MG is mild or affects ocular muscles alone; however, testing of a proximal muscle such as trapezius muscle along the spinal accessory nerve may be informative (27). Stimulation of the facial nerve is associated with movement artifact and is therefore technically more difficult. There are issues yet unresolved of the mathematic expression of decrement (25) and the separation of true decrement and facilitation from pseudofacilitation (28).

Several practical guidelines can be offered to enhance the accuracy of repetitive stimulation and to reduce false-positive decremental responses to as low as 3%. First, a true decremental response must be properly pathologic with a U-shaped response, that is, less decrement by the fourth shock. Second, it should be reproducible with the same pathologic decrement seen with varying percentages on several separate trials. Third, it should show a physiologic response to an increase in presynaptic termi-

**Single-Fiber Electromyography**

This is an electrophysiologic technique that analyzes the variability of NMJ transmission as a discharge-to-discharge variability in timing of single muscle fiber APs (5,29) (Fig. 6). This variability, called jitter, which is measured in microseconds, reflects slow EPP rise times and delayed generation of muscle fiber APs. When the EPP fails to reach threshold, jitter is infinite and the AP is blocked. It should be clear that SFEMG can detect abnormal jitter without blocking, whereas repetitive nerve stimulation only detects blocking (30,31). It requires a special concentric EMG needle with the exposed active electrode surface along the side of the needle and computer software to measure the variability. As most commonly measured, NMJ transmission is initiated by weak voluntary contraction, and the covariability of two NMJs is recorded. Alternatively, transmission can be initiated by electrical stimu-

![Fig. 6. Schematic diagram of single-fiber electromyography (SFEMG) recording technique and illustrative responses. Top diagram shows two muscle fibers (1 and 2) from the same motor unit. The SFEMG electrode, e, is positioned to record single fiber action potentials from each fiber. The higher amplitude of action potential 1 is used as the trigger potential. Lower traces show jitter from a normal and myasthenia gravis (MG) neuromuscular junction. Jitter is extreme in MG, with blocking of action potential 2. (Modified from Ref. 5, with permission.)](image-url)