CHAPTER

44

Disorders of Muscle Excitability

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ORGANIZATION OF THE NEUROMUSCULAR JUNCTION

Nerve and muscle communicate through neuromuscular junctions

The transmission of the nerve impulse to muscle to generate movement and force takes place at the neuromuscular junction (NMJ), a specialized type of synapse where the nerve action potential may trigger a muscle action potential through the intervention of a chemical relay: the release of acetylcholine (ACh) (Ch. 13). Each motor neuron in the brainstem and spinal cord innervates a variable group of muscle cells (called fibers)—which together constitute the motor unit—whose number depends on the precision of movement and strength of force for which the muscle is accustomed

(Figure 44-1). The entire pre- and postsynaptic apparatus, including the intercellular synaptic space, is contained in a differentiated area of significant complexity that measures about 40 μm in its largest dimension (Figure 44-2). Schwann cells delimit the surface of the NMJ by contributing a protective enveloping process devoid of myelin that contains specialized membrane proteins such as neural cell adhesion molecules. Entering slightly into the muscle, the nerve endings form boutons that are separated from a highly invaginated region of raised muscle plasma membrane by a synaptic space of 50 nm. The basal lamina of both nerve and muscle, which is mainly composed of type IV collagen, continues uninterrupted across the synaptic cleft, but differentiates at the NMJ by harboring specialized proteins such as acetylcholinesterase (AChE), laminin, agrin and neuregulins. Presynaptic terminal mitochondria provide ATP for the energetically demanding vesicular ACh turnover process, and a fraction of the synaptic vesicles in the terminal are associated with regions of increased membrane density called active or release zones (Figure 44-3). These zones are rich in voltage-gated Ca^{2+} channels of the P/Q type as well as calcium-activated K^+ channels, and are precisely located across from the infoldings of the postsynaptic membrane. Following nerve stimulation, vesicles available near these sites fuse with the plasma membrane and release their content of ACh directly over the postsynaptic receptor molecules on the adjacent folds. A significant fraction of synaptic vesicles, however, is directly associated with actin (see Chs. 6 and 7) away from the active zones and not available for immediate release. The postsynaptic muscle membrane is also highly specialized. Nicotinic ACh receptors (AChR) cluster in the infoldings and directly associate with 43 kDa rapsyn (receptor-aggregating protein at the synapse). Utrophin—a large protein similar to dystrophin—and α-dystrobrevin are thought to link the AChR complexes to actin filaments and to a much larger network of

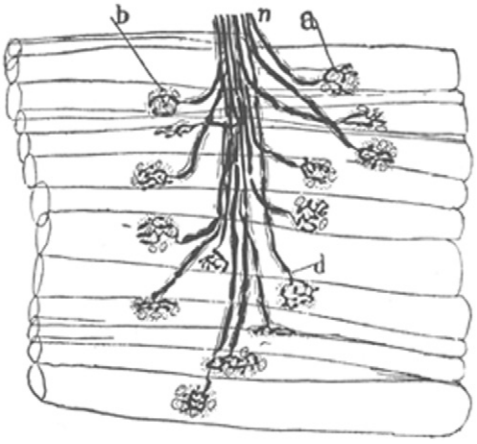

FIGURE 44-1 Motor endplates from rabbit intercostal muscle stained with Löwit's gold chloride method. *a*, terminal axonal arborization; *b*, nucleus; *d*, region where the myelin sheath ends; *n*, neural branch. (From Ramón y Cajal, S. *Textura del sistema nervioso del hombre y los vertebrados*. Madrid: N. Moya, 1899.)

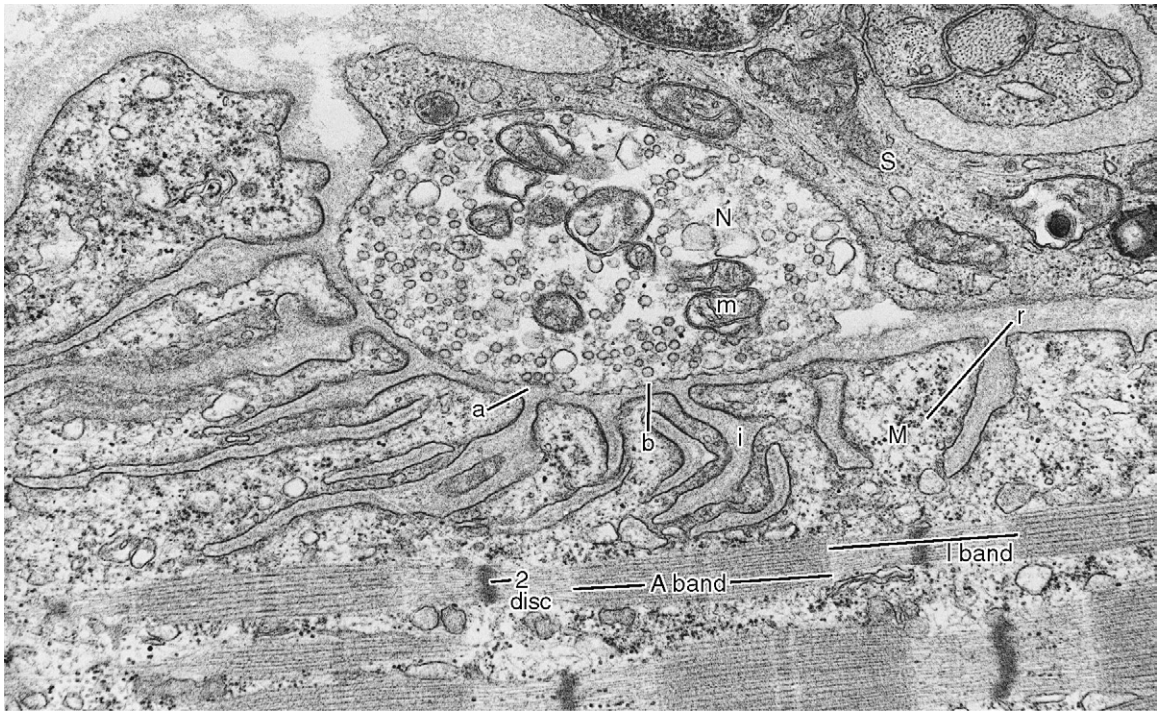

FIGURE 44-2 Photomicrograph of the human neuromuscular junction. In normal muscle, Ach receptors are associated with the terminal expansions of the junctional folds and the architecture of the postjunctional membrane follows closely the distribution of active zones in the presynaptic membrane. *b*, basal lamina; *I*, infoldings; *m*, mitochondria; *M*, myocyte; *N*, nerve terminal; *r*, ribosomes; *s*, synaptic space; *S*, Schwann cell. Courtesy of A. Engel.

proteins, many of which are coming to attention now that the molecular causes of an increasing number of human myopathies are being determined.

Acetylcholine acts as a chemical relay between the electrical potentials of nerve and muscle

ACh is synthesized in the nerve terminal by choline acetyltransferase (ChAT) from choline and acetyl-coenzyme A—a product of anaerobic glycolysis generated via the oxidation of pyruvate—much as in central nervous system synapses (see Ch. 11). ACh is released after the arrival of the nerve action potential to the resting terminal, whose potential is maintained by the Na^+/K^+ ATPase. Depolarization leads to activation of Ca^{2+} channels and to a surge of Ca^{2+} entry (see Ch. 24), reaching an impressive concentration of 0.1 mM for a few milliseconds. Later, Ca^{2+} is buffered in the terminal and extruded by the Na^+/Ca^{2+} exchanger, as the opening of voltage-gated K^+ channels also contributes to restore membrane potential. While still in the cytosol, Ca^{2+} interacts with the sensing vesicular protein synaptotagmin, priming other vesicular proteins for docking against and fusion with the plasma membrane. ACh then is freed to diffuse across the junctional cleft and interact with AChR (Karlin & Akabas, 1995) until it encounters AChE and is hydrolyzed into choline and acetate, later to be recovered by the terminal. AChE exists in a peculiar trimeric conformation composed of three groups of four active globular heads linked by a collagen strand named ColQ. An enzyme of great pharmacological significance, AChE is reversibly blocked by anticholinesterases of medical use, and irreversibly phosphorylated and disabled by organophosphate insecticides, among many other modulators. Following fusion and diffusion of ACh, the vesicular membrane is retrieved by endocytosis of clathrin-coated vesicles and refilled with ACh. While being refilled, vesicles are tethered to actin filaments as mentioned above via synapsin, a vesicular protein that loosens its attachment after Ca^{2+}-dependent phosphorylation by calmodulin kinase. Only then is a vesicle ready for localization near the active zone pending release.

The fidelity of signal transmission relies on the orchestration of innumerable stochastic molecular events

Neuromuscular transmission relies on the generation of a junctional muscle membrane depolarization of sufficient amplitude to sustain the formation of a propagated action potential; the difference between the magnitude of the first parameter and the threshold of the action potential is called the safety factor of neuromuscular transmission. Inexorably, impulse transmission must proceed across multiple conformational changes in the molecules that underlie NMJ excitability. An inherently stochastic (as opposed to a deterministic all-or-nothing) process, normal transmission necessitates a large safety factor in order to operate with fidelity. Among the processes that are non-deterministic, that is, best described by

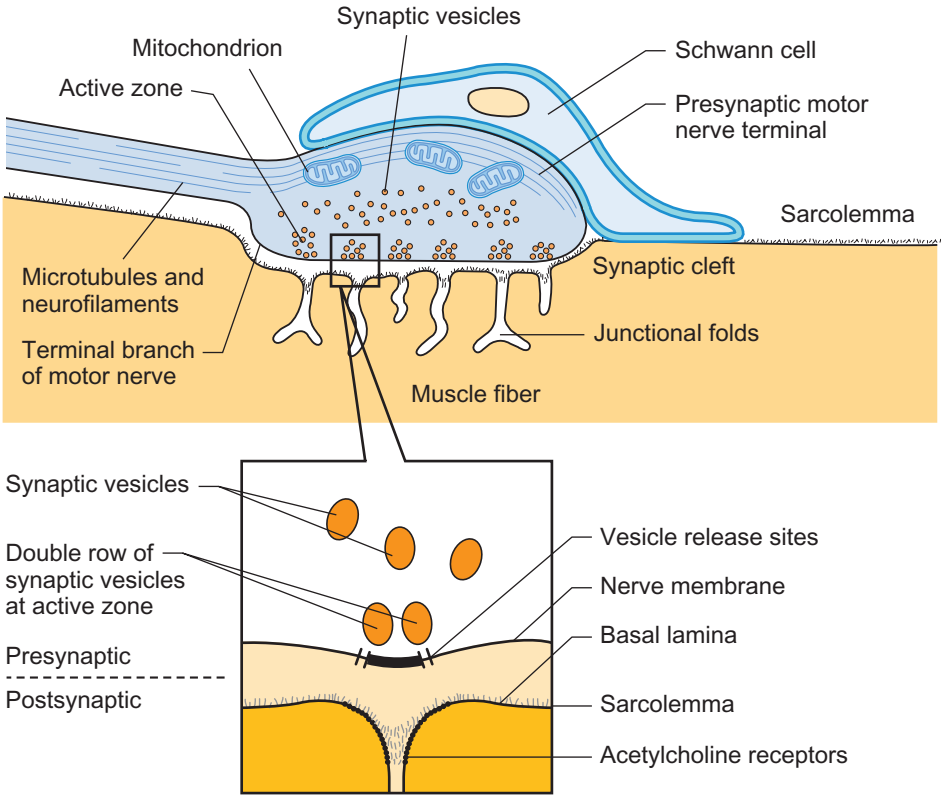

FIGURE 44-3 **Schematic representation of the key synaptic elements of the neuromuscular junction.**

probability distribution laws rather than individually, are the opening of voltage-gated Ca^{2+} channels, the number of ACh vesicles released, the diffusion of ACh in the cleft, the binding of two ACh molecules to the AChR, the onset of desensitization and closing of the AChR and the encounter of the neurotransmitter with AChE, to name but a few events for which probability distributions are known. The origin of this stochastic behavior is intrinsic to all molecules that change conformation or move, but it is accentuated by the limited dimensions of the NMJ, which includes a relatively small number of molecules. In contrast, an enzymatic reaction taking place, for example, in solution carried out by an ensemble of many more molecules behaves, as a whole, predictably. The limitation to the size of the NMJ may be due to the large energetic expense associated with its maintenance, particularly when it is subject to frequent use (think, for example, of a flying hummingbird) and, thus, energy may be safely spared by reducing synaptic size as long as the safety factor of transmission remains large. In line with the importance of neuromuscular transmission for the survival of the organism, all key molecular elements of the NMJ are encoded by highly conserved genes whose absence or severe dysfunction is incompatible with life. Instead, diseases of the NMJ tend to shift the probability distribution laws for the different conformations of its key molecules, and may be thus viewed as processes that decrease the safety factor of transmission. This is a fundamental principle in molecular neuromuscular pathology.

EXCITATION AND CONTRACTION OF THE MUSCLE FIBER

The excitable apparatus of muscle is composed of membranous compartments

The muscle cell membrane, together with connective tissue elements and collagen fibrils, forms the sarcolemma (Franzini-Armstrong, 1979). The interior of the resting cell is maintained at an electrical potential about 80 mV more negative than the exterior by the combined action of pumps and channels and the solutions bathing the membrane. Unlike nerve membranes, muscle membranes possess a high conductance (G) to chloride ions in the resting state such that G_{Cl^-} accounts for ~70% of the total membrane conductance. Potassium ion conductance accounts for most of the remainder, such that the membrane potential is normally close to the Nernst potential for these two ions (see Ch. 4). Asymmetrical concentration gradients for sodium and potassium ions are maintained at the expense of energy consumed by the membrane Na^+/K^+-ATPase. During the generation of a muscle action potential, a rapid and stereotyped membrane depolarization is produced by an increase in sodium ion conductance mediated by voltage-dependent Na^+ channels. The sodium conductance increase is self-limited due to channel inactivation, and membrane repolarization is also assisted by the delayed opening of potassium channels. Action potentials originating at the NMJ spread in a nondecremental fashion over the entire surface of the muscle and penetrate the interior of the muscle cell along transverse (T) tubules that are continuous with the outer membrane (Fig. 44-4). These tubules convey sarcolemmal depolarization to the center of the muscle fiber very effectively. As the T-tubular network extends inward into the cell, close associations are formed with specialized terminal elements of the sarcoplasmic reticulum (SR). At the electron microscope level of observation, the structure formed by a single tubule interposed between two terminal SR elements is called a triad. The SR stores Ca^{2+} in relaxed muscle and releases it into the sarcoplasm upon depolarization of the membrane of the T-tubular system.

Myofibrils are designed and positioned to produce movement and force

The physiological unit of muscle, the myocyte or muscle fiber, contains repeating structures known as sarcomeres that are separated from each other by dark lines (when observed by electron microscopy) called Z disks. Within each sarcomere, A and I bands can be identified. The A band, lying between two I bands, occupies the center of each sarcomere and is highly birefringent. Within the A band is a central, lighter zone, the H band, and in the center of the H band is the darker M band. The Z disk is at the center of the I band (Fig. 44-4). The difference in birefringence between the A and I bands produces the characteristic striated appearance of voluntary muscle when seen through the light microscope. The repeating optical patterns of A and I bands in each sarcomere reflect the regular arrangement of two sets of filaments: Thin filaments have a diameter of ~50–70 Å, appear to be attached to the Z bands and are found in the I band as well as part of the A band. Thick filaments have a diameter of ~150 Å, occupy the A band and are connected crosswise by material in the M band. When observed in cross-section, thick filaments are arranged in a hexagonal lattice and thin filaments occupy the centers of the triangles formed by the thick filaments. With the identification of two sets of discontinuous filaments in the sarcomere came the recognition that (i) the two kinds of filaments become cross-linked only on excitation and (ii) contraction of muscle does not depend on shortening the length of the filaments but rather on the relative motion of the two sets of filaments, termed the sliding-filament mechanism (Huxley, 1969). Thus, muscle length depends on sarcomere length, which, in turn, is determined by the degree of overlap between thin and thick filaments. In addition to actin and myosin, other proteins are found in the two sets of filaments. Tropomyosin and a complex of three subunits collectively called troponin are present in the thin filaments and play an important role in the regulation of muscle contraction. Among the proteins that constitute the M and the Z bands are α-actinin and desmin as well as the enzyme creatine kinase. A continuous elastic network of proteins, such as connectin, surround actin and myosin filaments, provide muscle with a parallel passive elastic element. Actin forms the backbone of the thin filaments (Pollard & Cooper, 1986) (Ch. 6). The thin filaments of muscle are linear polymers of slightly elongated, bilobar actin subunits, each about 4 nm × 6 nm, arranged in a helical fashion, with their longer dimension arranged approximately at a right angle relative to the filament axis. Each monomer has a molecular weight of ~42 kDa and contains a single nucleotide binding site. Hydrolysis of ATP to ADP takes place during

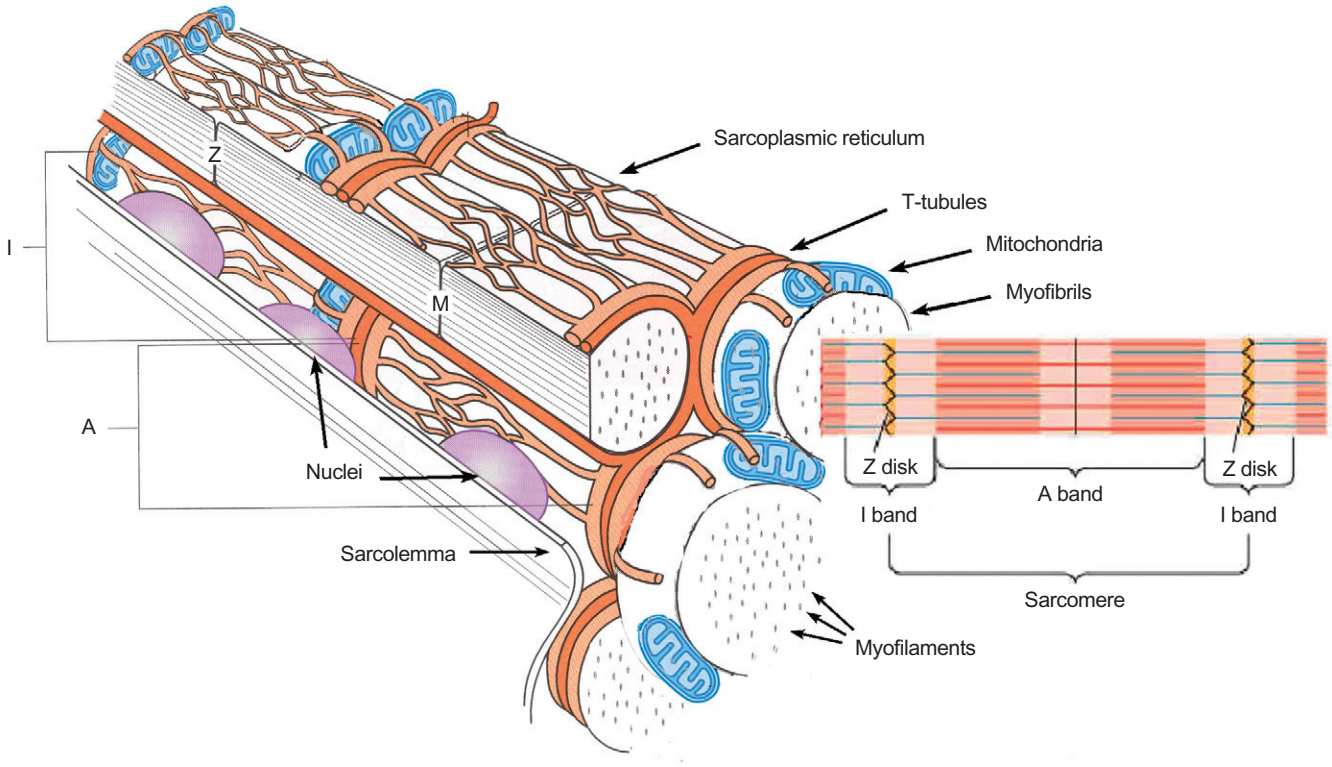

FIGURE 44-4 **Cellular and molecular organization of the skeletal myocyte.**

actin polymerization but does not determine muscle contraction. A wide variety of proteins interact with actin in both muscle and nonmotile cells. They may affect the polymerization–depolymerization of actin and are involved in the attachment of actin to other cellular structures, including the Z disks in muscle as well as membranes in both muscle and nonmuscle cells. Myosin II, the chief constituent of thick filaments, is an asymmetrical multisubunit protein of ~500 kDa with an overall length of ~150 nm. Its width varies between ~2 and 10 nm. In contrast to actin, myosin consists of several peptide subunits. Each myosin molecule contains two heavy chains of ~200 kDa that extend through the length of the molecule. Over most of their length, the two chains are intertwined to form a double α-helical rod and at one end they separate, each forming an elongated globular portion. The two globular portions contain the sites responsible for ATP hydrolysis and interaction with actin. In addition to the two heavy chains, each myosin molecule contains four light chains of ~20 kDa. These light chains modulate myosin activity and some of them can be phosphorylated by kinases. Myosin molecules form end-to-end aggregates involving the rod-like segments, which then grow into thick filaments. The polarity of the myosin molecules is reversed on both sides of the central portion of the filament. The globular ends of the molecules form projections, termed cross-bridges, on the aggregates that interact with actin. Conformational changes within this region, driven by ATP hydrolysis, provide the force that propels the movement of actin fibrils with respect to the myosin filament (Fig. 44-5). The ATPase activity of myosin itself is stimulated by Ca^{2+} and is low in Mg^{2+}-containing media. The hydrolysis of ATP releases sufficient free energy to induce conformational changes that are converted into cellular movement and, ultimately, mechanical work (Pollard & Cooper, 1986). Tropomyosin and troponin are proteins located in the thin filaments, and together with Ca^{2+} they regulate the interaction of actin and myosin (Fig. 44-3) (Hibberd & Trentham, 1986). Tropomyosin is an α-helical protein consisting of two polypeptide chains; its structure is similar to that of the rod portion of myosin. Troponin is a complex of three proteins. If the tropomyosin–troponin complex is present, actin cannot stimulate the ATPase activity of myosin unless the concentration of free Ca^{2+} increases substantially, while a system consisting solely of purified actin and myosin does not exhibit any Ca^{2+} dependence. Thus, the actin–myosin interaction is controlled by Ca^{2+} in the presence of the regulatory troponin-tropomyosin complex (Zot & Potter, 1987).

Calcium couples muscle membrane excitation to filament contraction

Important work has focused on the proteins present in the T-tubule/SR junction. One protein, an integral component of the T-tubular membrane, is a form of L-type, dihydropyridine-sensitive, voltage-dependent calcium channel. Another, the ryanodine receptor (RyR), is a large protein associated with the SR membrane in the triad that may couple the conformational changes in the Ca^{2+} channel protein induced by T-tubular depolarization to the Ca^{2+} release from the SR (Fig.

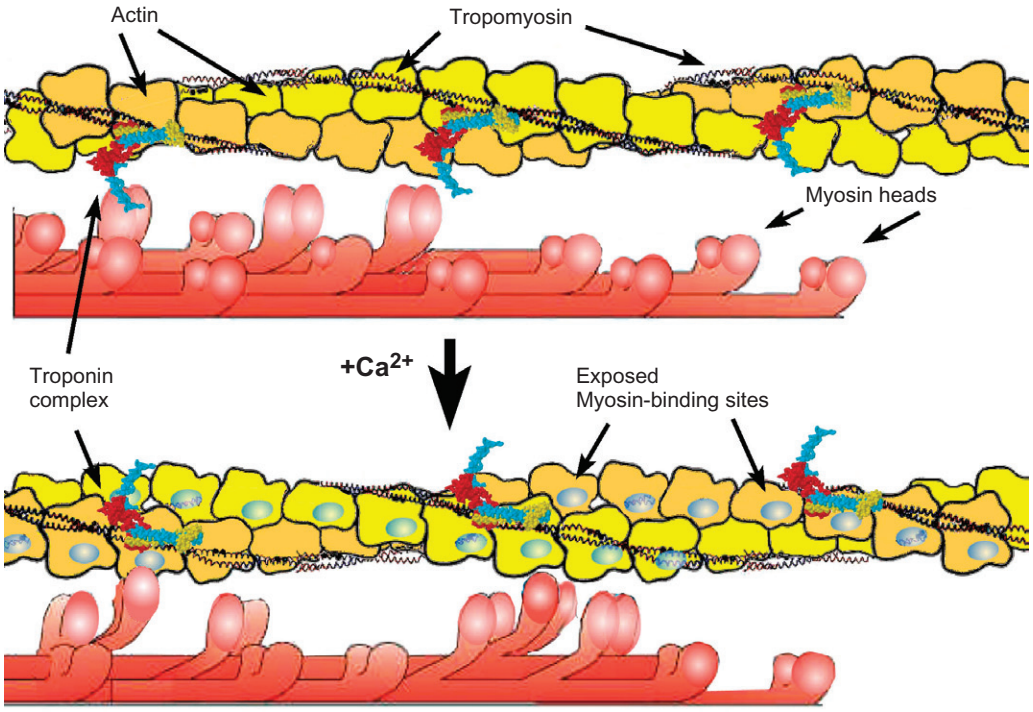

FIGURE 44-5 **Myofibrillar apparatus and muscular contraction**. Troponin is essential in Ca^{2} regulation of contraction and is represented as a small, boomerang-shaped trimer. It consists of three subunits (TnT (*yellow*), TnC (*red*) and TnI (*cyan*)) and, together with tropomyosin (drawn as a helicoidal dimeric string), is located in apposition to the actin filament (represented as an *orange-yellow* helical chain with its myosin binding sites in *blue*). Myosin filament heads, represented in the lower part of each panel, possesses ATPase activity. Redrawn from Takeda, S. *et al.* Structure of the core domain of human cardiac troponin in the Ca^{2+}-saturated form, *Nature* 424: 35–41 and Brown J. H. *et al*. Deciphering the design of the tropomyosin molecule, *PNAS* 98: 8496–8501, 2001.

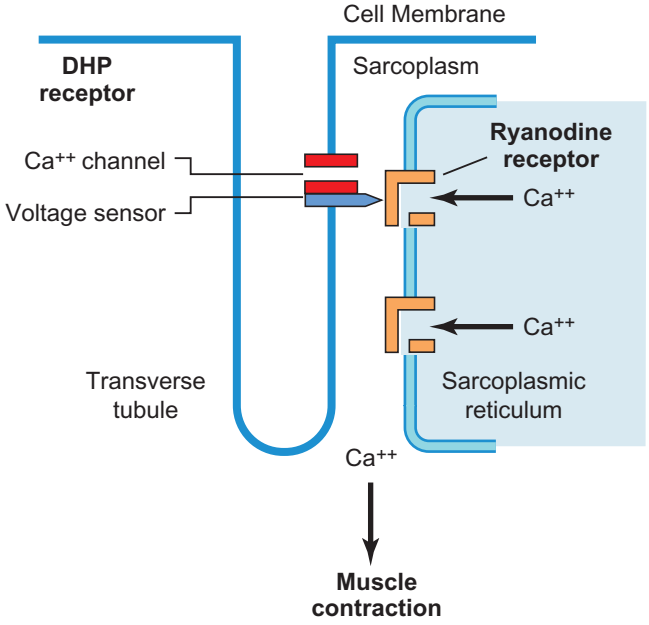

FIGURE 44-6 **Molecular physiology of muscle excitation–contraction coupling**.

44-.6). The first molecule, the Ca^{2+} channel, is required for coupling at the triad. Skeletal muscle contains higher concentrations of this L-type Ca^{2+} channel that can be accounted for on the basis of measured voltage-dependent Ca^{2+} influx because much of the Ca^{2+} channel protein in the T-tubular membrane does not gate calcium ion movement but, rather, acts as a voltage transducer that links depolarization of the T-tubular membrane to Ca^{2+} release through a receptor protein, the ryanodine receptor, situated in the SR membrane. The bar-like structures that connect the terminal elements of the SR with the T-tubular membrane in the triad are formed by a large protein that is the principal pathway for Ca^{2+} release from the SR. This protein, which binds the plant alkaloid ryanodine with high affinity (hence its name), is a large homotetramer of 565 kDa polypeptide subunits (Campbell et al., 1987). The purified complex, when incorporated into planar bilayers, exhibits Ca^{2+} channel activity that is modulated by Ca^{2+} itself, Mg^{2+}, and ATP among other regulators. The manner in which activation of the ryanodine receptor complex is coupled to events at the T-tubular membrane probably involves direct mechanical linkage through a conformational change exerted upon the dihydropyridine receptor protein (Wagenknecht et al., 1989). After diffusion towards the myofibrils, Ca^{2+}reuptake in the sarcoplasmic reticulum allows the relaxation of muscle and the

TABLE 44-1 **Disorders of Muscle Excitability**

INHERITABLE DISEASES	
Disorders of the neuromuscular junction: congenital myasthenic syndromes	
Choline acetyltransferase deficiency	MIM 254210
Acetylcholine receptor deficiency	MIM 254210
Rapsyn deficiency	MIM 601592
Slow channel syndrome	MIM 601462
Fast channel syndrome	MIM 254210
Acetylcholinesterase deficiency	MIM 603034
Disorders of the muscle membrane	
Hyperkalemic periodic paralysis	MIM 170500
Paramyotonia congenita	MIM 168300
Hypokalemic periodic paralysis	MIM 170400
Andersen syndrome	MIM 170390
Myotonic dystrophy (Steinert disease)	MIM 160900
Myotonia congenita (Thomsen disease and Becker myotonia)	MIM 160800 and 255700
Malignant hyperthermia	MIM 145600
Brody disease	MIM 601003
IMMUNOLOGICAL DISEASES	
Myasthenia gravis	
Lambert-Eaton syndrome	
Neuromyotonia (Isaac syndrome)	
INTOXICATIONS AND METABOLIC DISEASES	
Botulism	
Animal envenomations	
Electrolyte imbalances	

Mutations associated with inheritable diseases are numbered in MIM notation as described in McKusick, V.A., *Mendelian Inheritance in Man: A Catalog of Human Genes and Genetic Disorders*. Baltimore: Johns Hopkins University Press, 1998 (12th edition). An updated Internet version is found at Online Mendelian Inheritance in Man, OMIM(TM). McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), 2000. URL: http://www.ncbi.nlm.nih.gov/omim/

maintenance of a low resting intracellular Ca^{2+} concentration by means of an ATP-dependent Ca^{2+} pump, located in the SR membrane. The free energy of ATP hydrolysis is utilized here for the concentrative uptake of Ca^{2+} into the SR vesicle through a phosphorylated enzyme intermediate (see Ch. 3). Other SR proteins assist in Ca^{2+} uptake and storage. Phospholamban is prominent in cardiac muscle and slowly contracting muscle, where phosphorylation participates in the control of Ca^{2+}-ATPase and Ca^{2+} uptake activity. Another protein, calsequestrin, containing numerous low-affinity Ca^{2+}-binding sites, is present in the lumen of the SR and also participates in Ca^{2+} storage. Fast-twitch muscle contains a soluble Ca^{2+}-binding protein, parvalbumin, which is structurally related to troponin-C. Parvalbumin may also regulate the Ca^{2+} concentration in the initial stages of relaxation to facilitate rapid contraction.

The disorders of muscle excitability are best classified according to their cause (Jones et al., 2003) and are listed in Table 44-1.

GENETIC DISORDERS OF THE NEUROMUSCULAR JUNCTION

Congenital myasthenic syndromes impair the operation of the acetylcholine receptor

These disorders result in impaired neuromuscular transmission due to mutations of either presynaptic, synaptic basal lamina–associated, or postsynaptic proteins and cause fatigable weakness that follows predilect muscle distribution patterns (Engel et al., 2010). Traditionally termed "congenital myasthenia," they should be differentiated from acquired, immune-mediated myasthenia gravis, and it is recognized that additional types of congenital myasthenic syndromes may continue to be discovered. In fact, mutations of SCN4A—the gene that encodes the $Na_V1.4$ skeletal muscle sodium channel—leading to enhanced channel inactivation have been found in a patient with typical myasthenic symptoms.

ChAT Deficiency

The distinguishing clinical feature is sudden episodes of severe respiratory difficulty and oropharyngeal (bulbar) weakness leading to apnea (cessation of respiration) either precipitated by infections, fever or excitement or occurring spontaneously. In some patients, the disease presents at birth with hypotonia (diminished muscle resistance to mobilization) and severe bulbar and respiratory weakness, requiring ventilatory support that gradually improves through life, but is followed by episodes of apnea attacks and bulbar paralysis later. Other patients are normal at birth and may develop myasthenic symptoms and apneic attacks after infancy or childhood. Muscle action potentials decline abnormally when neuronal impulse flow is increased and recovers slowly, indicating an underlying defect in resynthesis or vesicular packaging of ACh. Several recessive mutations have been identified, all of which decrease the abundance or efficiency of ChAT. Although still largely unexplained, the selective neuromuscular -versus central nervous system- involvement in ChAT deficiency may be due to the enzyme rate-limiting characteristics for ACh synthesis at the NMJ.

AChR Deficiency

The degree of disease severity may vary from mild to very severe. In general, patients harboring low-expressor or even homozygous null mutations in the AChR ϵ subunit may experience mild symptoms, presumably owing to the compensatory replacement of this peptide by the analogous γ subunit. Conversely, patients with low-expressor mutations in the other non-interchangeable subunits are severely affected, and no patients with null mutations in both alleles of a non-ε subunit have been observed. AChR deficiency results from mutations that cause premature termination of the translational chain by frameshift, by altering splice sites, or by generating stop codons; from point mutations in the promoter region; from chromosomal microdeletion; and even from missense mutations, some of which affect the signal peptide or residues essential for assembly of the AChR. Patients respond moderately well to anti-AChE drugs.

Rapsyn deficiency

These patients manifest AChR deficiency with decreased rapsyn as well as secondary AChR abundance and resulting impaired postsynaptic morphologic development. None of the known rapsyn mutations hinders rapsyn self-association, but all diminish clustering of AChR with this protein. In addition to weakness, some patients manifest striking facial malformations. Patients are treated with anti-AChE drugs and with 3,4-diaminopyridine (3,4-DAP), a blocker of open K^+ channels. 3,4-DAP decreases presynaptic membrane resting conductance, causing hyperpolarization and therefore resetting more Ca^{2+} channels into their closed, non-inactivated state from which they can readily open, increasing Ca^{2+} influx into the terminal and facilitating a compensatory increase in ACh release.

Slow channel syndrome

Abnormally long-lived openings of mutant AChR channels result in prolonged endplate currents and potentials, which in turn elicit one or more repetitive muscle action potentials of lower amplitude that decrement. The morphologic consequences stem from prolonged activation of the AChR channel that causes cationic overload of the postsynaptic region—the endplate myopathy—with Ca^{2+} accumulation, destruction of the junctional folds, nuclear apoptosis, and vacuolar degeneration of the terminal. Some slow channel mutations in the transmembrane domain of the AChR render the channel leaky by stabilization of the open state, which is populated even in the absence of ACh. Curiously, some slow channel mutants can be opened by choline even at the concentrations that are normally present in serum. Quinidine, an open-channel blocker of the AchR, is used for therapy.

Fast channel syndrome

The clinical features resemble those of autoimmune myasthenia gravis (see below) with variable severity. Conversely to what is found in slow channel syndrome, the open state of the AChR is destabilized, manifesting as fast dissociation of ACh from the receptor and/or excessively reduced open times. One mutation has also caused multiple congenital joint contractures owing to fetal hypomotility in utero. In most cases, the mutant allele causing the kinetic abnormality is accompanied by a null mutation in the second allele, so that the kinetic mutation dominates the phenotype, but homozygous fast-channel mutations also exist. Therapy includes anti-AChE agents and 3,4-DAP.

Acetylcholinesterase deficiency

Inhibition of the AChE results in prolonged exposure of AChR to ACh, leading to prolonged endplate potentials, desensitization of AChR, and depolarization block. Endplate myopathy with loss of AChR may result. In most patients, the disease presents in the neonatal period and is highly disabling, but exceptions with mild neonatal onset and severe adult impairment exist. Some patients manifest an excessively slow pupillary response to light. An array of mutations may cause (a) reduced attachment of AChE to ColQ; (b) truncation of the collagen domain, rendering it insertion incompetent; or (c) hindrance of the triple-helical assembly of the collagen domain.

HEREDITARY DISEASES OF MUSCLE MEMBRANES

Mutations of the sodium channel cause hyperkalemic periodic paralysis and paramyotonia congenital

An important group of inherited muscle diseases called the periodic paralyses is characterized by intermittent episodes of skeletal muscle weakness or paralysis that occur in individuals who otherwise appear normal or are just mildly weak between attacks (Cannon, 2006). The periods of paralysis are often associated with changes in serum K^+ concentration; while the serum K^+ concentration may increase or diminish, the direction of change is usually consistent for a particular family and forms one basis for classifying these diseases as either hyperkalemic or hypokalemic. A variant of periodic paralysis, in which spells of weakness are less frequent and in which a form of muscle hyperexcitability is often seen, is called paramyotonia congenita. Recordings from muscle fibers isolated from patients during an attack of periodic paralysis have shown that the paralytic episodes are associated with acute depolarization of the sarcolemma. In all forms of periodic paralysis, this depolarization is due to an increase in membrane conductance to Na^+. In the case of hyperkalemic periodic paralysis and paramyotonia congenita, this abnormal conductance can be blocked by tetrodotoxin, a small polar molecule that is highly specific for the voltage-dependent Na^+ channel. Foods with a high K^+ content may trigger an attack, while carbohydrate-rich substances are abortive of the paralytic episode. Single-ion channel recordings in hyperkalemic periodic paralysis have revealed that some of the muscle membrane Na^+ channels show abnormal inactivation kinetics, intermittently entering a mode in which they fail to inactivate. These channels produce a persistent, noninactivating Na^+ current that in turn produces membrane depolarization. Because normal Na^+ channels enter an inactivated state after depolarization (see Ch. 4), the net result of long-term depolarization is loss of sarcolemmal excitability and paralysis. Both hyperkalemic periodic paralysis and paramyotonia are caused by mutation of the adult skeletal muscle Na^+ channel SkM1 gene SCN4A on chromosome 17. The hypokalemic form of periodic paralysis, however, is not linked to this Na^+ channel gene (see below). Numerous mutations alter the coding region of the SCN4A gene in families with hyperkalemic periodic paralysis or paramyotonia congenita. Although these mutations are distributed through a wide span of the channel coding region, a number of them are clustered in a cytoplasmic linker region known from biophysical studies to control inactivation. Others are located near the cytoplasmic ends of transmembrane domains S5 and S6, and these residues may in fact constitute the binding site for the closing inactivation gate. Mutations in these regions may destabilize this closed conformation, leading to abnormalities in channel inactivation. Mutants associated with the paramyotonia congenita phenotype show, on the other hand, a marked slowing in the major component of fast inactivation. In some cases, the voltage dependence of the inactivation rate constant, known as τ_h, is markedly reduced as well, and the mutations appear to uncouple the inactivation process from the voltage-dependent

channel conformational changes associated with inactivation. In some families with hyperkalemic periodic paralysis, the mutations cause a small, persistent inward Na^+ current in the myocyte that is the result of a shift in channel modal gating. Normal skeletal muscle Na^+ channels can shift between a fast and a slow inactivation gating mode and, usually, the channels are found in the fast inactivation mode. Channels with hyperkalemic periodic paralysis mutations, however, spend a greater percentage of the time in the slow inactivation gating mode, and late openings associated with this slow gating mode contribute to the persistent inward current seen in cells harboring these mutations. Under voltage-clamp conditions at the single-channel level, SkM1 channels with paramyotonia congenita mutations show multiple late openings and prolonged openings after depolarization. These late openings account for the slow inactivation of the Na^+ current observable in the cell. Hyperkalemic periodic paralysis mutations also show multiple late openings at the single-channel level, but these abnormal events are temporally clustered, consistent with an underlying shift in modal gating. Single-channel conductance is not altered by any of these mutations, but all sodium ion channel mutations in periodic paralysis produce dominant-negative effects. Although some mutant channels only intermittently exhibit abnormal inactivation, this small population of abnormally inactivating channels can modify the behavior of the remaining mutant and normal channels present in the membrane. Unlike the ClC-1 chloride channel mutations in myotonia congenita (see below), which produce dominant-negative effects within a single-channel multimer, these Na^+ channel mutations produce dominant-negative effects that reflect the relationship of normal channel inactivation to membrane potential. In either case, the persistent inward current carried by a small population of noninactivating channels, or the prolonged inward current resulting from mutant channels with slowed inactivation rates, results in a slight but long-lasting membrane depolarization. Since the relationship between voltage and inactivation in normal channels is very steep near the resting potential, this slight depolarization can produce inactivation of normal channels. If depolarization is sufficient, too few channels will remain in the noninactivated state to satisfy the requirements for a regenerative action potential and the muscle will become paralyzed.

Hypokalemic periodic paralysis is due to calcium channel mutations

Hypokalemic periodic paralysis, in which serum potassium drops during a paralytic episode, is the most common of the inherited periodic paralyses. Although paralysis is associated with membrane depolarization and increased resting Na^+ conductance, this conductance is not sensitive to tetrodotoxin. This disease is linked to a region of chromosome 1 that encodes the $\alpha 1$ subunit of the skeletal muscle, dihyropyridine-sensitive Ca^{2+} channel, designated CACNL1A3 (Ptacek et al., 1994). Although this channel in skeletal muscle contains at least five subunits, the channel-forming ⟨ subunit has a structure that bears strong sequence homology to the voltage-gated Na^+ channel. Several mutations have been identified in the Ca^{2+} channel gene in families with hypokalemic periodic paralysis and some of these are in locations where mutations in the homologous Na^+ channel also occur. The pathophysiological mechanism linking these L-type muscle Ca^{2+} channel mutations to the depolarization seen during episodes of weakness in hypokalemic periodic paralysis is less clear. This dihydropyridine-sensitive Ca^{2+} channel is present in the T-tubular membrane at the triadic junction with the terminal elements of the SR. There, it is thought to provide the coupling between T-tubular depolarization and activation of the SR ryanodine-sensitive Ca^{2+} release channel with which it interacts, instead of conducting ionic currents. Perhaps another uncharacterized form of the sarcolemmal Ca^{2+} channel, in which CACNL1A3 is complexed with different accessory subunits, is responsible for the abnormal Na^+ currents seen in this disorder.

Abnormal potassium channels in Andersen syndrome cause more than periodic paralysis

Andersen syndrome includes periodic paralysis, prolongation of the electrocardiographic QT interval causing susceptibility to cardiac ventricular arrhythmias, and characteristic physical features including low-set ears, a small jaw, and malformation of the digits; it is inherited in an autosomal-dominant fashion. The disease is unique in that it involves the combination of both a skeletal and a cardiac muscle phenotype and may be caused by mutations in KCNJ2, a gene that encodes the inward rectifier K^+ channel Kir2.1, which is expressed in both cardiac and skeletal muscles. Kir2.1 is an important contributor to the cardiac inward rectifier K^+ current, I_{K1}, which provides substantial current during the repolarization phase of the cardiac action potential (Plaster et al., 2001). All KCNJ2 mutations cause a dominant negative effect on channel function, and a reduction in I_{K1} prolongs the terminal repolarization phase, rendering the myocardium prone to repetitive ectopic action potentials. Attacks of paralysis can be associated with hypo-, hyper- or normokalemia and, although serum potassium levels during attacks differed among kindreds, they are consistent within an individual kindred. Mutations in Kir2.1 may sufficiently reduce the muscle resting K^+ conductance such that the membrane depolarizes, leading to inactivation of Na^+ channels making them unavailable for initiation and propagation of action potentials.

Ribonuclear inclusions are responsible for the multiple manifestations of myotonic dystrophy

Myotonic dystrophy (DM; Steinert disease) is a multisystemic disorder that is now recognized as one of the most common forms of muscular dystrophy in adults. In addition to hereditary muscular dystrophy and myotonia (involuntary persistence of muscle contraction), DM causes a constellation of seemingly unrelated clinical features including cardiac conduction defects, cataracts, and endocrine and immunological abnormalities. Two clinical and genetic types of DM exist (Lee & Cooper, 2009). The genetic features of DM type 1 (DM1) include variable penetrance, anticipation (a tendency for the disease to worsen in subsequent generations), and a maternal transmission bias for congenital forms despite the location of the causative gene on chromosome 19. The cause of DM1 is a

$(CTG)_n$ repeat in the 3-UTR of a protein kinase (DMPK) gene. Type 2 DM, in contrast, predominantly causes pelvic girdle weakness, and is often referred to as proximal myotonic myopathy, ascribed to the genetic locus encoding zinc finger protein 9 on chromosome 3. DM2 is caused by a $(CCTG)_n$ expansion. Nevertheless, all individuals affected by DM1 and DM2 experience weakness, pain and myotonia, and cardiac involvement may lead to conduction defects, arrhythmias and sudden death. Endocrine abnormalities in both DM1 and DM2 result in hyperinsulinemia, hyperglycemia and insulin insensitivity, with type 2 diabetes occurring in each disorder. Testicular failure is also common, with associated hypotestosteronism, elevated follicle-stimulating hormone (FSH) levels and oligospermia. Other serological abnormalities in both disorders include reduced levels of immunoglobulins G and M. The brain is also affected as assessed by magnetic resonance imaging (MRI), but mental retardation is only a feature of DM1.

The unusual multisystemic clinical parallels between DM1 and DM2 suggest a similar pathogenic mechanism. The discovery that DM2 mapped to chromosome 3 and not to the DM1 region of chromosome 19 makes it unlikely that specific gene expression defects cause the common clinical features of the disease. The discovery that a CCTG repeat expansion located on chromosome 3 that is expressed at the RNA (but not at the protein level) causes DM2, and the observation that both CUG and CCUG repeat-containing foci accumulate in affected muscle nuclei, suggests that a gain-of-function RNA mechanism underlies the clinical features common to both diseases. This is, in fact, but one of an emerging class of disorders. RNA-binding proteins, including CUG-binding protein (CUG-BP) and *muscleblind* isoforms bind to—being sequestered—or are dysregulated by the repeat-containing RNA transcripts, resulting in specific trans-alterations in pre-mRNA splicing. Specific changes in pre-mRNA splicing have been associated with several genes, including the insulin receptor, the chloride channel ClC-1, and cardiac troponin T, and are probably correlated with insulin resistance, myotonia and cardiac abnormalities. For example, CUG-BP, which is elevated in DM1 skeletal muscle, binds to the ClC-1 pre-mRNA, causing an aberrant pattern of ClC-1 splicing. Thus, altered splicing regulation of ClC-1 decreases its abundance in the muscle plasma membrane causing hyperexcitability and leading to the DM feature of myotonia. Further, mutant RNA transcripts bind and sequester transcription factors leading to the depletion of as high as 90% for some factors, resulting in secondary depletion of proteins such as the chloride channel ClC-1.

Congenital myotonia is caused by mutant Cl^- channels

In two diseases, dominant myotonia congenita (Thomsen disease) and recessive myotonia congenita (Becker myotonia), myotonia is the major presenting symptom and often the only abnormality, although the muscles may be overdeveloped (conferring individuals a herculean appearance) in Thomsen disease. Patients afflicted with these diseases have difficulty relaxing their muscles normally: doorknobs and handshakes are difficult to release, clumsiness is a problem and falls often occur. In both Thomsen and Becker myotonia, multiple mutations have been found in chromosome 7 and the sarcolemma exhibits a severe reduction in membrane Cl^- conductance (Steinmeyer et al., 1991). This locus encodes the ClC-1 skeletal muscle Cl^- channel family, whose members control anion flux in a number of tissues and are closely related in structure, forming dimers of two ClC subunits. The fact that mutations in the gene encoding ClC-1 can produce either dominant or recessive effects is surprising. Mutants that introduce frameshifts of stop codons early in the coding sequence produce a nonfunctional protein product. With one defective gene copy, wild-type channels encoded by the second allele should produce a net Cl^- conductance about 50% of normal. When both gene copies carry the mutation, expression of the functional channels will be very low or absent and, as a recessive disorder, the myotonia can be severe. Point mutations can also lead to the alteration of a single amino acid in the primary structure of an otherwise full-length channel monomer, and channels formed from this protein may not function normally. The possibility also exists that channels containing even a single mutant subunit may fail to function even though the other subunits are encoded by a normal copy of the gene. Such a dominant-negative effect, which has been demonstrated for a number of myotonia congenita mutations, leads to a dominant transmission of the disease phenotype. Since mixed channels containing different numbers of mutant subunits may have different levels of residual activity, the resulting membrane Cl^- conductance may be more variable and the disease phenotype less severe than in the recessive form of the disease.

Malignant hyperthermia caused by mutant ryanodine receptor calcium release channels

A rare complication of general inhalation anesthesia is a syndrome characterized by muscle stiffness and fever due to a state of elevated skeletal muscle metabolism that occurs in genetically predisposed individuals. If untreated, this syndrome, called malignant hyperthermia (MH), can be rapidly fatal. Although the inheritance pattern of the disease is difficult to trace, it is likely to be transmitted from generation to generation as an autosomal dominant trait. A similar disease occurs in a strain of pigs, and this experimental animal model has proven useful in studying the physiology of the disease. Measurements on isolated muscle from affected pigs or individuals show that the defect is at the level of excitation–contraction coupling. Specifically, the muscles release Ca^{2+} when exposed to caffeine at concentrations much lower than those required for Ca^{2+} release from normal muscle. Once released, this Ca^{2+} produces persistent activation of tropomyosin and sustained contraction, which associates with hypermetabolism. Analysis of the ryanodine receptor (RyR) gene sequence in affected individuals has uncovered several mutations in the coding region of the protein that cosegregates with the MH phenotype (Durham et al., 2007). All of the RyR mutations result in amino acid substitutions in the myoplasmic portion of the protein, with the exception of the mutation in the C-terminus, which resides in the luminal/transmembrane region. Functional analysis shows that MH and central core disease—a related myopathy—mutations produce RyR abnormalities that alter the channel

kinetics for calcium inactivation and make the channel hyper- and hyposensitive to activating and inactivating ligands, respectively. The deciding factors in determining whether a particular RyR mutation results in MH alone or MH and central core disease are the sensitivity of the RyR mutant proteins to agonists, the degree of abnormal channel-gating caused by the mutation, the extent of decrease in the size of the releasable calcium store and increase in resting concentration of calcium, and the level of compensatory calcium homeostasis achieved by the muscle.

Calcium channel mutations may also cause malignant hyperthermia

At least five loci other than the RyR have been associated with MH, indicating great genetic heterogeneity. One such gene, CACNA1, encoding the main subunit of the voltage-gated dihydropyridine receptor, has a confirmed role in MH, so that this type of MH is allelic to hypokalemic periodic paralysis. In contrast to the mutations specific for hypokalemic periodic paralysis, the mutations so far described for MH are situated in the myoplasmic loop connecting repeats III and IV, the function of which is unknown but which may serve as a link to RyR.

Brody disease is an unusual disorder of the sarcoplasmic reticulum calcium ATPase

Brody disease, a dominant or recessive disorder, is characterized by exercise-induced delay in skeletal muscle relaxation, which is most pronounced in the legs, arms and eyelids. The muscles stiffen during sustained strenuous muscular activities, leading to painless or only mildly painful cramping. Early studies provided evidence for reduced ATP-dependent Ca^{2+} uptake and Ca^{2+}-ATPase activities in the sarcoplasmic reticulum (SERCA) of Brody disease patients. Several mutations in ATP2A1, the gene encoding SERCA1, have been found in families afflicted by the recessive form of the disease, and all have been predicted to truncate SERCA1 (Hovnanian, 2007). These mutant enzymes would be inactive and degraded in situ. The disease may also cosegregate in an autosomal-dominant fashion with an apparently balanced constitutional chromosome translocation (2;7)(p11.2;p12.1), providing evidence for another causative locus.

IMMUNE DISEASES OF MUSCLE EXCITABILITY

Myasthenia gravis is caused by antibodies that promote premature AChR degradation

Myasthenia gravis (MG), a frequent disorder of neuromuscular transmission, is an acquired autoimmune disease affecting the AChR on the postsynaptic membrane. Clinically, the disorder is characterized by weakness and abnormal fatigability. Most patients have circulating antibodies against the AChR in their serum and many of them are directed against the main immunogenic region of the receptor, located extracellularly (Farrugia & Vincent, 2010). Patients with MG typically show fluctuating symptoms; weakness and fatigability may be worse in the evenings and usually become more severe with exercise. Weakness may involve only the extraocular muscles, producing diplopia (double vision), or may be so extensive as to cause quadriparesis (weakness of all limbs) and respiratory compromise. Although spontaneous remissions can occur, the untreated disease is often progressive and can eventually lead to death from respiratory failure. The classic electrophysiological observation in MG is a decremental response in the extracellularly recorded compound muscle action potential with repeated nerve stimulation (Fig. 44-7). The decrease in action potential amplitude is due to a reduction in the number of AChR molecules present in the postsynaptic membrane combined with a pathological alteration in the architecture of the postsynaptic membrane. The density of receptors in the myasthenic postsynaptic membrane may be as low as 20% of normal. In addition, the architecture of the postsynaptic membrane is lost. The distance between pre- and postsynaptic membrane is often increased, and the postsynaptic membrane is highly simplified. Antibodies directed against the AChR mediate the destruction seen at the postsynaptic membrane. The major effects of anti-AChR antibodies seem to be twofold. First, antibodies cross-link receptor proteins and increase their rates of endocytosis and lysosomal degradation. In the absence of an increased rate of receptor synthesis, this results in a net decrease in receptor density in the postsynaptic membrane. Second, these antibodies target the postsynaptic membrane for complement fixation and activation of the lytic phase of the complement reaction cascade. Although the mechanism of chronic neuromuscular damage in this disease has been defined, the nature of the initial triggering event is unclear. One hypothesis involves the role of muscle-like cells that exist in the thymus gland. These cells express AChRs on their surface membranes. An initial inflammatory response in the thymus may trigger the generation of cross-reacting antibodies that subsequently target AChRs on muscle. This hypothesis would help to explain the beneficial role of the removal of the thymus (thymectomy) as well as that of immunosuppressive steroids in patients with MG. AChE inhibitors and removal of antibodies by plasma filtration are also of therapeutic value. Careful reduction of endplate AChE activity by administration of inhibitors can prolong the action of released ACh sufficiently to increase the amplitude of an abnormally low endplate potential above that required for successful neuromuscular transmission. Excessive inhibition, however, produces long-term postsynaptic depolarization, receptor inactivation and transmission block.

Antibodies against MuSK mimic myasthenia gravis

Another salient antigenic target of the NMJ is the muscle-specific receptor kinase MuSK (see Box). During development, MuSK participates in the orchestration of agrin-dependent clustering of AChR and of other components that make up the postsynaptic architecture of the NMJ. It is unclear how the reaction of this cytoplasmic target with antibodies causes the symptoms of myasthenia in adults. One possibility is that these antibodies bind complement and cause

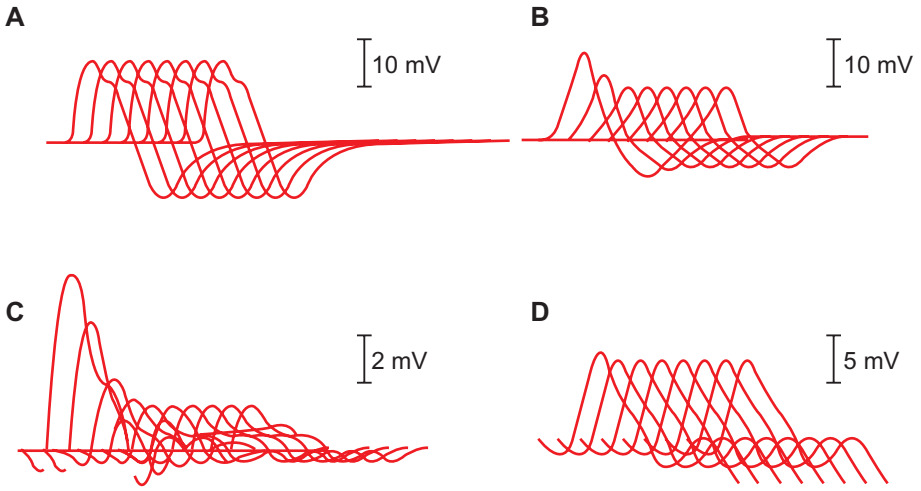

FIGURE 44-7 **Block of the acetylcholine receptor in myasthenia gravis**. Compound muscle action potentials recorded from the abductor digiti minimi of human subjects during repetitive stimulation of the ulnar nerve at three stimuli per second. **(A)** A recording from a normal individual shows no change in the amplitude of the muscle electrical response during the stimulation interval. **(B)** In a patient with myasthenia gravis, the same stimuli produce a 40% decrement in response amplitude over the first five stimuli, with a slow partial recovery during subsequent stimulation. Similar studies from a patient with severe myasthenia gravis before **(C)** and 2 minutes after **(D)** intravenous administration of 10 mg edrophonium, a short-acting acetylcholinesterase inhibitor. Note the change in amplitude scale between the two recordings and the reversal of the abnormal decrement by edrophonium.

loss of AChR-containing postsynaptic membrane, similar to the action of AChR antibodies. Another possible explanation is that the antibodies do penetrate the postsynaptic cytoplasm and alter the function of MuSK at the NMJ. Exposure of myocytes to plasma containing MuSK antibodies can inhibit agrin-dependent AChR clustering substantially and, in the absence of added agrin, they can cause a modest clustering of AChR, suggesting that they are able to cross-link and activate MuSK on the cell surface. Therefore, it is possible that the antibodies lead to reduced clustering of AChRs and reduced stability of the adult postsynaptic membrane architecture (Farrugia & Vincent, 2010).

Antibodies cause calcium channel dysfunction in Lambert-Eaton syndrome

Some patients with cancer, especially small-cell carcinoma of the lung, develop a syndrome of weakness associated with autonomic dysfunction as part of the Lambert-Eaton myasthenic syndrome (LEMS). Complaints often begin with progressive proximal muscle weakness and fatigue; unlike myasthenia gravis, bulbar involvement is usually mild and respiratory compromise is unusual.

These patients demonstrate a remarkable reduction in the amplitude of the compound muscle action potential produced by single supramaximal stimulus to the motor nerve of a resting muscle. Repeated stimulation of the same nerve, however, results in progressive improvement in response amplitude, often returning to nearly normal levels. These clinical findings are indicative of a defect in presynaptic neurotransmitter release. When analyzed at the cellular level, LEMS is characterized by a reduction of ACh release, often to 10% or less of normal values. Repetitive stimulation causes a progressive increase in ACh release, consistent with the improvement seen in the compound muscle action potential. LEMS, like MG, is an autoimmune disease. The autoantibodies in LEMS are heterogeneous and target a number of presynaptic proteins. Antibodies are most commonly observed against P/Q-type voltage-gated Ca^{2+} channels, but antibodies against L- and N-type channels are also present (Farrugia & Vincent, 2010). Antibodies against the synaptic vesicle protein synaptotagmin and the small cell cancer antigen SOX1 have also been identified (Sabater et al., 2008). The S5-6 loop in the channel α subunit is a particularly active target for LEMS autoantibodies. The large intramembranous particles that form the synaptic active zones are disrupted in the disease. The number of these arrays is reduced, with many particles found instead in irregular aggregates. In small-cell lung carcinomatous cells, voltage-gated Ca^{2+} channels are expressed on their surface, and Ca^{2+} currents in these cells are inhibited by LEMS serum. It is possible that an immune response against the voltage-gated Ca^{2+} channel population in these tumor cells causes additional pathology in LEMS by cross-reaction with similar epitopes present in the presynaptic channels of autonomic and NMJ nerve terminals.

Potassium channel antibodies in Isaac syndrome cause neuromyotonia

Dysfunction of voltage-gated K^+ channels is a potential cause of acquired neuronal hyperexcitability disorders, as patients with different point mutations in the potassium channel Kv1.1 can manifest varying combinations of episodic ataxia, neuromyotonia and seizures. Antibodies directed against presynaptic voltage-gated K^+ channels have been found in acquired neuromyotonia, also called Isaac disease, a disorder associated with peripheral nerve hyperactivity that results in muscle twitching (myokymia) and cramps, increased sweating and other

autonomic nervous system abnormalities (Sabater et al., 2008). Approximately 20% of patients with neuromyotonia have an associated thymoma and, occasionally, patients may also experience central nervous system disturbances including insomnia, hallucinations and confusion. Application of the antibodies to the NMJ mimics the electrophysiological effect of 3,4-DAP exposure, with enhanced excitation of muscle owing to pronounced ACh release from hyperpolarized nerve terminals.

TOXINS AND METABOLITES THAT ALTER MUSCULAR EXCITATION

Bacterial botulinum toxin blocks presynaptic ACh release

Botulism is caused by intoxication with an exotoxin with metalloprotease activity synthesized and excreted by bacterial strains from the genus *Clostridium (botulinum, barati and butirycum)*. The effect of botulinum toxin on the neuromuscular junction is one of the most potent processes known in biology and recovery from intoxication may persist for months. A dose of 1 ng toxin/Kg of body weight is lethal to mice. In infancy, the toxin gains access to the neuromuscular junction after bacterial colonization of the gut by *C. botulinum*, whereas foodborne toxin ingestion and wound contamination give rise to more rapid forms of the intoxication. In all its forms, botulism causes a severe and persistent blockade of endplate potentials, rendering the postsynaptic membrane unexcitable after depolarization of the nerve terminal. The neurotoxin greatly reduces the frequency, but not the amplitude, of evoked miniature endplate potentials, indicating that the number of vesicles capable of undergoing fusion and release of ACh is diminished, without affecting the ACh quantum, which indicates an unaltered neurotransmitter synthesis, uptake and storage. In fact, the only structural abnormality of the synapse is an increase in the number of synaptic vesicles near the presynaptic membrane. A structurally related toxin synthesized by *C. tetani* produces tetanus, a process that causes rigid paralysis—often resulting in death from diaphragmatic spasm—due to retrograde transport of this toxin beyond the neuromuscular junction and transcytosis into inhibitory spinal cord interneurons. Another member of this infamous genus, *C. perfringens*, is associated with gas gangrene, an often-lethal complication of wound infection in the pre-antibiotic era.

Seven serotypes (A–G) of botulinum toxin exist. All share a degree of structural homology—different amino acid sequences can be part of each serotype—but differ in potency and duration of action, a phenomenon that has allowed the beneficial use of type A toxin in conditions associated with persistent muscular contraction like dystonia or spasticity and with excessive autonomic cholinergic activity. At high doses, however, some of the specificity of action is lost and type A toxin may be transported retrogradely, acting on the central nervous system. Botulinum toxins, synthesized as single polypeptides of ~150 kDa (comprising 1,295 amino acids in the case of type A toxin), are encoded in the genome of a bacteriophage virus that infects the bacterium lysogenically (Montecucco et al., 2009). Prior to acquiring its active conformation, the peptide is cleaved into a light (LC) and a heavy (HC) chain that weigh about 50 and 100 kDa, respectively, and remain linked via a disulfide bond. The LC domain is a potent zinc endopeptidase designed against specific elements inside the presynaptic nerve terminal, while the HC region contains regions for binding of the toxin to the membrane of the cholinergic motor neuron and for translocation of the LC domain into the terminal. The molecular targets of LC are several synaptic members of the SNARE (soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) attachment protein receptor) family (see Chs. 7 and 12), the facilitators of all membrane fusion events in eukaryotic cells.

Upon exposure of the neuromuscular junction to toxin and binding to the neural membrane, a chain of events comprising internalization into synaptic vesicles, translocation into the cytoplasm and inhibition of acetylcholine release unfolds inexorably. Once internalized, the toxin cannot be neutralized by antiserum. The first step of intoxication—involving contact between the carbohydrate-binding, lectin-like HC domain and the terminal membrane—is highly specific. It requires the presence of gangliosides, in particular GD1b, GT1b and GQ1b, together with a protein receptor—possibly synaptotagmin (see Chs. 7 and 12)—all embedded and crowded in lipid rafts that help anchor HC with high affinity. Thus, multivalent binding of HC is accomplished by several near-simultaneous molecular recognition events in a restricted two-dimensional plane, taking place between multiple carbohydrate-binding sites on HC and selected sialic acid–containing gangliosides and glycoproteins. Consequently, the toxin coats the terminal in a punctate distribution and different toxin types bind independently of each other. The presence of the glycosylated portion of synaptotagmin I and II in the synaptic space, however, is contingent upon the fusion of synaptic vesicles with the plasma membrane during neurotransmission and exposure of the luminal aspect of the vesicle to the extracellular medium. Upon binding, the toxins are internalized via endocytosis and reach vesicular or endosomal compartments that undergo acidification as part of their normal recycling process. Targeting to the appropriate compartment is attributed to the protein receptor present in the endocytic vesicle. The reduction in pH is believed to facilitate a conformational change in the toxin, increasing the exposure of hydrophobic residues on its surface and facilitating its penetration through the membrane to reach the cytosol. Two histidine residues (H551 and H560) lie on a suitably exposed location on botulinum toxin type A, the "translocation belt" portion of HC, and may therefore be susceptible to titration by pH. The HC domain undergoes conformational changes at low pH *in vitro* and forms somewhat cation-selective channels in artificial membranes of ~15 Å in diameter as judged from their unitary conductance of 110 pS. It is thought that LC also experiences a structural change inside the vesicle or the endosome that allows it to translocate via—or next to—the small transmembrane HC channel in an unfolded conformation. This is the least-understood event of the intoxication process, as it is very difficult to envision the ~ 55 Å × 55 Å × 62 Å folded, intact LC domain cross the membrane. Nevertheless, protein-translocating channels are part of the repertoire used by other toxins such as diphtheria and cholera and by the mitochondrial protein import system, the latter with a pore diameter of 13 Å. After translocation and

refolding of LC, the toxin is preactivated by proteolysis of a surface-exposed loop accomplishable by a variety of cellular proteases that separate the peptidic continuity after the first 419 to 449 residues of LC, giving rise to the LC and HC chains proper, and which remain bound by the disulfide bond between C430 and C454 and by noncovalent interactions. Disulfide reduction then releases active LC into the cytosol. The ultimate localization of LC depends on toxin type, with, for example, type A residing near the plasma membrane and type E remaining in the cytosol, a phenomenon that may explain the differential stability of the toxin isoforms and the persistence of their action. Nevertheless, the LC fragment is always effectively sequestered from the degradative cycle of the terminal. Tyrosines in both LC and HC may be phosphorylated by Src (see Ch. 26), enhancing the stability and the proteolytic activity of LC. The targets of LC proteolysis are SNARE proteins including SNAP25, synaptobrevin and syntaxin, all of which share repeats of a motif that is recognized by LC. The SNARE proteins exist in a complex stabilized by a bundle of tightly packed α-helices, each containing the SNARE motif (distinct from the recognition motif above), which complex is disassembled following transmitter release. Binding of LC occurs only after individual SNARE members dissociate and results in their proteolysis at the level of the SNARE motif, preventing reassembly and thus causing impairment of synaptic vesicular release. Residues H222, E223 and H226 form part of the LC active site and are typical of zinc proteases (which display the sequence HEXXH), with H222 and H226 and E261 coordinating zinc and E223 coordinating a water molecule. Additionally, after cleavage, the complex LC-SNAP25 may not dissociate, reentering the otherwise intact SNARE complex and continuing to interfere with neurotransmission.

The long-term effects of botulism are reversible, as both nerve and muscle remain alive. The muscle undergoes a transient atrophy with loss of acetylcholinesterase and dispersion of ACh receptors from the endplate over the course of several weeks. During recovery, the release of trophic factors from paralyzed muscle fibers causes enlargement of the endplate, and nerve sprouts develop. The number of endplates per muscle fiber also increases and some muscle fibers may be innervated by more than one motor axon. After axonal sprouting and reformation of functional neuromuscular junctions, the muscle eventually regains its normal size and then redundant sprouts degenerate. The muscle atrophy induced by botulism in animals and man is therefore largely reversible.

Snake, scorpion, spider, fish and snail peptide venoms act on multiple molecular targets at the neuromuscular junction

β-neurotoxins from the venoms of snakes and scorpions are endowed with phospholipase A2 (PLA2) activity. PLA2 subtypes are essential in phospholipid turnover and in the production of inflammatory mediators (see Chs. 5 and 36). PLA2-directed toxic potency measured as mouse LD50 ranges from 1 mg/Kg for taipoxin (present in the venom of the Australian snake *Oxyuranus scutellatus*) to 750 mg/Kg for pseudexin B (produced by *Pseudechis porphyriacus*), although this wide difference is partly due to toxin pharmacokinetics. There are over 50 neuromuscular toxins with PLA2 activity and although their effects differ to some extent, all tend to cause death from respiratory failure due to paralysis of respiratory muscles. This final event may be preceded by hyperexcitability (as in the intoxication with β-bungarotoxins) or by flaccid paralysis of the limbs (after intoxication with crotoxin) that evolves over at least one hour, regardless of toxin dose. Muscle use accelerates paralysis and, in the end, the synaptic membrane appears to have experienced massive fusion of ACh vesicles with inhibition of their recycling. ACh release from the nerve terminal, however, undergoes three phases: (a) a short initial phase displaying either decreased or unchanged ACh release, (b) a longer phase (10–30 min) of stimulation of evoked release, and (c) complete and irreversible inhibition (30–120 min). In addition, some toxins with PLA2 activity also inhibit voltage-gated K^+ channels at the synaptic terminal. In the late stage of intoxication, severe alterations of plasma and of synaptic organelle—including mitochondrial—membrane permeability become apparent.

Four types of PLA2 toxin structure exist. Class I is comprised of single-chain toxins of ~14 kDa containing seven disulfide bridges. This class includes agkistrodotoxin from *Agkistrodon* snakes, ammodytoxin from *Vipera ammodytes ammodytes*, caudotoxin from *Bitis caudalis*, notexin *from Notechis scutatus scutatus*, OS toxin from *Oxyuranus scutellatus scutellatus* and pseudexin from *Pseudechis porphyriacus*. Class II includes toxins with two noncovalently linked homologous subunits, such as crotoxin and related rattlesnake neurotoxins produced by the genus *Crotalus*. Class III includes heterodimers composed of unrelated subunits, such as the most studied of PLA2 toxins, β-bungarotoxin. This toxin is manufactured by *Bungarus multicinctus* (together with the ACh receptor-specific α-bungarotoxin). β-bungarotoxin is similar to dendrotoxin, a neurotoxin that binds to the voltage-gated potassium channels of presynaptic membranes. Class IV toxins comprise noncovalently associated oligomers of homologous subunits and include taipoxin and paradoxin, both of which are heterotrimers. Textilotoxin, produced by *Pseudonaja textilis textilis,* is the most complex of these neurotoxins and includes five homologous subunits, all of them endowed with PLA2 activity.

Binding of PLA2 toxin to the nerve membrane, although selective, is less well understood than that of clostridial toxins. A limited number of high-affinity binding sites exist along with unsaturable low-affinity binding sites. While lipids of the presynaptic membrane may be involved in low-affinity interactions, proteins or glycoproteins are thought to constitute the high-affinity sites. For example, taipoxin may bind the neuronal pentraxin, facilitating its access to the endoplasmic reticulum, where the toxin would be retained via its binding to other specific proteins. But even less well understood is how phospholipid hydrolysis in the endoplasmic reticulum (located in the cellular body) would lead to inhibition of ACh release at the nerve terminal. The structure of the simpler, class I-toxin notexin, however, sheds some light into the PLA2 catalytic mechanism. The toxin is folded into three α-helices with two β-strands and it is both stabilized and activated by Ca^{2+}. The divalent metal binding site consists of the consensus sequence $YGC^{Y}/_{F}CXGG$ and may be occupied, instead of Ca^{2+}, by Sr^{2+}, Ba^{2+} and Zn^{2+}, all of which inhibit PLA2 activity potently. The toxin also contains a hydrophobic

channel that accommodates and secures the fatty acid chains of the phospholipid molecule and places the ester bond to be cleaved into the active site. The activity of the toxin is much higher against membrane-inserted phospholipids than against isolated ones due to the higher efficiency of interfacial catalysis, which depends on the absorption of the enzyme onto the lipid-water interface, facilitating the seamless diffusion of the phospholipid molecule from the membrane to the active site channel.

Elapidae and Hydrophiidae snake α-neurotoxins, in contradistinction, block postsynaptic AChR, inhibiting neuromuscular transmission in a manner analogous to (+)-tubocurare (see Ch. 11). Some 100 α-neurotoxins have been isolated, the most used being α-bungarotoxin, synthesized by the Asian krait *Bungarus multicinctus*. The LD50 for these toxins is ~ 100μg/Kg in mice. Exposure of humans to low doses of toxin that induce partial blockade of junctional receptors can produce weakness and fatigability that resemble acquired myasthenia gravis. Higher doses can lead to complete neuromuscular block, paralysis, respiratory failure and death. The α-toxins responsible for the postsynaptic curarimimetic activity are proteins of 7 to 8 kDa. Structurally, they fall into two groups: long toxins, which are comprised of 71 to 74 amino acids and five internal disulfide bonds, and short toxins, with 60 to 62 amino acids and four internal disulfide bonds. All of the α-toxins have about the same equilibrium dissociation constant for the AChR, but they differ markedly in their binding kinetics. The short toxins bind to and dissociate from the AChR five to nine times faster than the long toxins, explaining the near irreversibility of long toxin binding. The stability of the binding of the long α-neurotoxins to the AChR, especially that of α-bungarotoxin, has made them valuable tools for the purification and characterization of the receptor. α-bungarotoxin binds to a site on each α subunit of the AChR that sterically overlaps and interferes with ACh binding at the interfaces between the two α and the γ and δ subunits. All α-toxins exhibit a high degree of homology and are shaped as concave disks with a small projection at one end. Their elliptical dimensions are approximately 3.8 nm × 2.8 nm × 1.5 nm, and for the most part, the structure is only a single polypeptide chain thick. The reactive site of the protein is on the concave surface and involves the regions encompassed by residues 32 to 45 and 49 to 56 as well as isolated residues from other regions of the molecule.

In contrast with toxins that block neuromuscular transmission, latrotoxins (LTX) cause excessive ACh release and excitation. These toxins are produced by black or brown widow spiders from the genus *Latrodectus* and are directed predominantly against vertebrates (α-LTX), although five homologous insect-specific latrotoxins and one crustacean-specific latrotoxin also coexist in the venom of *Latrodectus mactans tredecimguttans*. This family of toxins may have arisen through a process of gene duplication in the spider. Envenomation by spiders of the genus *Latrodectus* causes lactrodectism, a generalized poisoning syndrome that develops within an hour of being bitten, with pain first localized at regional lymph nodes. Rapidly, generalized muscle cramps and rigidity develop, together with hypertension and transient tachycardia followed by bradycardia, profuse sweating and oliguria. Stonefish stings cause a similar envenomation with prominent autonomic nervous system dysfunction. All of these symptoms can be ascribed to hyperexcitability of various nerve terminals. Trachynilysin, a toxin of ~150 kDa, has been isolated from the venom of the stonefish *Synanceia trachynis*, and similar-sized stonustoxin and verructoxin are synthesized by *S. horrida* and *S. verrucosa*, respectively. Poisoned neuromuscular junction terminals exhibit enlarged and swollen synaptic boutons depleted of ACh vesicles and containing swollen mitochondria. Exocytosis of synaptic vesicles takes place at active zones and is followed by unaltered vesicular recycling.

Two types of high-affinity LTX receptors have been distinguished in the neuronal membrane on the basis of their Ca^{2+} dependence, with affinity in the nM range. Binding of LTX to the plasmalemma of neuronal cells causes a large influx of Ca^{2+}. After a delay of a few dozens of seconds, LTX induces the formation of long-lasting channels, which are nonselective for cations and display a large conductance of tens of pS, leading to influx of Ca^{2+} and Na^{+} and consequent membrane depolarization. Ion influxes through LTX channels are such that they can well account for the massive neurotransmitter release. However, LTX is capable of inducing neurotransmitter release even in the absence of extracellular Ca^{2+}, provided that the terminal is supplemented with millimolar concentrations of other divalent cations. Unlike the Ca^{2+}-dependent LTX-induced neurotransmitter release, which is mediated via SNARE proteins, LTX may cause Ca^{2+}-independent phospholipase C stimulation leading to exocytosis. In addition, neurexin Ia and a 120-kDa integral membrane protein associated with the G protein α-subunit (see Ch. 19), syntaxin and synaptotagmin, both bind LTX. If these interactions were indeed functional, binding of LTX could activate Ca^{2+} channel/syntaxin complexes coupled to neurexin Ia and to the 120-kDa LTX receptor via a G protein.

LTX consists of 1,381 residues distributed in four different regions: part I is a poorly conserved signal sequence, which is removed during toxin maturation, whereas part II is highly conserved and contains two putative membrane-inserting segments, which may account for the channel-forming activity of these neurotoxins; part III is less conserved and includes many ankyrin repeats, suggestive of an adaptor role linking membrane proteins to the cytoskeleton (see Ch. 6). Part IV is of variable size and is not conserved but instead is removed during maturation and activation of latrotoxin.

Other fish toxins are yet to be characterized, but it appears that all fish venoms act both pre- and post-junctionally to cause depolarization and that the venoms possess cytolytic activity. In contrast, venoms produced by the marine predatory snails of the genus *Conus* are significantly more diverse, the total number of peptides in the venom of a single Conus species ranging from 50 to 200. About 50,000 different *Conus* peptide toxins are estimated to exist, owing to a synthetic strategy that amounts to a combinatorial library scheme (Terlau & Olivera, 2004). The most prominent venoms belong to the conotoxin type, a class of peptides containing multiple disulfide bonds usually targeted against ion channels. A-conotoxins, produced by the fish-, snail- and worm-hunting marine snails *C. striatus*, *C. magus* and *C. purpurascens*, are competitive antagonists of the AChR with nM affinity. In particular, subtypes α-conotoxin GI and MI inhibit all AChR types from vertebrate muscle without affecting neuronal receptors. *C. purpurascens* also secretes ψ-conotoxin PIIIE, an unrelated noncompetitive

AChR antagonist structurally similar to the μ-conotoxins (the potent Na^+ channel blockers), which probably acts as AChR channel blocker. α-conotoxins bind preferentially to the α:δ subunit interface of the AChR versus the α:γ interface (see Chapter 13) with an affinity difference of 10,000-fold between the two sites in some cases. AChR residues γK34, γS111 and γF172, at the interface with the α subunit, confer lower affinity than δS36, δY113 and δI178, at the equivalent α subunit interface.

The overall structure of α-conotoxin GI is triangular, with both the positively charged amino group of the first residue (E1) and the guanidino group of R9 located at two of the corners, and a central hydrophobic scaffold held together by the disulfide bonds. Not unexpectedly, conotoxin ψ-conotoxin PIIIE is shaped very differently—as a flat disc with five exposed positive charges that may penetrate the AChR channel pore when its intrinsic electrostatic potential becomes less positive due to opening (Pascual & Karlin, 1998).

Electrolyte imbalances alter the voltage sensitivity of muscle ion channels

While the effect of changing ionic concentrations across cellular membranes on membrane potential and excitability is dictated by the Nernst equation (see Chapter 4), plasmatic electrolyte disturbances affect excitable tissues differently depending on the rate of exchange of the extracellular solution and the mobility of ions across the often narrow intercellular spaces (Jones et al., 2003). Hypercalcemia causes divalent metal ionic screening of membrane surface charges and is associated with weakness and fatigability due to muscle dysfunction caused by offsetting of the extracellular potential to which the voltage sensors of all ion channels are exposed, and which can eventually lead to chronic myopathy. Effects on the brain include altered consciousness ranging from apathy to agitation and seizures. Common causes of hypercalcemia are hyperparathyroidism, metastatic disease and vitamin D intoxication. Hypocalcemia, a feature of hypoparathyroidism, malabsorption, vitamin D deficiency and—rarely—of thyroid and parathyroid surgery, initially causes numbness of mouth, hands and feet, followed by tetany or spasms in the same distribution. The encephalopathy associated with hypocalcemia is more dramatic, with hallucinations, psychosis and seizures. Effects remote from the cerebral cortex include parkinsonism and chorea (see Chapter 49) and even spinal cord dysfunction. The effects of magnesium electrolyte imbalance resemble those of calcium, except that hypomagnesemia may escape analytical detection because magnesium is predominantly an intracellular ion. Renal tubular acidosis may cause hypomagnesemia, while renal failure commonly causes hypermagnesemia.

Alterations in potassium concentration are worse tolerated, owing to the fundamental role of potassium in setting resting membrane potential in almost all excitable cells of the organism. Hyperkalemia usually causes cardiac arrhythmia before nerve and muscle dysfunction. The latter abnormalities are manifested as weakness preceded by burning sensation (paresthesia) and are sometimes accompanied by mental changes. Hypokalemia, on the contrary, causes primarily neuromuscular disturbance, with fatigability, weakness of large (proximal) muscles and, ultimately, lysis of the muscle membrane (rhabdomyolysis with release of myoglobin to the plasma). When accompanied by alkalosis, hypokalemia causes tetany.

The neuromuscular junction and muscle are more resistant to changes in sodium concentration, to which they are minimally permeable at rest. In fact, the consequences of sodium disturbance relate instead to the role of this ion in maintaining the osmotic equilibrium between the brain and plasma and range from depression of consciousness, coma and seizures caused by hyponatremia, to brain shrinkage and tearing of superficial blood vessels due to excessive serum osmolarity due to hypernatremia.

MUSK AS THE MASTER ORGANIZER OF NEUROMUSCULAR JUNCTION DEVELOPMENT

Scott T. Brady

Although myasthenia gravis was originally identified as an autoimmune disease in which the acetylcholine receptor (AChR) was the primary antigenic target (Meriggioli, 2009), other antigens can produce a similar clinical phenotype. As noted in the text, another antigenic target that can produce the clinical symptoms of myasthenia is MuSK, a muscle specific receptor tyrosine kinase (Shigemoto, 2007) (Chapter 26). In both cases, there is a loss of normal clustering of AChR at the neuromuscular junction. An appreciation of the role played by MuSK in organizing the neuromuscular junction (Wu et al., 2010) illuminates why two antigens that do not stably interact can result in similar clinical presentations.

MuSK is a receptor tyrosine kinase with a cytoplasmic tyrosine kinase domain and an extracellular domain with immunoglobulin-like repeats and a cysteine-rich domain (Forrester, 2002). Clustering of AChRs at the neuromuscular junction requires the interaction of agrin, MuSK and a member of the low-density lipoprotein receptor family (Lrp4). Agrin does not bind directly to MuSK, but Lrp4 appears to be a coreceptor interacting with both agrin and MuSK (Kim et al., 2008; Zhang et al., 2008). This interaction dimerizes and activates the MuSK tyrosine kinase activity as well as triggering endocytosis of the MuSK complex (Zhu et al., 2008).

However, MuSK has a much larger set of interacting proteins, involving both physical (i.e., acting as a scaffold) and functional (i.e., downstream signaling) interactions. Examples of the MuSK scaffolding functions include an interaction with rapsyn, which can also interact with and aggregate AChRs, and with ColQ, which is important for localization of acetylcholinesterase to the synaptic cleft (chapter 13). Activation of MuSK by agrin stimulates the interaction of rapsyn with surface AChRs and with the actin cytoskeleton through α-actinin. Interestingly, rapsyn

MUSK AS THE MASTER ORGANIZER OF NEUROMUSCULAR JUNCTION DEVELOPMENT (*cont'd*)

mediates phosphorylation of AChR by MuSK after agrin activation, a step required for aggregation (Lee et al., 2008. Activation of the MuSK pathway also leads to tyrosine phosphorylation of rapsyn carboxy terminal. Rapsyn may also activate Src family nonreceptor tyrosine kinases, linking it to the downstream signaling functions of MuSK in the neuromuscular junction.

Examples of downstream signaling pathways associated with MuSK include activation of Abl and Src family nonreceptor tyrosine kinases, CK2, Pak1, and Rho GTPases (Wu et al., 2010). Abl activates Rho GTPase, which can alter actin dynamics through the WASP pathway (Chapter 6), but Rho GTPase can also activate Pak1 kinase and ROCK (Chapter 25). Pak1 also affects actin filament dynamics through activation of the serine/threonine LIM kinase 1, which inhibits the ADF/Cofilin pathway. ROCK affects myosin function. These represent only a fraction of the physiological interactions and related signaling pathways mediated directly or indirectly by the MuSK complex, but they illustrate the intricate network required to generate and maintain the AChR clusters in the NM.

While the combined functions of activating these signaling pathways downstream of MuSK kinase activity and of scaffolding lead to clustering and stabilization of AChRs, many of the participating proteins are rapidly turned over at the NMJ. Loss of MuSK signaling leads to a rapid degradation of rapsyn and other elements that stabilize AChR clusters. For example, denervation and associated secretion of agrin from the motor neuron leads to a dispersal of AChR from the NMJ. Similarly, antibodies against MuSK that interfere with agrin binding or with other aspects of MuSK signaling would be expected to produce the characteristic muscle weakness of myasthenia gravis (Shigemoto, 2007). Patients with anti-MuSK antibodies represent about 6% of all myasthenia cases. Unlike the situation with anti-AChR antibodies (Meriggioli, 2009), patients with anti-MuSK antibodies have a similar number of AChR in the muscle, but these receptors fail to cluster at the NMJ in the junctional folds.

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