Neuromuscular junctions provide the essential link between the nervous system and muscles. In healthy humans, neuromuscular junctions reliably convert every impulse in a motor neuron into a corresponding action potential in each muscle fibre innervated by that particular motor neuron. However, the structural and molecular complexity of neuromuscular junctions means that they contain many potential targets for deleterious mutations. In addition, their location at the relatively unprotected periphery of the nervous system heightens the vulnerability of neuromuscular junctions to injury from a variety of chemical and mechanical agents. Detailed investigations of patients with impaired neuromuscular transmission have provided important insights into how a variety of pathological processes may conspire to impair neuromuscular transmission.

The reliability of neuromuscular transmission depends both on how much acetylcholine is released from the nerve terminal and how it acts to excite the muscle fibre membrane (Slater, 2008). Two papers in this issue of Brain add to the list of myasthenic conditions in which the action of acetylcholine is impaired (Klooster et al., 2012; Webster et al., 2012). Taken together, these results emphasize that effectiveness of the transmitter depends on both the detailed molecular properties of the ion channels at the core of the acetylcholine receptors (AChRs) and how many of those receptors are within the range of transmitter molecules released from the nerve terminal.

In recent years, many mutations have been found in genes encoding key proteins at neuromuscular junctions that account for inherited defects of neuromuscular transmission, conditions known collectively as congenital myasthenic syndromes (Engel et al., 2010). The targets of these mutations are often the genes encoding the five homologous subunits of the AChR itself. Most of these mutations affect either the detailed kinetics of the opening and closing of ion channels gated by acetylcholine or the abundance of AChR.

The paper by Webster et al. (2012) describes an adult patient with lifelong weakness of a number of muscle groups. EMG studies confirmed an impairment of neuromuscular transmission. Sequence analysis of the patient’s DNA revealed two mutations of the gene encoding the epsilon subunit (ε-ε) of the AChR, each in a different copy of the gene. One mutation (deletion, ε-εF266), when present in AChRs expressed in cultured human embryonic kidney (HEK) 293 cells, has little effect on AChR channel kinetics but reduces the flow of current through the open channels to ~60% of its normal value. The mutation results in the deletion of a phenylalanine from the M2 transmembrane helix of the epsilon AChR subunit, one of five that line the channel pore and regulate its conductance. However, it is not yet clear how this leads to reduced ion conductance.

Since this ‘low-conductance’ mutation is only present in one of the two copies of the ε-εAChR gene, its defect would be expected to be compensated by its wild-type partner, particularly in the light of the normally high safety factor for neuromuscular transmission. However the second, previously described (Ishigaki et al., 2006), mutation in this patient (missense, p.εP282R) also causes changes in channel conductance and kinetics. Additionally, and more importantly in this case, it substantially reduces the number of functional AChR molecules expressed in HEK cells. Assuming that the same reduced expression of the p.εP282R AChRs occurs in vivo, the low-conductance p.εF266 form would be expected to have a much greater, and probably dominant, functional impact. This case thus appears to be the first recognized where a clinical defect results from decreased conductance of the AChR channel. It also exemplifies how two mutations, that on their own may have minimal functional effects, can combine to cause a significant functional deficit.

While all systems of the body are vulnerable to the deleterious effects of genetic mutations, the neuromuscular junction, located as it is beyond the protection of the blood–brain barrier, runs the additional risk of attack by blood-borne pathogens. These include both natural toxins, such as those that cause botulism, and autoantibodies. Some of the best known autoantibodies are directed against the AChRs themselves and are the causative agents in ~90% of cases of myasthenia gravis. They bind the extracellular surface of the AChRs at the neuromuscular junction, which triggers activation of the complement system. This, in turn, causes localized cytolysis of the muscle fibre, disrupting the specialized subcellular structures that allow an effective response to the transmitter and causing a dramatic reduction in the number of AChRs.

In recent years it has become clear that circulating autoantibodies may also attack other molecular components of the neuromuscular junction.
neuromuscular junction. In particular, a number of myasthenic patients who lack antibodies to AChRs (so-called ‘seronegative’ patients with myasthenia gravis) have been found to have antibodies directed against a muscle-specific membrane kinase (MuSK) that is closely associated with the neuromuscular junction (Hoch et al., 2001). When activated by agrin released from the motor nerve terminal, MuSK triggers a signalling cascade that plays an essential role in maintaining the local molecular differentiation of the postsynaptic membrane. A central part of this process is the clustering of AChRs into high-density domains that are essential for effective transmitter action. Thus, patients with these antibodies often have a reduction of AChR numbers, resulting in impaired neuromuscular transmission. However, most of the anti-MuSK antibodies are members of the IgG4 subclass, which do not activate the complement system. Until now the basis for their pathogenesis has remained enigmatic.

In an effort to clarify how their effect is exerted, Klooster et al. (2012) transferred anti-MuSK autoantibodies passively from seronegative myasthenic patients into mice. They became paralysed and EMG studies revealed clear impairment of neuromuscular transmission. When neuromuscular junctions were studied in vitro, the amplitudes of the postsynaptic potentials were found to be significantly reduced, suggesting lowered sensitivity of the muscle to acetylcholine. A reduction in the intensity of AChR labelling by fluorescent C11-bungarotoxin supported this view. In further studies, binding of the anti-MuSK IgG4, but not that of key components of the complement system, was seen to be concentrated at the neuromuscular junctions. This study thus provides clear support for the hypothesis that autoantibodies, which bind proteins involved in localizing AChRs, may cause impaired neuromuscular transmission without activating the complement system. Additional studies will be needed to establish the details of their action.

A further notable feature of these mice is the absence of an effective adaptive response by their neuromuscular junctions. In many situations involving impaired efficacy of transmission, myasthenia gravis among them, the nerve responds by increasing the amount of acetylcholine released by each nerve impulse. No such increase was observed in these mice, suggesting that this normal protective behaviour of the neuromuscular junction is also blocked by the anti-MuSK IgG4. As a result, the functional effect of lowered numbers of AChRs is fully expressed.

Taken together, these two studies highlight the diverse, and sometimes subtle, ways in which pathogenic processes may interact to cause clinically significant impairment of neuromuscular transmission. These include both alterations of the functional properties of the AChR and decreases in their number, each of which can result from a number of different molecular events. The studies further emphasize that a satisfactory understanding of clinical impairment of neuromuscular transmission, one that has a chance of suggesting appropriate treatment and genetic counselling, can only come from detailed investigations of the structure, function and molecular status of the neuromuscular junctions in each patient.

Clarke Slater
Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK

Correspondence to: Clarke Slater, Professor of Neuroscience (Emeritus), Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE2 4HH, UK
E-mail: c.r.slater@ncl.ac.uk

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References

Huntington’s disease: fighting on many fronts

The discovery in 1993 of the genetic abnormality that causes Huntington’s disease triggered an explosion in our understanding of this inherited neurodegenerative disorder (The Huntington’s Disease Collaborative Research Group, 1993). Since the Huntington’s disease mutation is fully penetrant, everyone with the same underlying CAG triplet repeat expansion in the huntingtin (HTT) gene will develop a devastating combination of motor, cognitive and psychiatric disturbances. The monogenic nature of Huntington’s disease suggests that disease modifying therapies should be within reach. Currently, however, no such treatments