Chapter 22 – Neuromuscular Physiology and Pharmacology

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The **physiology** of **neuromuscular** transmission could be analyzed and understood at the most simple level by using the classic model of nerve signaling to muscle through the acetylcholine receptor. The mammalian **neuromuscular** junction is the prototypical and most extensively studied synapse. Research has provided more detailed information on the processes that, within the classic scheme, can modify neurotransmission and response to drugs. One example of this is the role of qualitative or quantitative changes in acetylcholine receptors modifying neurotransmission and response to drugs.[1][2] In myasthenia gravis, for example, the decrease in acetylcholine receptors results in decreased efficiency of neurotransmission (and therefore muscle weakness)[3] and altered sensitivity to **neuromuscular** relaxants.[1][2] Another example is the importance of nerve-related (prejunctional) changes that alter neurotransmission and response to drugs.[1][4] At still another level is the evidence that muscle relaxants act in ways that are not encompassed by the classic scheme of unitary site of action. The observation that muscle relaxants can have prejunctional effects[5] or that some nondepolarizers can also have agonist-like stimulatory actions on the receptor[6] while others have effects not explainable by purely postsynaptic events[7] has provided new insights into some previously unexplained observations. Although this multifaceted action-response scheme makes the **physiology** and **pharmacology** of neurotransmission more complex, these added insights also bring experimentally derived knowledge much closer to clinical observations.

Crucial to the seminal concepts that have developed relative to the neurotransmitter acetylcholine and its receptor systems has been the introduction of powerful and contemporary techniques in molecular biology, immunology, and electrophysiology, as well as more elegant techniques for observations of **neuromuscular** junction in vivo.[8] These have augmented the more traditional pharmacologic, protein chemical, morphologic, and cytologic approaches.[9] Research has elucidated the manner in which the nerve ending regulates the synthesis and release of transmitter and the release of trophic factors, both of which control muscle function, and how these processes are influenced by exogenous and endogenous substances.[8][9][10][11] Research continues into how receptors are synthesized and anchored at the end plate, the role of the nerve terminal in the maturation process, and the synthesis and control of acetylcholinesterase, the enzyme that breaks down acetylcholine. Several reviews that provide detailed insights into these areas are available.[8][9][10][11][12][13]

NEUROMUSCULAR TRANSMISSION

Overview

Neuromuscular transmission occurs by a fairly simple and straightforward mechanism. The nerve synthesizes acetylcholine and stores it in small, uniformly sized packages called *vesicles*. Stimulation of the nerve causes these vesicles to migrate to the surface of the nerve, rupture, and discharge acetylcholine into the cleft separating nerve from muscle. Acetylcholine receptors in the end plate of the muscle respond by opening its channels for influx of sodium ions into the muscle to depolarize the muscle. The end-plate potential

created is continued along the muscle membrane by the opening of the sodium channels present throughout the muscle membrane, initiating a contraction.[13] The acetylcholine immediately detaches from the receptor and is destroyed by acetylcholinesterase enzyme, which also is in the cleft. Drugs, notably depolarizing relaxants or carbachol (a synthetic analog of acetylcholine not destroyed by acetylcholinesterase), can also act on these receptors to mimic the effect of acetylcholine and cause depolarization of the end plate. These drugs are therefore called *agonists* of the receptor, because to a greater or lesser extent, at least initially, they stimulate the receptor. Nondepolarizing relaxants also act on the receptors, but they prevent acetylcholine from binding to the receptor and so prevent depolarization by agonists. Because these nondepolarizers prevent the action of agonists (e.g., acetylcholine, carbachol, succinylcholine), they are referred to as *antagonists* of the acetylcholine receptor. Other compounds, frequently called *reversal agents* or *antagonists of* ***neuromuscular*** *paralysis* (e.g., neostigmine), inhibit acetylcholinesterase enzyme and therefore impair the hydrolysis of acetylcholine. The increased accumulation of undegraded acetylcholine can effectively compete with nondepolarizing relaxants, displacing the latter from the receptor (i.e., law of mass action), antagonizing the effects of nondepolarizers.

Morphology

The **neuromuscular** junction is specialized on the nerve side and on the muscle side to transmit and receive chemical messages.[8][9][10][11][12] Each motor neuron runs without interruption from the ventral horn of the spinal cord to the **neuromuscular** junction as a large, myelinated axon. As it approaches the muscle, it branches repeatedly to contact many muscle cells, to gather them into a functional group known as a *motor unit*. The architecture of the nerve terminal is quite different from that of the rest of the axon. As the terminal reaches the muscle fiber, it loses its myelin to form a spray of terminal branches against the muscle surface and is covered by Schwann cells.[10] This arrangement conforms to the architecture on the synaptic area of muscle membrane (Fig. 22-1). The nerve is separated from the surface of the muscle by a gap of approximately 20 nm, called the *junctional cleft*. The nerve and muscle are held in tight alignment by protein filaments called basal lamina, which span the cleft between nerve and end plate. The muscle surface is heavily corrugated, with deep invaginations of the junctional cleft—the primary and secondary clefts—between the folds in the muscle membrane; the end plate's total surface area is very large. The depths of the folds also vary between muscle types and species. The human **neuromuscular** junctions, relative to muscle size, are smaller than those of the mouse, although the junctions are located on muscle fibers that are much larger. Human junctions have longer junctional foldings and deeper gutters.[11] The functional significance of these folds is unclear. The shoulders of the folds are densely populated with acetylcholine receptors, about 5 million of them in each junction. These receptors are sparse in the depths between

the folds. Instead, these deep areas contain sodium channels.

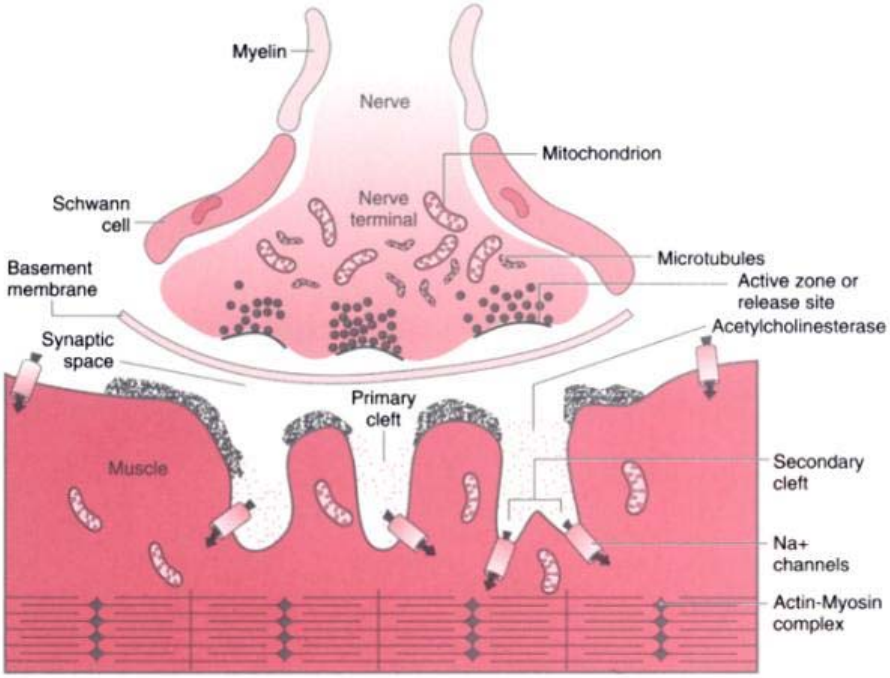

Figure 22-1 Adult **neuromuscular** junction with the three cells that constitute the synapse: the motor neuron (i.e., nerve terminal), muscle fiber, and Schwann cell. The motor neuron from the ventral horn of the spinal cord innervates the muscle. Each fiber receives only one synapse. The motor nerve loses its myelin to terminate on the muscle fiber. The nerve terminal, covered by a Schwann cell, has vesicles clustered about the membrane thickenings, which are the active zones, toward its synaptic side and mitochondria and microtubules toward its other side. A synaptic gutter, made up of a primary and many secondary clefts, separates the nerve from the muscle. The muscle surface is corrugated, and dense areas on the shoulders of each fold contain acetylcholine receptors. The sodium channels are present at the clefts and throughout muscle membrane.

The trophic function of the nerve is vital for the development and maintenance of adequate **neuromuscular** function. Before birth, each muscle cell commonly has contacts with several nerves and has several **neuromuscular** junctions.[14] At birth, all but one of the nerves retract, and a single end plate remains. Once formed, the nerve-muscle contact, especially the end plate, is durable. Even if the original nerve dies, the one replacing it innervates exactly the same region of the muscle. The nerve endings on fast muscles are larger and more complicated than those on slow muscles. The reason for this is unclear. These differences in the nerve endings on the muscle surfaces may play a role in the differences in the response to muscle relaxants of fast and slow muscles.

Because all the muscle cells in a unit are excited by a single neuron, stimulation of the nerve electrically or by an action potential originating from the ventral horn or by any agonist, including depolarizing relaxants (e.g., succinylcholine), causes all muscle cells in the motor

unit to contract synchronously. The synchronous contraction of the cells in a motor unit is fasciculation and often is vigorous enough to be observed through the skin. Although most adult human muscles have only one **neuromuscular** junction per cell, an important exception is some of the cells in the extraocular muscles. The extraocular muscles are "tonic" muscles, and unlike other mammalian striated muscles, they are multiply innervated, with several **neuromuscular** junctions strung along the surface of each muscle cell.[15] These muscles contract and relax slowly, rather than quickly as other striated muscles do; they can maintain a steady contraction, or contracture, whose strength is proportional to the stimulus received. Physiologically, this specialization apparently holds the eye steadily in position. These muscles are important to an anesthetist because depolarizing relaxants affect them differently than they do most skeletal muscles. Instead of causing a brief contraction followed by paralysis, the drugs cause a long-lasting contracture response, which pulls the eye against the orbit and contributes to an increase in the pressure of the intraocular fluid[16] (see Chapter 65). The clinical significance of this has been questioned. Although many textbooks invoke the reported extrusion of intraocular content with succinylcholine, the basis for this seems to be anecdotal.[17]

The perijunctional zone is the area of muscle immediately beyond the junctional area, and it is critical to the function of the **neuromuscular** junction. The perijunctional zone contains a mixture of the receptors, which include a smaller density of acetylcholine receptors and high-density sodium channels (see Fig. 22-1). The admixture enhances the capacity of the perijunctional zone to respond to the depolarization (i.e., end-plate potential) produced by acetylcholine receptors and to transduce it into the wave of depolarization that travels along the muscle to initiate muscle contraction. The density of sodium channels in the perijunctional area is richer than in more distal parts of the muscle membrane.[18] The perijunctional zone is close enough to the nerve ending to be influenced by transmitter released from it. Moreover, special variants (i.e., isoforms) of receptors (see "Biology of Prejunctional and Postjunctional Nicotinic Receptors") and sodium channels can appear in this area at different stages of life and in response to abnormal decreases in nerve activity. Congenital abnormalities in the acetylcholine receptor[3] or the sodium channels (i.e., mutations)[19] are also known. These variabilities seem to contribute to the differences in response to relaxants that are seen in patients with different pathologic conditions and ages.[1][20] Such qualitative differences may also play a role in altered muscle function (see "Myopathy of Critical Illness and Acetylcholine Receptors").

Quantal Theory

The contents of the nerve ending are not homogeneous. As shown in Figure 22-1 , the vesicles are congregated in the portion toward the junctional surface, whereas the microtubules, mitochondria, and other support structures are located toward the opposite side. The vesicles containing transmitter are ordered in repeating clusters alongside small, thickened, electron-dense patches of membrane, referred to as *active zones* or *release sites*. This thickened area is a cross section of a band running across the width of the synaptic surface of the nerve ending, believed to be the structure to which vesicles attach (active zones) before they rupture into the junctional cleft (see "Process of Exocytosis"). High-resolution scanning electron micrographs reveal small protein particles arranged alongside

the active zone between vesicles. These particles are believed to be special channels, the voltage-gated calcium channels, that allow calcium to enter the nerve and cause the release of vesicles.[21] The rapidity with which the neurotransmitter is released (200 μsec) suggests that the voltage-gated calcium channels are close to the release sites.

When observing the electrophysiologic activity of a skeletal muscle, small, spontaneous, depolarizing potentials at **neuromuscular** junctions can be seen. These potentials have only one-hundredth the amplitude of the evoked end-plate potential produced when the motor nerve is stimulated. Except for amplitude, these potentials resemble the end-plate potential in the time course and the manner in which they are affected by drugs. These small-amplitude potentials are called *miniature end-plate potentials* (MEPPs). Statistical analysis led to the conclusion that they are unitary responses; that is, there is a minimum size for the MEPP, and the sizes of all MEPPs are equal to or multiples of this minimum size. Because MEPPs are too big to be produced by a single molecule of acetylcholine, it was deduced that they are produced by uniformly sized packages, or *quanta*, of transmitter released from the nerve (in the absence of stimulation). The stimulus-evoked end-plate potential is the additive depolarization produced by the synchronous discharge of quanta from several hundred vesicles. The action potential that is propagated to the nerve ending allows entry of calcium into the nerve through voltage-gated calcium channels, and this causes vesicles to migrate to the active zone, fuse with the neural membrane, and discharge their acetylcholine into the junctional cleft.[21][22] Because the release sites are located immediately opposite the receptors on the postjunctional surface, little transmitter is wasted, and the response of the muscle is coupled directly with the signal from the nerve.

The alignment of the presynaptic receptor site is achieved by adhesion molecules or specific cell-surface proteins located on both sides of the synapse that grip each other across the synaptic cleft and hold the prejunctional and postjunctional synaptic apparatuses together.[23] One such protein implicated in synapse adhesion is neurexin, which binds to neuroligins on the postsynaptic membrane. The amount of acetylcholine released by each nerve impulse is large, at least 200 quanta of about 5000 molecules each, and the number of acetylcholine receptors activated by transmitter released by a nerve impulse also is large, about 500,000. The ions (mostly Na^+ and some Ca^{2+}) that flow through the channels of the activated receptors cause a maximum depolarization of the end plate, which causes an end-plate potential that is greater than the threshold for stimulation of the muscle. This is a very vigorous system. The signal is carried by more molecules of transmitter than are needed, and they evoke a response that is greater than needed. At the same time, only a small fraction of the available vesicles and receptors or channels are used to send each signal. Consequently, transmission has a substantial margin of safety, and at the same time, the system has substantial capacity in reserve.[24]

THE **NEUROMUSCULAR** JUNCTION

Formation of Neurotransmitter at Motor Nerve Endings

The axon of the motor nerve carries electrical signals from the spinal cord to the muscles and has all of the biochemical apparatus needed to transform the electrical signal into a chemical one. All the ion channels, enzymes, other proteins, macromolecules, and

membrane components needed by the nerve ending to synthesize, store, and release acetylcholine and other trophic factors are made in the cell body and are transmitted to the nerve ending by axonal transport[10][25] (Fig. 22-2). The simple molecules choline and acetate are obtained from the environment of the nerve ending, the former by a special system that transports it from the extracellular fluid to the cytoplasm and the latter in the form of acetyl coenzyme A from mitochondria. The enzyme choline acetyltransferase brings about the reaction of choline and acetate to form acetylcholine, which is stored in cytoplasm until it is transported into vesicles, which are in a better position for release.

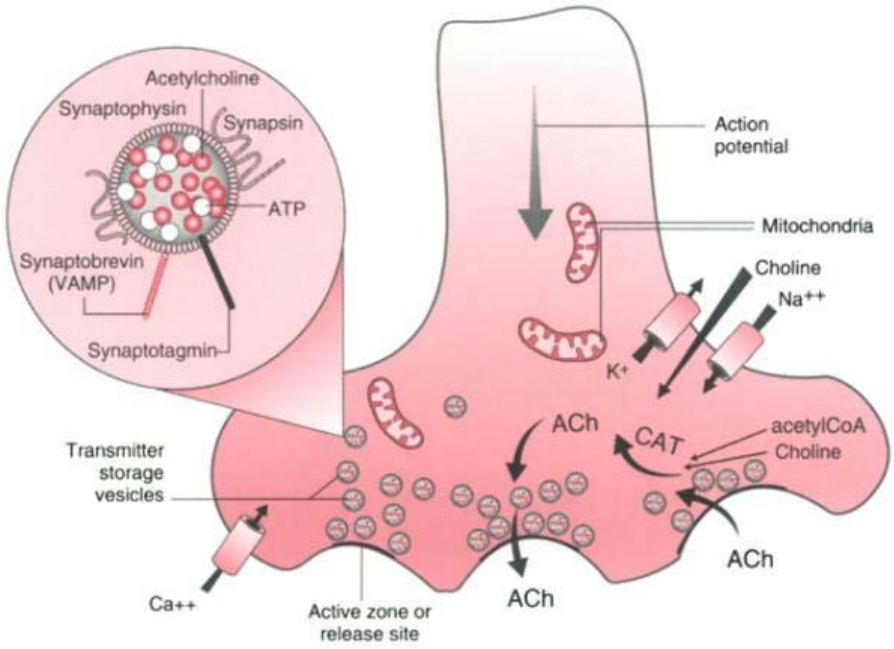

Figure 22-2 The working of a chemical synapse, the motor nerve ending, including some of the apparatus for transmitter synthesis. The large, intracellular structures are mitochondria. Acetylcholine, synthesized from choline and acetate by acetylcoenzyme A, is transported into coated vesicles, which are moved to release sites. A presynaptic action potential, which triggers calcium influx through specialized proteins (Ca^{2+} channels), causes the vesicles to fuse with the membrane and discharge transmitter. Membrane from the vesicle is retracted from the nerve membrane and recycled. Each vesicle can undergo various degrees of release of contents—from incomplete to complete. The transmitter is inactivated by diffusion, catabolism, or reuptake. The *inset* provides a magnified view of a synaptic vesicle. Quanta of acetylcholine together with ATP are stored in the vesicle and covered by vesicle membrane proteins. Synaptophysin is a vesicle membrane component glycoprotein. Synaptotagmin is the vesicle's calcium sensor. Phosphorylation of another membrane protein, synapsin, facilitates vesicular trafficking to the release site. Synaptobrevin (VAMP) is a SNARE protein involved in attaching the vesicle to the release site (see Fig. 22-3). ACh, acetylcholine, acetyl CoA, acetyl coenzyme A; CAT, choline acetyltransferase.

Nerve Action Potential

During a nerve action potential, sodium from outside flows across the membrane, and the resulting depolarizing voltage opens calcium channels, which allow entry of the calcium ions into the nerve and cause the release of acetylcholine. A nerve action potential is the normal activator that releases the transmitter acetylcholine. The number of quanta released

by a stimulated nerve is greatly influenced by the concentration of ionized calcium in the extracellular fluid. If calcium is not present, depolarization of the nerve, even by electrical stimulation, will not produce release of transmitter. Doubling the extracellular calcium results in a 16-fold increase in the quantal content of an end-plate potential. The calcium current persists until the membrane potential is returned to normal by outward fluxes of potassium from inside the nerve cell. With the calcium channels on the nerve terminal are the potassium channels, including the voltage-gated and calcium-activated potassium channels, whose function is to limit calcium entry into nerve and therefore depolarization.[13] The calcium current can be prolonged by potassium channel blockers (e.g., 4-aminopyridine, tetraethylammonium), which slow or prevent potassium efflux out of the nerve. The increase in quantal content produced in this way can reach astounding proportions.[10][26] An effect of increasing the calcium in the nerve ending is also seen clinically as the so-called post-tetanic potentiation, which occurs after a nerve of a patient paralyzed with a nondepolarizing relaxant is stimulated at high, tetanic frequencies. Calcium enters the nerve with every stimulus, but because it cannot be excreted as quickly as the nerve is stimulated, it accumulates during the tetanic period. Because the nerve ending contains more than the normal amount of calcium for some time after the tetanus, a stimulus applied to the nerve during this time causes the release of more than the normal amount of acetylcholine. The abnormally large amount of acetylcholine antagonizes the relaxant and causes the characteristic increase in the size of the twitch (see Chapter 30 and Chapter 39).

Calcium enters the nerve through specialized proteins called *calcium channels*.[13][25] Of the several types of calcium channels, two seem to be important for transmitter release, the P channels and the slower L channels. The P channels, probably the type responsible for the normal release of transmitter, are found only in nerve terminals.[10][27] In motor nerve endings, they are located immediately adjacent to the active zones (see Fig. 22-2). They are voltage-dependent; they are opened and closed by the changes in membrane voltage caused by the nerve action potential. Alterations in calcium entry into nerve ending can also alter release of transmitter. Eaton-Lambert myasthenic syndrome is an acquired autoimmune disease in which antibodies are directed against voltage-gated calcium channels at nerve endings.[4][28] In this syndrome, the decreased function of the calcium channel causes decreased release of transmitter, resulting in inadequate depolarization and muscle weakness. Patients with myasthenic syndrome exhibit increased sensitivity to depolarizing and nondepolarizing relaxants.[1]

Higher than normal concentrations of bivalent inorganic cations (e.g., magnesium, cadmium, manganese) can also block calcium entry through P channels and profoundly impair **neuromuscular** transmission. This is the mechanism for muscle weakness in the mother and fetus when magnesium sulfate is administered to treat preeclampsia. The P channels, however, are not affected by calcium entry-blocking drugs, such as verapamil, diltiazem, and nifedipine. These drugs have profound effects on the slower L channels present in the cardiovascular system. As a result, the L-type calcium channel blockers at therapeutic doses have no significant effect on the normal release of acetylcholine or on the strength of normal **neuromuscular** transmission. There have been a few reports,

however, that calcium entry-blocking drugs may increase the block of **neuromuscular** transmission induced by nondepolarizing relaxants. The effect is small, and not all investigators have been able to observe it. The explanation may lie in the fact that nerve endings also contain L-type calcium channels.

Synaptic Vesicles and Recycling

There seem to be two pools of vesicles that release acetylcholine, a readily releasable store and a reserve store, sometimes called VP2 and VP1, respectively.[25][29] The vesicles in the former are a bit smaller and are limited to an area very close to the nerve membrane, where they probably are bound to the active zones. These vesicles are the ones that ordinarily release transmitter. The release seems to occur when calcium ion enters the nerve through the P channels lined up on the sides of the active zones.[29] This calcium needs to move only a very short distance (i.e., a few atomic radii) to encounter a vesicle and to activate the proteins in the vesicle wall involved in a process known as docking[30](see "Process of Exocytosis"). The activated protein seems to react with the nerve membrane to form a pore, through which the vesicle discharges its acetylcholine into the junctional cleft. Studies using fluorescent proteins have visualized how synaptic vesicles fuse with release sites and release their contents, which are then retrieved.[31] Some vesicles stay open briefly before retrieval and do not completely collapse into the surface membrane ("kiss and run"). Others stay open longer and probably do not completely collapse ("compensatory"). Still others completely collapse and are not retrieved until another stimulus is delivered ("stranded").[31]

Most vesicles in the nerve ending are the larger reserve (VPI) vesicles. These are firmly tethered to the cytoskeleton by many proteins, including actin, synapsin (an actinbinding protein), and spectrin.[32] From their position on the cytoskeleton, they may be moved to the readily releasable store to replace worn-out vesicles or to participate in transmission when the nerve is called on to work especially hard (e.g., when it is stimulated at very high frequencies or for a very long time). Under such strenuous circumstances, calcium may penetrate more deeply than normal into the nerve or may enter through L channels to activate calcium-dependent enzymes, which cause breakage of the synapsin links that hold the vesicles to the cytoskeleton, thereby allowing the vesicles to be moved to the release sites. Repeated stimulation requires the nerve ending to replenish its stores of vesicles filled with transmitter, a process known as mobilization. The term commonly is applied to the aggregate of all steps involved in maintaining the nerve ending's capacity to release transmitter—everything from the acquisition of choline and the synthesis of acetate to the movement of filled vesicles to the release sites. The uptake of choline and the activity of choline acetyltransferase, the enzyme that synthesizes acetylcholine, probably are the rate-limiting steps.[10][11][21][22][23][24][25][26][27]

Process of Exocytosis

The readily releasable pool of synaptic vesicles constitutes those vesicles readily available

for release. During an action potential and calcium influx, neurotransmitter is released. Studies have shed some light on the inner workings by which the vesicle releases its contents. A conserved set of membrane proteins known as SNAREs (soluble *N*-ethylmaleimide-sensitive attachment protein receptors) are involved in the fusion, docking, and release of acetylcholine at the active zone. The whole process is called *exocytosis*. The SNAREs include the synaptic-vesicle protein synaptobrevin and the plasmalemma-associated proteins synataxin and synaptosome-associated protein of 25 kd (SNAP-25).[33] The current model for protein-mediated membrane fusions in exocytosis is as follows. Syntaxin and SNAP-25 are complexes attached to plasma membrane. After initial contact, the synaptobrevin on the vesicle forms a ternary complex with syntaxin/SNAP-25. Synaptotagmin is the protein on the vesicular membrane that acts as a calcium sensor and localizes the synaptic vesicles to synaptic zones rich in calcium channels, stabilizing the vesicles in the docked state.[34] The assembly of ternary complex forces the vesicle close to the underlying nerve terminal membrane (i.e., active zone), and the vesicle is then ready for release (Fig. 22-3). The vesicle can release part or all of its contents, some of which can be recycled to form new vesicles.[31] Botulinum toxin and tetanus neurotoxins, which selectively digest one or all of these three SNARE proteins, blocks exocytosis.[35] The result is muscle weakness or paralysis. These toxins in effect produce a partial or complete chemical denervation. Botulinum toxin is used therapeutically to treat spasticity or spasm in several neurologic and surgical diseases and cosmetically to correct wrinkles.

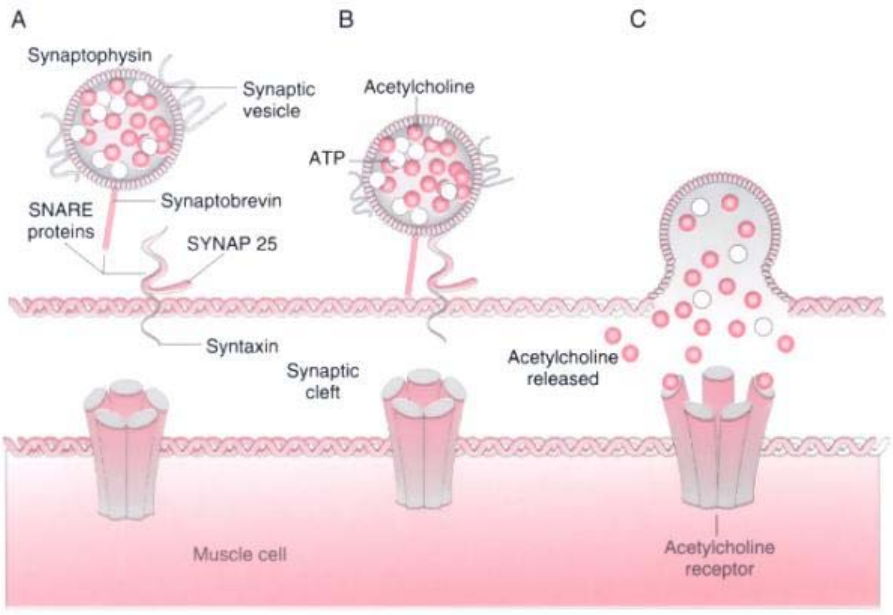

Figure 22-3 Model for protein-mediated membrane fusion and exocytosis. **A,** The release of acetylcholine from the vesicles is mediated by a series of proteins collectively called SNARE proteins. Synaptotagmin is the neuronal Ca^{2+} receptor detecting Ca^{2+} entry. Synaptobrevin (i.e., vesicle-associated membrane protein [VAMP]) is a filament-like protein on the vesicle. **B,** During depolarization and calcium entry, synaptobrevin on the vesicle unfolds and forms a ternary complex with syntaxin/SNAP-25. This process is facilitated by phosphorylation of synapsin, also present on the vesicle membrane. **C,** Assembly of the ternary complex forces the vesicle in close apposition to the nerve membrane at

the active zone with release of its contents, acetylcholine. The fusion is disassembled, and the vesicle is recycled.

Acetylcholinesterase

The acetylcholine released from the nerve diffuses across the junctional cleft and reacts with specialized receptor proteins in the end plate to initiate muscle contraction. Transmitter molecules that do not react immediately with a receptor or those released after binding to the receptor are destroyed almost instantly by the acetylcholinesterase in the junctional cleft. Acetylcholinesterase at the junction is the asymmetric or A12 form protein made in the muscle, under the end plate. The acetylcholinesterase (enzyme classification 3.1.1.7) is a type-B carboxylesterase enzyme. There is a smaller concentration of it in the extrajunctional area. The enzyme is secreted from the muscle but remains attached to it by thin stalks of collagen fastened to the basement membrane.[12][25] Most of the molecules of acetylcholine released from the nerve initially pass between the enzymes to reach the postjunctional receptors, but as they are released from the receptors, they invariably encounter acetylcholinesterase and are destroyed. Under normal circumstances, a molecule of acetylcholine reacts with only one receptor before it is hydrolyzed. Acetylcholine is a potent messenger, but its actions are very short lived because it is destroyed in less than 1 millisecond after it is released.

There are congenital and acquired diseases related to altered activity of acetylcholinesterase enzyme. Congenital absence of the secreted enzyme (in knock-out mice), leads to impaired maintenance of motor neuronal system and organization of nerve terminal branches.[36] Many syndromes due to congenital abnormalities of cholinesterase enzymes have been described and result in **neuromuscular** disorders whose symptoms and signs usually resemble those of myasthenia.[37] Denervation decreases the acetylcholinesterases at the junctional and extrajunctional areas.[2] Other acquired diseases of cholinesterases are related to chronic inhibition of acetylcholinesterase by organophosphate pesticides or nerve gas (e.g., sarin) or to chronic pyridostigmine therapy given as prophylaxis against nerve gas poisoning.[38] Symptoms from chronic fatigue to muscle weakness have been attributed to chronic cholinesterase inhibition, underscoring the importance of acetylcholinesterase in normal and abnormal **neuromuscular** function.

Postjunctional Receptors

The similarity of the acetylcholine receptors among many species and the abundance of acetylcholine receptors from the *Torpedo* electric fish have greatly facilitated research in this area. The availability of the messenger ribonucleic acids (mRNAs) of humans and other species and of deoxyribonucleic acids (DNAs) has allowed the study of the receptor in artificial systems such as oocytes from frogs and mammalian cells that do not express the receptor, such as COS or fibroblast cells. It is also possible by molecular techniques to mutate receptors to simulate pathologic states and then study receptor function in these artificial systems. By using these and related techniques, much has been learned about the synthesis, composition, and biologic

function and the mechanisms that underlie physiologic and pharmacologic responses in the acetylcholine receptors.[1][7][39][40][41] It is evident that two isoforms of postjunctional receptors exist, a junctional or mature and an extrajunctional or immature receptor (see "Biology of Prejunctional and Postjunctional Nicotinic Receptors").[1][25] The differences between receptor subtypes, however, can be neglected in a general discussion of the role of receptors in **neuromuscular** transmission.

The acetylcholine receptors are synthesized in muscle cells and are anchored to the end-plate membrane by a special 43-kd protein known as rapsyn. This cytoplasmic protein is associated with acetylcholine receptor in a 1:1 ratio.[8] The receptors, formed of five subunit proteins, are arranged like the staves of a barrel into a cylindrical receptor with a central pore for ion channeling. The key features are sketched in Figure 22-4 . The receptor protein has a molecular mass of about 250,000 daltons. Each receptor has five subunits, which are designated α, β, δ, and ε or γ; there are two subunits of α and one of each of the others.[1][2] Each of the subunits consists of approximately 400 to 500 amino acids. The receptor protein complex passes entirely through the membrane and protrudes beyond the extracellular surface of the membrane and into the cytoplasm. The binding site for acetylcholine is on each of the α-subunits, and these are the sites of competition between the receptor agonists and antagonists. Agonists and antagonists are attracted to the binding site, and either may occupy the site, which is located near cysteine residues (unique to the α-chain) at amino acid positions 192–193 of the α-subunit.[42] Radiolabeled α-bungarotoxin from the cobra, used to quantitate the receptor, binds to heptapeptide region 189–199 of the α-subunit.[43]

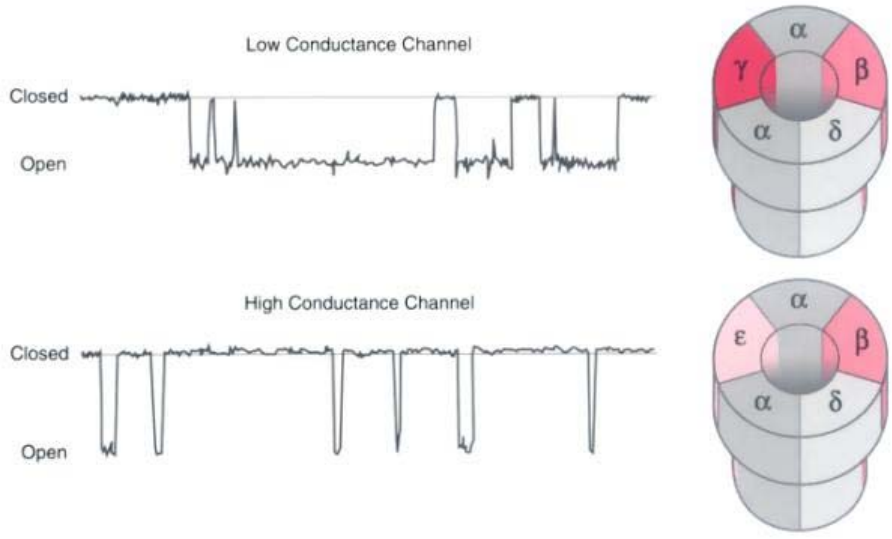

Figure 22-4 Sketch of acetylcholine receptor channels *(right)* and tracings of cell-patch records of receptor channel openings *(left)*. The mature, or junctional, receptor consists of two α-subunits and one each of β-, δ, and ε-subunits. The immature, extrajunctional or fetal form consists of two α- and one each of β, δ, and γ-subunits. These

subunits are arranged around the central cation channel. The immature isoform containing the γ-subunit shows long open times and low-amplitude channel currents. The mature isoform containing the ε-subunit shows shorter open times and high-amplitude channel currents. Substitution of the ε-subunit for the γ-subunit gives rise to the fast-gated, high-conductance channel type.

Synthesis and Stabilization of Postsynaptic Receptors

Muscle tissue is formed from the mesoderm and initially appear as myoblasts. The myoblasts fuse to produce myotubes, which therefore have multiple nuclei. As the myotubes mature, the sarcomere, which is the contractile element of the muscle consisting of actin and myosin, develops. The protein, β-integrin, seems essential for myoblast fusion and sarcomere assembly.[44] Shortly after, the motor nerve axons grow into the developing muscle, and these axons bring in nerve-derived signals (i.e., growth factors), including agrin, that are key to maturation of the myotubes to muscle.[45] Agrin is a protein from the nerve that stimulates postsynaptic differentiation by activating muscle-specific kinase (MuSK), a tyrosine kinase expressed selectively in muscle. With signaling from agrin, the acetylcholine receptors, which have been scattered throughout the muscle membrane, cluster at the area immediately beneath the nerve. Agrin together with other growth factors called neuregulins also induce the clustering of other critical muscle-derived proteins, including MUSK, rapsyn, and ERBB proteins, all of which are necessary for maturation and stabilization of the acetylcholine receptors at the junction (Fig. 22-5). Just before and shortly after birth, the immature, γ-subunit-containing acetylcholine receptors are converted to the mature, ε-subunit-containing receptors. Although the mechanism of this change is unclear, a neuregulin (growth factor) called ARIA (for acetylcholine receptor-inducing activity), which binds to one of the ERBB receptors, seems to play a role.[46]

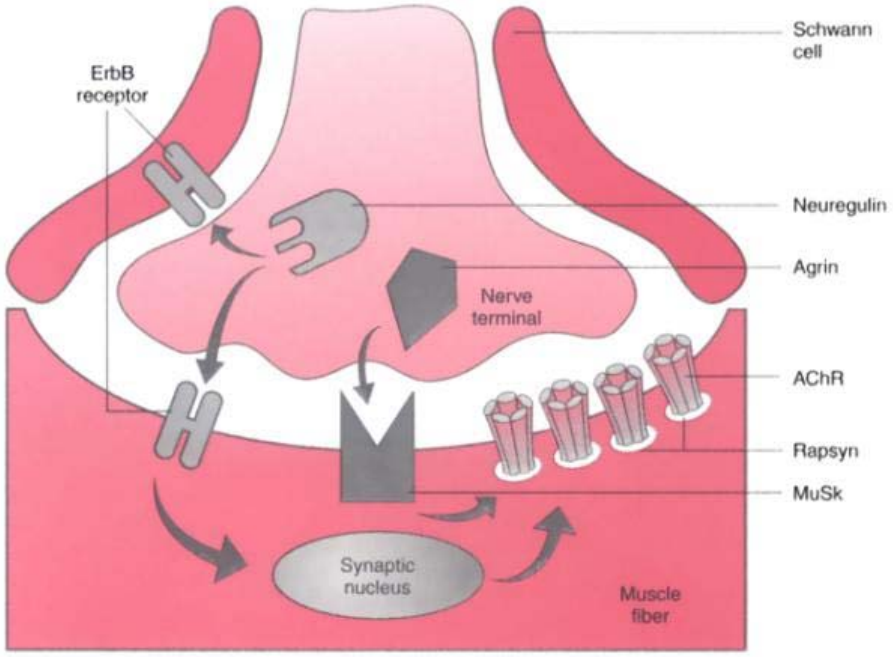

Figure 22-5 Diagram of agrin- and ARIA/neuregulin-dependent events during **neuromuscular** junction maturation. After establishment of a nerve on the muscle, growth factors, including agrin and ARIA (acetylcholine receptor-inducing activity), are released. Agrin interacting with its receptor MuSK (muscle-specific kinase) enhances the clustering of the synaptic proteins, including acetylcholine receptors (AChRs), rapsyn, and ERBB receptors. ARIA is the best candidate for involvement in the conversion of γ-subunit-containing immature receptor to ε-subunit-containing mature (innervated) receptor, which is synapse specific and therefore not inserted in the extrajunctional area.

Basic Electrophysiology of Neurotransmission

Progress in electrophysiologic techniques has moved at an equal pace with advances in molecular approaches to study the receptor. Patch-clamping is a technique in which a glass micropipette is used to probe the membrane surface until a single functional receptor is encompassed. The tip of the pipette is pressed into the lipid of the membrane, and the electronic apparatus is arranged to keep the membrane potential clamped (i.e., fixed) and to measure the current that flows through the channel of the receptor. The solution in the pipette can contain acetylcholine, tubocurarine, another drug, or a mixture of drugs. By application of these drugs to the receptor through the micropipette, electrical changes can be monitored.

Figure 22-6 illustrates the results of the classic depolarizing action of acetylcholine on end-plate receptors. Normally, the pore of the channel is closed by the approximation of the cylinders (i.e., subunits). When an agonist occupies both α-subunit sites, the protein molecule undergoes a conformational change that forms a channel in the center through which ions can flow along a

concentration gradient (see Fig. 22-6). When the channel is open, there is a flow of sodium and calcium from the outside of the cell to the inside and of potassium from the inside to the outside. The channel in the tube is large enough to accommodate many cations and electrically neutral molecules, but it excludes anions (e.g., chloride). The current carried by the ions depolarizes the adjacent membrane. The net current is depolarizing and creates the end-plate potential that stimulates the muscle to contract. In this instance, downward-going (i.e., depolarizing) rectangular pulses (see Fig. 22-4) can be recorded by the electrophysiologic technique described previously.

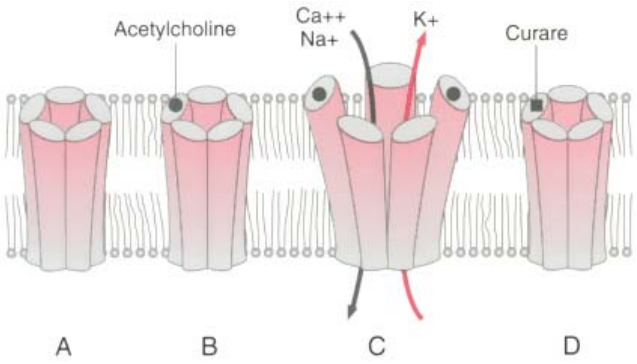

Figure 22-6 The actions of acetylcholine or curare on end-plate receptors. **A,** The ion channel is inactive and does not open in the absence of acetylcholine. **B,** Even the binding of one acetylcholine molecule *(filled circle)* to one of two binding sites does not open the channel. **C,** When acetylcholine binds to recognition sites of both α-subunits simultaneously *(filled circle)*, it triggers a conformation change that opens the channel and allows ions to flow across the membrane. **D**, The action of antagonists such as curare *(filled square)*. Acetylcholine is in competition with tubocurarine for the receptor's recognition site but may also react with acetylcholinesterase. Inhibiting the enzyme increases the lifetime of acetylcholine and the probability that it will react with a receptor. When one of the two binding (recognition) sites is occupied by curare, the receptor will not open, even if the other binding site is occupied by acetylcholine.

The pulse stops when the channel closes and one or both agonist molecules detach from the receptor. The current that passes through each open channel is minuscule, only a few picoamperes (about 10^4 ions/msec). However, each burst of acetylcholine from the nerve normally opens about 500,000 channels simultaneously, and the total current is more than adequate to produce depolarization of the end plate and contraction of muscle. The opening of a channel causes the conversion of chemical signals from a nerve to current flows to end-plate potentials, leading to muscle contraction. We are used to thinking of the end-plate potential as a graded event, which may be reduced in magnitude or extended in time by drugs, but in reality, the end-plate potential is

the summation of many all-or-nothing events occurring simultaneously at myriad ion channels. It is these tiny events that are affected by drugs.

Receptors that do not have two molecules of agonists bound remain closed. Both α-subunits must be occupied simultaneously by agonist; if only one of them is occupied, the channel remains closed (see Fig. 22-6). This is the basis for the prevention of depolarization by antagonists. Drugs such as tubocurarine act by binding to either or both α-subunits, preventing acetylcholine from binding and opening the channel. This interaction between agonists and antagonists is competitive, and the outcome—transmission or block—depends on the relative concentrations and binding characteristics of the drugs involved (see "Drug Effects on Postjunctional Receptors").

Individual channels are also capable of a wide variety of conformations.[47][48] They may open or stay closed, affecting total current flow across the membrane, but they can do more. They may open for a longer or shorter time than normal, open or close more gradually than usual, open briefly and repeatedly (i.e., chatter), or pass fewer or more ions per opening than they usually do. Their function also is influenced by drugs, changes in the fluidity of the membrane, temperature, the electrolyte balance in the milieu, and other physical and chemical factors.[49] Receptor channels are dynamic structures that are capable of a wide variety of interactions with drugs and of entering a wide variety of current-passing states. All these influences on channel activity ultimately are reflected in the strength or weakness of **neuromuscular** transmission and the contraction of a muscle.

DRUG EFFECTS ON POSTJUNCTIONAL RECEPTORS

Classic Actions of Nondepolarizing Muscle Relaxants

Neurotransmission occurs when the action potential releases acetylcholine and binds to the receptor. All non-depolarizing relaxants impair or block neurotransmission by competitively preventing the binding of acetylcholine to its receptor. The final outcome (i.e., block or transmission) depends on the relative concentrations of the chemicals and their comparative affinities for the receptor. Figure 22-6 shows a system exposed to acetylcholine and tubocurarine. One receptor has attracted two acetylcholine molecules and opened its channel, where current will flow to depolarize that segment of membrane. Another has attracted one tubocurarine molecule; its channel will not open, and no current will flow, even if one acetylcholine molecule binds to the other site. The third receptor has acetylcholine on one α-subunit and nothing on the other. What will happen depends on which of the molecules binds. If acetylcholine binds, the channel will open, and the membrane will be depolarized; if tubocurarine binds, the channel will stay closed, and the membrane will not be depolarized. At other times, one or two tubocurarine

molecules may attach to the receptor, in which case the receptor is not available to agonists; no current flow is recorded. In the presence of moderate concentrations of tubocurarine, the amount of current flowing through the entire end plate at any instant is reduced from normal, which results in a smaller end-plate potential and, if carried far enough, a block of neurotransmission or production of **neuromuscular** paralysis.

Normally, acetylcholinesterase enzyme destroys acetylcholine and removes it from the competition for a receptor, so that tubocurarine has a better chance of inhibiting transmission. If, however, an inhibitor of the acetylcholinesterase such as neostigmine is added, the cholinesterase cannot destroy acetylcholine. The concentration of agonist in the cleft remains high, and this high concentration shifts the competition between acetylcholine and tubocurarine in favor of the former, improving the chance of two acetylcholine molecules binding to a receptor even though tubocurarine is still in the environment. Cholinesterase inhibitors overcome the **neuromuscular** paralysis produced by nondepolarizing relaxants by this mechanism. The channel opens only when acetylcholine attaches to both recognition sites. A single molecule of antagonist, however, is adequate to prevent the depolarization of that receptor. This modifies the competition by biasing it strongly in favor of the antagonist. Mathematically, if the concentration of tubocurarine is doubled, the concentration of acetylcholine must be increased fourfold if acetylcholine is to remain competitive. Paralysis produced by high concentrations of antagonist is more difficult to reverse than those produced by low concentrations. After large doses of nondepolarizing relaxants, reversal drugs may be ineffective until the concentration of the relaxant in the perijunctional area decreases to a lower level by redistribution or elimination of the drug.

Classic Action of Depolarizing Muscle Relaxants

Depolarizing relaxants, at least initially, simulate the effect of acetylcholine and therefore can be considered agonists despite the fact that they block neurotransmission after initial stimulation. Structurally, succinylcholine is two molecules of acetylcholine bound together. It is therefore not surprising that it can mimic the effects of acetylcholine. Succinylcholine or decamethonium can bind to the receptor, open the channel, pass current, and depolarize the end plate. These agonists, similar to acetylcholine, attach only briefly; each opening of a channel is of very short duration, 1 millisecond or less. The response to acetylcholine, however, is over in milliseconds because of its rapid degradation by acetylcholinesterase, and the end plate resets to its resting state long before another nerve impulse arrives. In contrast, the depolarizing relaxants characteristically have a biphasic action on muscle—an initial contraction, followed by relaxation lasting minutes to hours. The depolarizing relaxants, because they are not susceptible to hydrolysis by acetyl-cholinesterase, are not

eliminated from the junctional cleft until after they are eliminated from the plasma. The time required to clear the drug from the body is the principal determinant of how long the drug effect lasts. Whole-body clearance of the relaxant is very slow compared with acetylcholine, even when the plasma cholinesterase is normal. Because relaxant molecules are not cleared from the cleft quickly, they react repeatedly with receptors, attaching to one almost immediately after separating from another, thereby repeatedly depolarizing the end plate and opening channels.

The quick shift from excitation of muscle contraction to blockade of transmission by depolarizing relaxants occurs because the end plate is continuously depolarized. This comes about because of the juxtaposition at the edge of the end plate on the muscle membrane—a different kind of ion channel, the sodium channel, that does not respond to chemicals but opens when exposed to a transmembrane voltage change. The sodium channel is also a cylindrical transmembrane protein through which sodium ions can flow. Two parts of its structure act as gates that allow or stop the flow of sodium ions.[50] Both gates must be open if sodium is to flow through the channel; the closing of either cuts off the flow. Because these two gates act sequentially, a sodium channel has three functional conformation states and can move progressively from one state to another (Fig. 22-7).

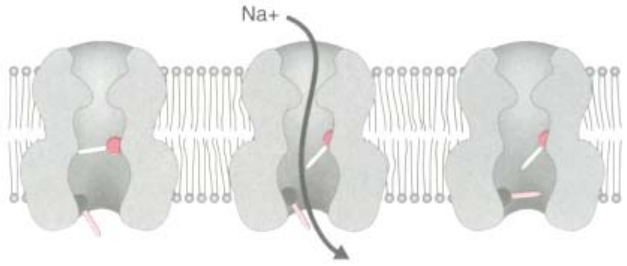

Figure 22-7 Sketch of sodium channel. The *bars* represent parts of the molecule that act as gates. The upper bar is voltage dependent; the lower bar is time dependent. The left side of the drawing represents the resting state. Once activated by a voltage change, the molecule and its gates progress as illustrated *(left to right)*.

When the sodium channel is in its resting state, the lower gate (i.e., the time-dependent or inactivation gate) is open, but the upper gate (i.e., the voltage-dependent gate) is closed, and sodium ions cannot pass. When the molecule is subject to a sudden change in voltage by depolarization of the adjacent membrane, the top gate opens, and because the bottom (time-dependent) gate is still open, sodium flows through the channel. The voltage-dependent gate stays

open as long as the molecule is subject to a depolarizing influence from the membrane around it; it will not close until the depolarization disappears. However, shortly after the voltage-dependent gate opens, the bottom gate closes and again cuts off the flow of ions. It cannot open again until the voltage-dependent gate closes. When the depolarization of the end plate stops, the voltage-dependent gate closes, the time-dependent one opens, and the sodium channel returns to its resting state. This whole process is short lived when depolarization occurs with acetylcholine. The initial response of a depolarizing muscle relaxant resembles that of acetylcholine, but because the relaxant is not hydrolyzed rapidly, depolarization of the end plate is not brief.

Depolarization of the end plate by the relaxant initially causes the voltage gate in adjacent sodium channels to open, causing a wave of depolarization to sweep along the muscle, producing muscle contraction. Shortly after the voltage-dependent gate opens, the time-dependent inactivation gate closes. Because the relaxant is not removed from the cleft, the end plate continues to be depolarized. Because the sodium channels immediately adjacent to the end plate are influenced by the depolarization of the end plate, their voltage-dependent gates stay open, and their inactivation gates stay closed. Because sodium cannot flow through a channel that has a closed inactivation gate, the perijunctional muscle membrane does not depolarize. When the flow of ions though the sodium channels in the perijunctional zone stops because the inactivation gates have closed, the channels down-stream (beyond the perijunctional zone) are freed of depolarizing influence. In effect, the perijunctional zone becomes a buffer that shields the rest of the muscle from events at the end plate. Consequently, the muscle membrane is separated into three zones: the end plate, which is depolarized by succinylcholine; the perijunctional muscle membrane, in which the sodium channels are frozen in an inactivated state; and the rest of the muscle membrane, in which the sodium channels are in the resting state. Because a burst of acetylcholine from the nerve cannot overcome the inactivated sodium channels in the perijunctional zone, **neuromuscular** transmission is blocked. This phenomenon is also called *accommodation*. During accommodation, when the synapse is inexcitable through the nerve (transmitter), direct electrical stimulation of muscle causes muscle contraction because the sodium channels beyond the junctional area are in the resting excitable state.

The extraocular muscles contain tonic muscle, which is multiply innervated and chemically excitable along most of its surface.[15] Accommodation does not occur, and these muscles can undergo a sustained contracture in the presence of succinylcholine. The tension so developed forces the eye against the orbit and accounts for part of the increase in intraocular pressure produced by depolarizing relaxants. There is also evidence that the extraocular muscles contain a special type of receptor that does not become desensitized (discussed

later) in the continued presence of acetylcholine or other agonists.[51]

Nonclassic and Noncompetitive Actions of Neuromuscular Drugs

Several drugs can interfere with the receptor, directly or through its lipid environment, to change transmission. These drugs react with the **neuromuscular** receptor to change its function and to impair transmission but do not act through the acetylcholine-binding site. These reactions cause drug-induced changes in the dynamics of the receptor, and instead of opening and closing sharply, the modified channels are sluggish. They open more slowly and stay open longer, or they close slowly and in several steps, or both. These effects on channels cause corresponding changes in the flow of ions and distortions of the end-plate potential. The clinical effect depends on the molecular events. For example, procaine, ketamine, inhaled anesthetics, or other drugs that dissolve in the membrane lipid may change the opening or closing characteristics of the channel.[52][53] If the channel is prevented from opening, transmission is weakened. If, however, the channel is prevented from or slowed in closing, transmission may be enhanced. These drugs do not fit the classic model, and the impaired **neuromuscular** function is not antagonized by increasing perijunctional acetylcholine concentrations with cholinesterase inhibitors. Such drugs can be involved in two clinically important reactions: receptor desensitization and channel blockade. The former occurs in the receptor molecule, and the latter occurs in the ion channel.

Desensitization Block

The acetylcholine receptor, because of its flexibility and the fluidity of the lipid around it, is capable of existing in a number of conformational states.[49][52][53][54] Because the resting receptor is free of agonist, its channel is closed. The second state exists when two molecules of agonists are bound to the α-subunit of the receptor, and the receptor has undergone the conformation change that opens the channel and allows ions to flow. These reactions are the bases of normal **neuromuscular** transmission. Some receptors that bind to agonists, however, do not undergo the conformation change to open the channel. Receptors in these states are called *desensitized* (i.e., they are not sensitive to the channel-opening actions of agonists). They bind agonists with exceptional avidity, but the binding does not result in the opening of the channel. The mechanisms by which desensitization occurs are not known. The receptor macromolecule, 1000 times larger by weight than most drugs or gases, provides many places at which the smaller molecules may act. The interface between lipid and receptor protein provides additional potential sites of reaction. Several different conformations of the protein are known, and because acetylcholine cannot cause the ion channel to open in any of them, they all are included in the functional term *desensitization*. Some evidence suggests that

desensitization is accompanied by phosphorylation of a tyrosine unit in the receptor protein.[55][56]

Although agonists (e.g., succinylcholine) induce desensitization, the receptors are in a constant state of transition between resting and desensitized states whether agonists are present or not. Agonists to promote the transition to a desensitized state or, because they bind very tightly to desensitized receptors, trap a receptor in a desensitized state. Antagonists also bind tightly to desensitized receptors and can trap molecules in these states. This action of antagonists is not competitive with that of acetylcholine; it may be augmented by acetylcholine if the latter promotes the change to a desensitized state. Desensitization can lead to significant misinterpretations of data. Superficially, the preparation seems to be normal, but its responsiveness to agonists or antagonists is altered. One variety occurs very rapidly, within a few milliseconds after application of an agonist. This may explain the increased sensitivity to nondepolarizers after prior administration of succinylcholine. There also is the phenomenon caused by prolonged administration of depolarizing relaxants and known as *phase II block* (see "Phase II Block"). This frequently is referred to as a desensitization blockade but should not be, because desensitization of receptors is only one of many phenomena that contribute to the process.

Many other drugs used by anesthetists also promote the shift of receptors from a normal state to a desensitized state.[52][53][54] These drugs, some of which are listed in Table 22-1 , can weaken **neuromuscular** transmission by reducing the margin of safety that normally exists at the **neuromuscular** junction, or they can cause an apparent increase in the capacity of nondepolarizing agents to block transmission. These actions are independent of the classic effects based on competitive inhibition of acetylcholine. The presence of desensitized receptors means that fewer receptor channels than usual are available to carry transmembrane current. The production of desensitized receptors decreases the efficacy of **neuromuscular** transmission. If many receptors are desensitized, insufficient normal ones are left to depolarize the motor end plate, and **neuromuscular** transmission will not occur. Even if only some receptors are desensitized, **neuromuscular** transmission will be impaired, and the system will be more susceptible to block by conventional antagonists such as tubocurarine or pancuronium.

Table 22-1 -- Drugs that can cause or promote desensitization of nicotinic cholinergic receptors

Volatile anesthetics
Halothane
Methoxyflurane
Isoflurane
Antibiotics
Polymyxin B
Cocaine
Alcohols
Ethanol
Butanol
Propanol
Octanol
Barbiturates
Thiopental
Pentobarbital
Agonists
Acetylcholine
Decamethonium
Carbachol
Succinylcholine
Acetylcholinesterase inhibitors
Neostigmine
Pyridostigmine
Difluorophosphate (DFP)
Local anesthetics
Dibucaine
Lidocaine
Prilocaine
Etidocaine
Phenothiazines

Chlorpromazine
Trifluoperazine
Prochlorperazine
Phencyclidine
Ca^{2+} channel blockers
Verapamil

Channel Block

Local anesthetics and calcium-entry blockers block the flow of sodium or calcium through their respective channels, explaining the term *channel-blocking drugs*. Similarly, a block to the flow of ions can occur at the acetylcholine receptor with concentrations of drugs used clinically and may contribute to some of the phenomena and drug interactions seen at the receptor. Two major types, closed channel and open channel block, can occur.[57][58] In a closed channel block, certain drugs can occupy the mouth of the channel, preventing ions from passing through the channel to depolarize the end plate. The process can take place even when the channel is not open. In an open channel block, a drug molecule enters a channel that has been opened by reaction with acetylcholine but does not necessarily penetrate all the way through. Open channel blockade is a use-dependent block, which means that molecules can enter the channel only when it is open. In open and closed channel blocks, the normal flow of ions through receptor is impaired, resulting in prevention of depolarization of the end plate and a weaker or blocked **neuromuscular** transmission. However, because the action is not at the acetylcholine recognition site, it is not a competitive antagonism of acetylcholine and is not relieved by anticholinesterases that increase concentrations of acetylcholine. Increasing the concentration of acetylcholine may cause the channels to open more often and thereby become more susceptible to blockade by use-dependent compounds. There is evidence that neostigmine and related cholinesterase inhibitors can act as channel-blocking drugs.[57]

Channel blockade is believed to play a role in some of the antibiotics, cocaine, quinidine, piperocaine, tricyclic antidepressants, naltrexone, naloxone, and histrionicotoxin-induced alterations in **neuromuscular** function. Muscle relaxants, in contrast, can bind to the acetylcholine recognition site of the receptor and occupy the channel. Pancuronium preferentially binds to the recognition site. Gallamine seems to act equally at the two sites. Tubocurarine is in between; at low doses, those that produce minimal blockage of transmission clinically, the drug is essentially a pure antagonist at the

recognition site; at larger doses, it also enters and blocks channels. Decamethonium and succinylcholine as agonists can open channels and, as slender molecules, also enter and block them. Decamethonium and some other long, thin molecules can penetrate all the way through the open channel and enter the muscle cytoplasm. Whether prolonged administration of nondepolarizers, as in the intensive care situation, can result in entry of the relaxant, occupation of the channel, and entry of drug into the cytosol is unknown. This effect may partially explain the muscle weakness associated with relaxant therapy in the intensive care unit.

Phase II Block

Phase II block is a complex phenomenon that occurs slowly at junctions continuously exposed to depolarizing agents. The junction is depolarized by the initial application of a depolarizing relaxant, but then the membrane potential gradually recovers toward normal, even though the junction is still exposed to drug. **Neuromuscular** transmission usually remains blocked throughout the exposure. Several factors are involved. The repeated opening of channels allows a continuous efflux of potassium and influx of sodium, and the resulting abnormal electrolyte balance distorts the function of the junctional membrane. Calcium entering the muscle through the opened channels can cause disruption of receptors and sub-end-plate elements themselves. The activity of a sodium-potassium adenosine triphosphatase pump in the membrane increases with increasing intracellular sodium and, by pumping sodium out of the cell and potassium into it, works to restore the ionic balance and membrane potential toward normal. As long as the depolarizing drug is present, the receptor channels remain open, and ion flux through them remains high.[59]

Factors influencing the development phase II block include the duration of exposure to the drug, the particular drug used and its concentration, and even the type of muscle (i.e., fast or slow). Interactions with anesthetics and other agents also affect the process. All of these drugs may also have prejunctional effects on the rate and amount of transmitter release and mobilization. With so many variables involved in the interference with **neuromuscular** transmission, phase II block is a complex and ever-changing phenomenon. The reversal response of a phase II block produced by a depolarizing muscle relaxant to administration of cholinesterase inhibitors is difficult to predict. It is therefore best that reversal by cholinesterase inhibitors is not attempted, although the response to tetanus or train-of-four stimulation resembles that produced by nondepolarizers.

BIOLOGY OF PREJUNCTIONAL AND POSTJUNCTIONAL NICOTINIC RECEPTORS

Immature or Extrajunctional versus Mature or Junctional Isoforms

There are two variants of the postjunctional acetylcholine receptors. The acetylcholine receptor isoform present in the innervated, adult **neuromuscular** junction is referred to as the adult, mature, or junctional receptor. Another isoform is expressed when there is decreased activity in muscle, as seen in the fetus before innervation; after lower or upper motor neuron injury, burns, or sepsis; or after other events that cause increased muscle protein catabolism.[60][61] To contrast with the mature or junctional receptors, the other isoform is referred to as the immature, extrajunctional, or fetal form of acetylcholine receptor. Some evidence suggests that the immature isoform is not seen in muscle protein catabolism and wasting occurring with malnutrition.[61] The differences in the protein structure of the two isoforms cause significant qualitative variations among the responses of individual patients to relaxants and seem to be responsible for some of the anomalous results that are observed when administering relaxants to particular individuals. These qualitative differences in the isoforms can also cause variations in function of muscle (see "Myopathy of Critical Illness and Acetylcholine Receptors").[37]

In addition to their structural compositions, the two isoforms have other characteristics that are different.[1][8][60] At the molecular level, both types of receptors consists of five subunits (see Fig. 22-4). The mature junctional receptor is a pentamer of two α-subunits and one each of the β-, δ-, and ε-subunits. The immature receptor consists of two α-subunits and one each of β-, δ-, and γ-subunits; that is, in the immature receptor, the γ-subunit is present instead of the ε-subunit. The γ- and ε-subunits differ from each other very little in amino acid homology, but the differences are great enough to affect the **physiology** and **pharmacology** of the receptor and its ion channel. Although the names junctional and extrajunctional imply that each is located in the junctional and extrajunctional areas, this is not strictly correct. Junctional receptors are always confined to the end plate (perijunctional) region of the muscle membrane. The immature, or extrajunctional, receptor may be expressed anywhere in the muscle membrane. Despite the name *extrajunctional*, they are not excluded from the end plate. During development and in certain pathologic states, the junctional and extrajunctional receptors can coexist in the perijunctional area of the muscle membrane (Fig. 22-8).

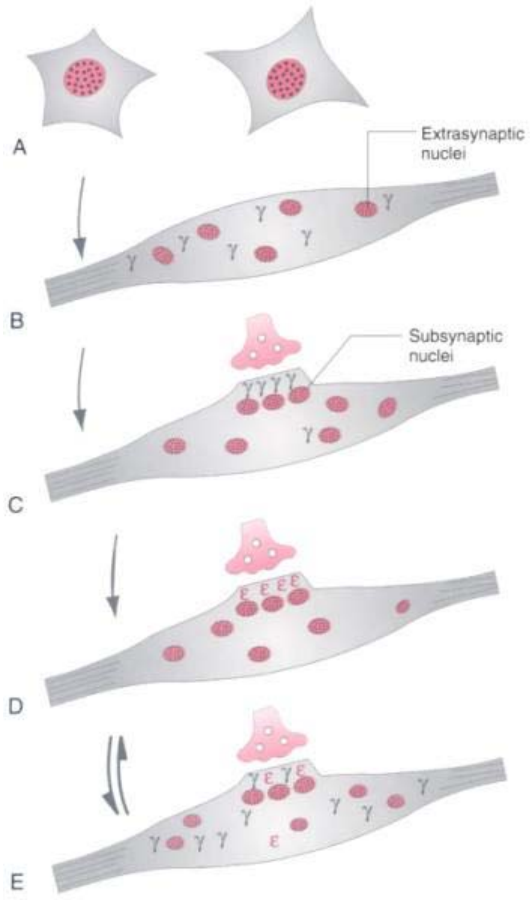

Figure 22-8 Distribution of acetylcholine receptors in developing adult, mature, and denervated muscle. **A** and **B,** In the early fetal stage, mononucleated myoblasts, derived from the mesoderm, fuse to form multinucleated myotubes. The γ-subunit-containing immature acetylcholine receptors are scattered throughout the muscle membrane. **C,** As the nerve makes contact with muscle, clustering of the receptors occurs at the synapse and is associated with some loss of extrasynaptic receptors. **D,** Maturation of the junction is said to occur when ε-subunit-containing receptors replace the γ-subunit-containing receptors. Even mature muscle is multinucleated, but it is devoid of extrasynaptic nuclei. **E,** Denervation or another pathologic state (e.g., burns, immobilization, chronic muscle relaxant therapy, sepsis) leads to re-expression of the γ-subunit receptor at the junctional and the extrajunctional areas. The latter changes are potentially reversible.

Quite unlike other cells, muscle cells are unusual in that they have many, usually hundreds of, nuclei per cell. Each of these nuclei has the genes to make both types of receptors. Multiple factors, including electrical activity, growth factor signaling (e.g., insulin, agrin, ARIA), and the presence or absence of innervation, control the expression of the two types of receptor isoforms.[8][10][25][46] This is most clearly seen in the developing embryo as the

neuromuscular junction is formed. Before they are innervated, the muscle cells of a fetus synthesize only the immature receptors—hence the term *fetal isoform of receptor*. The synthesis is directed by nearly all the nuclei in the cell, and the receptors are expressed throughout the membrane of the muscle cell (see Fig. 22-8). As the fetus develops and the muscles become innervated, muscle cells begin to synthesize the mature isoform of receptors, which are inserted exclusively into the developing (future) end plate area. The nerve releases several growth factors that influence the synthetic apparatus of the nearby nuclei. First, nerve-supplied factors induce the subsynaptic nuclei to increase synthesis of the acetylcholine receptors. Next, the nerve-induced electrical activity results in repression of receptors in the extrajunctional area. The nerve-derived growth factors, including agrin and ARIA/neuregulin, cause the receptors to cluster in the subsynaptic area and prompt expression of the mature isoform[8][46](see Fig. 22-5). In conditions associated with insulin resistance, there seems to be proliferation of acetylcholine receptors beyond the junctional area. Conditions in which insulin resistance (i.e., decreased growth factor signaling) has been observed include immobilization, burns, and denervation.[62][63][64] In these conditions, there is associated upregulation of the acetylcholine receptors and expression of the immature isoforms.[61][65][66]

Before innervation, acetylcholine receptors are present throughout the muscle membrane. After innervation, the acetylcholine receptors become more and more concentrated at the postsynaptic membrane and are virtually absent in the extrasynaptic area at birth. The innervation process progresses somewhat slowly during fetal life and matures during infancy and early childhood.[8][20][25] With time, the immature receptors diminish in concentration and disappear from the peripheral part of the muscle. In the active, adult, normal, innervated muscle, only the nuclei under and very near the end plate direct the synthesis of receptor; only the genes for expressing the mature receptors are active. The nuclei beyond the junctional area are not active, and therefore no receptors are expressed anywhere in the muscle cells beyond the perijunctional area. Conversion of all of the γ-subunit- to ε-subunit-containing acetylcholine receptors in the perijunctional area continues to take place after birth. In the rat, it takes about 2 weeks.[8] In humans, this process takes longer. At birth, the postsynaptic membrane itself is also not as specialized; the newborn junction has simplified synaptic folds, a widened synaptic space, and reduced numbers of acetylcholine receptors. Morphologically, the postsynaptic membrane of the newborn and that from a patient with myasthenia gravis are not too different. In patients with myasthenia gravis, the receptor numbers are usually decreased because of autoantibodies directed against the acetylcholine receptor.[3] It is not surprising therefore that neurotransmission is not as efficient in the newborn and patient with myasthenia gravis. A child is usually about 2 years old before nerve-muscle contacts are mature.

Proteins implicated in the linking of the mature receptors to the cytoskeleton include utrophin, α- and β-dystroglycan, and rapsyn. Several lines of evidence indicate that the clustering, expression, and stabilization of the mature receptors are triggered by at least three growth factors: agrin, ARIA, and calcitonin gene-related peptide.[8][11] Agrin is also released from the muscle, but muscle-derived agrin does not seem to be as important in the clustering and maturation of the receptor. ARIA is made in the nerve and seems to play a role in the maturation of vesicular arrangement and conversion of the γ to ε switch.[46][67] All of these growth factors interact with distinct membrane and cytosolic receptor proteins, causing phosphorylation, activation of nuclear (gene) transcriptional systems. Agrin signals through MuSK and ARIA through ERBB receptors (see Fig. 22-5). These receptors control qualitative and quantitative changes at the junction. Once begun, the process is very stable, and the nuclei in the junctional area continue to express mature receptors.

The extrajunctional receptors can reappear soon after upper and lower motor denervation and in certain pathologic states (e.g., burns, immobilization, chronic muscle relaxant therapy, loss of electrical activity). Stimulating a denervated muscle with an external electrical stimulus can prevent the appearance of the immature receptors. It has been suggested that the calcium that enters the muscle during activity is important to the suppression process.[68] In the pathologic states previously enumerated, if the process is severe and prolonged, extrajunctional receptors are inserted all over the surface of the muscle, including the perijunctional area (see Fig. 22-8). The junctional nuclei also continue to make mature receptors. The end plates consist of mature and immature receptors. The synthesis of immature receptors is initiated within hours of inactivity, but it takes several days for the whole muscle membrane to be fully covered with receptors. This upregulation of receptors has implications for the use of depolarizing and nondepolarizing relaxants.

The changes in subunit composition (γ versus ε) in the receptor confer certain changes in electrophysiologic (functional), pharmacologic, and metabolic characteristics.[1][25] The mature receptors are metabolically stable, with half-life approximating 2 weeks, whereas the immature receptor has a metabolic half-life of less than 24 hours. Immature receptors have a smaller single-channel conductance and a 2- to 10-fold longer mean channel open time than mature receptors (see Fig. 22-4). The changes in subunit composition may also alter the sensitivity or affinity, or both, of the receptor for specific ligands. Depolarizing or agonist drugs such as succinylcholine and acetylcholine depolarize immature receptors more easily, resulting in cation fluxes; one-tenth to one-hundredth doses, necessary for mature receptors, can effect depolarization.[69] Potency of nondepolarizers is also reduced, demonstrated as resistance to nondepolarizers documented in burns, denervation, and immobilization.[1][61][65][66] This resistance may be related to decreased affinity

of the receptor to nondepolarizers and to the upregulation of receptors in the perijunctional area. Data suggest that some nondepolarizers may also cause a partial agonist response in immature receptors, explaining the decreased potency.[6] The altered sensitivities for cholinergic ligands may also result from changes in composition of the lipid membrane surrounding the receptor that is known to occur with some pathologic states.[49]

The sensitivity to muscle relaxants may occur in only certain parts of the body or certain muscles if only some muscles are affected by the diminution of nerve activity (e.g., after a stroke). The sensitivity to relaxants can begin to change between 24 and 72 hours after an injury or hospitalization. The most serious side effect with the use of succinylcholine in the presence of upregulated receptors in one or more muscle is hyperkalemia.[1][2][69] In these subjects, the receptors can be scattered over a large surface of the muscle. Immature receptors are especially sensitive to succinylcholine. The channels opened by the agonist allow potassium to escape from the muscle and enter the blood. If a large part of the muscle surface consists of upregulated (immature) receptor channels, each of which stays open for a longer time, the amount of potassium that moves from muscle to blood can be very large. The resulting hyperkalemia can cause dangerous disturbances in cardiac rhythm, including ventricular fibrillation. Moreover, it is difficult to prevent the hyperkalemia by the prior administration of nondepolarizers because extrajunctional receptors are not very sensitive to block by nondepolarizing relaxants.[1] Larger than normal doses of nondepolarizers may attenuate the increase in blood potassium but cannot completely prevent it. However, hyperkalemia and cardiac arrest can occur after succinylcholine administration, even in the absence of denervation states. This is seen in certain congenital muscle dystrophies, in which the muscle membrane is prone to damage by succinylcholine releasing potassium into the circulation.[69]

Myopathy of Critical Illness and Acetylcholine Receptors

Critical illnesses (see Chapter 75 and Chapter 76) such as sepsis, trauma, and burns induce functional and pharmacologic aberrations at the skeletal muscle, similar to that seen with upper or lower motor neuron injuries. The aberrant pharmacologic responses consist of a hyperkalemic response to succinylcholine and resistance to nondepolarizers.[1][2] The important functional change in muscle associated with critical illness is muscle weakness, resulting in hypoventilation, dependence on respirators, and decreased mobilization.[70][71] The pathognomic biochemical feature in all of these critical illnesses is the upregulation of acetylcholine receptors with expression of the immature (γ-subunit) isoform of receptors.[60][61][65][66]

The immature isoform has different electrophysiologic characteristics from

those of the mature form, including prolonged open-channel time. In some clinical conditions, the presence of a prolonged open-channel time (due to congenital mutations in the receptor) is associated with muscle weakness.[37][72][73] These conditions present as progressive muscle weaknesses and impaired **neuromuscular** transmission without overt degeneration of the motor end plate. The receptor numbers typically are not significantly reduced. In all of these congenital conditions with prolonged open-channel time, there is delayed closure of the acetylcholine receptor ion channel. This leads to increased calcium load into the cytosol, progressive widening, and accumulation of debris in the synaptic cleft, resulting in reduced efficiency of released neurotransmitter and reduced safety factor. In the pathologic state of burns, sepsis, and trauma, in which muscle weakness is a concomitant finding, the expression of the immature isoform at the perijunctional membrane may have a role in muscle weakness. The presence of immature isoform can lead to prolonged open-channel time.[25] Whether these immature receptors play a role in the myopathy by this mechanism is unclear. The expression of the immature isoform may decrease the number of mature isoforms, a situation akin to myasthenia gravis, in which the mature receptor number at the junction is decreased.

In mice, deletion of the mature ε-subunit-containing receptors causes muscle weakness, despite the expression of immature receptors at the postjunctional membrane.[74] When there is de novo expression of immature isoforms of the receptor containing the γ-subunit, signaling through receptor tyrosine kinases or through growth factors (e.g., insulin) seems to be impaired.[62][63][64] Decreased signaling of growth factors such as agrin and ARIA may account for the dispersion of the acetylcholine receptors from the junctional area to areas throughout the muscle membrane with concomitant expression of the immature isoform of receptor, even in the junctional area.[75] These changes may play a role in the inefficient neurotransmission. The deficiency or absence of growth factor signaling (by means of insulin) leads to decreased anabolism of protein and enhanced muscle protein breakdown, including apoptosis, resulting in the loss of contractile elements. Apoptosis occurring in cardiac muscle contributes significantly to myocardial dysfunction.[76] The loss of muscle mass from apoptosis and decreased protein synthesis due to the decreased anabolic effect of insulin and other growth factors[77][78] may compound the skeletal muscle weakness related to ineffective neurotransmission related to expression of immature receptor. Signaling through receptor kinases and its effects on acetylcholine expression and apoptosis are intense areas of research by many groups. Correction of the altered signaling mechanism may reverse the expression of the immature to mature isoform, attenuate the loss of muscle mass due to apoptosis in muscle, and correct the muscle weakness associated with critical illness.

Prejunctional Receptors

Acetylcholine receptors exist as a variety of forms separate from that seen in muscle.[79] These receptors are expressed in peripheral neurons, autonomic and sensory ganglia, and in the central nervous system. There is also direct and indirect evidence for their existence in lymphocytes, fibroblasts, chondrocytes, macrophages, and granulocytes. Sixteen acetylcholine subunit genes have been cloned from vertebrates. They include various combinations of α-subunits (α_1 through α_9) and β-subunits (β_1 through β_4) and one each of γ-, δ-, and ε-subunits.

Prejunctional- or nerve terminal-associated cholinergic receptors have been demonstrated pharmacologically and by molecular biology techniques, but their form and functions are not well understood compared with those in the postjunctional area. Many drugs with an abundance of potential targets for drug action can affect the capacity of the nerve terminal to carry out its functions. The trophic function to maintain the nerve-muscle contact involves release and replenishment of acetylcholine together with trophic factors that require signaling through many receptors, of which the prejunctional nicotinic receptor is just one. Succinylcholine produces fasciculations that can be prevented by nondepolarizing relaxants. Because a fasciculation is, by definition, the simultaneous contraction of the multitude of muscle cells in a single motor unit and because only the nerve can synchronize all the muscles in its motor unit, it became apparent that succinylcholine must also act on nerve endings. Because nondepolarizing relaxants prevent fasciculation, it was concluded that they acted on the same prejunctional receptor. Since then, it has been shown many times that very small doses of cholinergic agonists (e.g., succinylcholine) and antagonists (e.g., curare) affect nicotinic receptors on the nerve ending, the former by depolarizing the ending and sometimes inducing repetitive firing of the nerve and the latter by preventing the action of agonists.[5]

Another clue to differences between prejunctional and postjunctional acetylcholine receptors was the finding that although both receptors can bind α-bungarotoxin, prejunctional binding was reversible, whereas postjunctional binding was not. Additional clues were found in the many demonstrations of quantitative differences in the reaction of prejunctional and postjunctional nicotinic receptors to cholinergic agonists and antagonists.[79][80] For instance, it was known that tubocurarine binds very poorly to the recognition sites of ganglionic nicotinic cholinoceptors and is not a competitive antagonist of acetylcholine at this site. Decamethonium is a selective inhibitor of the muscle receptor, and hexamethonium is a selective inhibitor of the nicotinic receptors in the autonomic ganglia.[79] Instead, D-tubocurarine and hexamethonium can block the opened channels of these receptors and owe their ability to block ganglionic transmission to this property. The functional characteristics of the

prejunctional receptor channels may also be different. For example, the depolarization of motor nerve endings initiated by administration of acetylcholine can be prevented by tetrodotoxin, a specific blocker of sodium flux with no effect on the end plate.

Specific information on the molecular organization of the neuronal nicotinic receptors on motor neuron terminal is lacking, but work on other parts of the nervous system such as the brain and ganglia indicate that they are structurally quite different from those found on the postjunctional muscle membrane.[79][80] Some of the subunit composition is similar, but other subunits do not resemble that of the postjunctional receptor. Of the 16 different nicotinic acetylcholine receptors gene product identified, only 11 (α_2 to α_9 and β_2 to β_4) are thought to contribute nicotinic receptors expressed in neurons. Most strikingly, nervous tissue does not contain genes for γ-, δ-, or ε-receptor subunits; it contains only the genes for the α- and β-subunits. The α- and β-subunit genes in nerve and muscle are not exactly the same; they are variants. Muscle contains only one gene for each of the subunits, which are called α_1 and α_1-subunit. In contrast, nervous tissue contains neither of these, but rather contains a number of related genes designated α_2 through α_9. To emphasize the distinction between neural and muscle nicotinic receptors, the former sometimes are designated *Nn* and the latter *Nm*. With so many different subunits available, there are many possible combinations, and it is not known which combinations are found in motor nerves. Their physiologic roles have also not been completely characterized. Expression of neuronal nicotinic acetylcholine receptors in vitro systems has confirmed that muscle relaxants and their metabolites can bind to these receptors.[81] Whether adverse effects observed during prolonged administration of relaxants could be attributed to interaction of relaxant with neuronal acetylcholine receptors is unclear.

The nicotinic receptor in the nerve ending of the **neuromuscular** junction may serve the function of regulator of transmitter release, as shown in other parts of the nervous system. The nicotinic receptor on the junctional surface of the nerve senses transmitter in the cleft and, by means of a positive-feedback system, causes the release of more transmitter. In other parts of the nervous system, this positive feedback is complemented by a negative-feedback system, which senses when the concentration of transmitter in the synaptic cleft has increased appropriately and shuts down the release system. Indirect evidence suggests that these receptors are muscarinic cholinergic receptors. Convincing data that motor nerve endings contain muscarinic receptors or a negative feedback system are not available for the motor neuron. The nerve ending is also known to bear several other receptors, such as opioid, adrenergic, dopamine, purine, and adenosine receptors and receptors for endogenous hormones, neuropeptides, and a variety of proteins. The physiologic roles of these receptors or the effects of anesthetics on them are unknown.

The motor nerves take up choline, synthesize acetylcholine, store it in vesicles, and move the vesicles into position to be released by a nerve action potential, a series of processes known collectively as *mobilization*. Muscle relaxants to a greater or lesser extent seem to influence this mobilization process by acting on the prejunctional nicotinic acetylcholine receptor. Tubocurarine and related muscle relaxants have a profound effect in decreasing the nerve's capacity to prepare more acetylcholine for release. Tubocurarine has no direct effect on the release process for acetylcholine; the amount of transmitter released is controlled by the availability of releasable acetylcholine and the amount of calcium that enters the nerve. Although it has frequently been observed that nondepolarizing relaxants do not diminish the transmitter released by a single nerve impulse or the first in a high-frequency train of impulses, they sharply decrease the release triggered by subsequent nerve pulses in the train. The most common manifestation of this is the so-called tetanic fade commonly seen after a nondepolarizing relaxant is administered. This effect is thought to result from inhibition of the process that replenishes releasable acetylcholine.[5]

ANTAGONISM OF **NEUROMUSCULAR** BLOCK

Mechanism of Antagonism

The nondepolarizing relaxants block (see Chapter 13) **neuromuscular** transmission predominantly by competitive antagonism of acetylcholine at the postjunctional receptor. The most straightforward way to overcome their effects is to increase the competitive position of acetylcholine. Two factors are important, the first of which is the concentration of acetylcholine. Increasing the number of molecules of acetylcholine in the junctional cleft changes the agonist-to-antagonist ratio and increases the probability that agonist molecules will occupy the recognition sites of the receptor. It also increases the probability that an unoccupied receptor will become occupied. Normally, only about 500,000 of the 5 million available receptors are activated by a single nerve impulse, and a large number of receptors is in "reserve" and could be occupied by an agonist. The second factor important to the competitive position of acetylcholine is the length of time acetylcholine is in the cleft. Acetylcholine must wait for the antagonist to dissociate spontaneously before it can compete for the freed site. The nondepolarizing relaxants bind to the receptor for slightly less than 1 millisecond, which is longer than the normal lifetime of acetylcholine. The destruction of acetylcholine normally takes place so quickly that most of it is destroyed before any significant number of antagonist molecules have dissociated from the receptor. Prolonging the time during which acetylcholine is in the junction allows time for the available acetylcholine to bind to receptor when the antagonist dissociates from the receptors.

Classes of Drugs Used

Three classes of drugs, potassium channel-blocking drugs, acetylcholinesterase inhibitors, and the γ-cyclodextrin derivatives, can be used clinically to reverse nondepolarizer-induced paralysis.[82][83] The best known of the potassium blocking drugs is 4-aminopyridine. Its actions are predominantly prejunctional; it impedes the efflux of potassium from the nerve ending. Because the efflux of potassium is the event that normally ends the action potential of the nerve ending, this action prolongs the depolarization of the nerve. Because the flux of calcium into the nerve continues for as long as the depolarization lasts, drugs of this class indirectly increase the flux of calcium into the nerve ending. The nerve releases more acetylcholine and for a longer time than usual, conditions that are effective in antagonizing nondepolarizing relaxants. Because they act prejunctionally, these drugs can antagonize a block produced by certain antibiotics that act on the nerve ending, notably the polymyxins. Although 4-aminopyridine and drugs like it can be used clinically, their use is severely restricted because they are not specific. They affect the release of transmitters by all nerve endings, including motor nerves, autonomic nerves, and central nervous system components. Their use is accompanied by a variety of undesirable effects, and in practice, they are used only in special circumstances. A most serious side effect of potassium channel blockers is seizures.

The more commonly used antagonists of **neuromuscular** block (e.g., neostigmine, pyridostigmine, edrophonium) inhibit acetylcholinesterase by mechanisms that are similar but not identical.[82] Neostigmine and pyridostigmine are attracted by an electrostatic interaction between the positively charged nitrogen in the molecules and the negatively charged catalytic site of the enzyme. This produces a carbamylated enzyme, which is not capable of further action (i.e., the catalytic site is blocked and the enzyme is inhibited). Edrophonium has neither an ester nor a carbamate group, but it is attracted and bound to the catalytic site of the enzyme by the electrostatic attraction between the positively charged nitrogen in the drug and the negatively charged acetylcholinesterase site of the enzyme. Edrophonium also seems to have prejunctional effects, enhancing the release of acetylcholine from the nerve terminal. This effect is therefore useful when deep **neuromuscular** block needs reversal. Of the three commonly used anticholinesterases, edrophonium shows by far the greatest selectivity between acetylcholinesterase and butyrylcholinesterase, the serum esterase that hydrolyzes succinylcholine and mivacurium. It greatly favors the former enzyme and therefore seems to be the most desirable agent to reverse mivacurium. However, if the patient has normal serum esterase, pharmacokinetic factors are the principal determinants of the duration of blockade, and the activity of serum esterase or the lack of it plays only a minor role in the recovery. There is little reason to prefer one or another reversal drug on these grounds. Anticholinesterases are administered for prolonged periods in the treatment of myasthenia gravis[3] and as

prophylaxis in cases of nerve gas poisoning.[38] Ironically, prolonged administration of cholinesterase inhibitors can also lead to a myasthenia-like state with muscle weakness.[84]

The cholinesterase inhibitors act preferentially at the **neuromuscular** junction and act at other synapses that use the same transmitter, including muscarinic receptors. An atropine-like drug should be administered with the cholinesterase inhibitor to counter the effects of the acetylcholine that accumulates in the muscarinic synapses of the gut, bronchi, and cardiovascular system. These three anticholinesterase inhibitors do not affect synapses in the central nervous system because all are quaternary ammonium ions, which do not easily penetrate the blood-brain barrier. A quaternary ammonium derivative of atropine, such as glycopyrrolate, which does not diffuse through the blood-brain barrier, frequently is used to limit the anticholinergic effects to the periphery. Other cholinesterase inhibitors, notably physostigmine and tacrine, are not quaternary ammonium compounds, and they have profound effects in the central nervous system. These may be antagonized by atropine but not by its quaternary ammonium analog derivatives. Unlike the other cholinesterase inhibitors, physostigmine and tacrine are also potent inhibitors of the enzyme phosphodiesterase, which plays an important role in the regulation of transmitter release at many synapses in the central nervous system. This action may be related to the reported efficacy of these two drugs in the treatment of Alzheimer's dementia.

Cholinesterase inhibitors also have actions at the postjunctional membrane independent of its effects on the enzyme. Several of these compounds contain methyl groups on a positively charged nitrogen, and they can act as agonists on the receptor channels, initiating ion flow and enhancing **neuromuscular** transmission. Neostigmine, physostigmine, and certain organophosphates can increase the frequency of MEPPs and increase the quantal content of end-plate potentials, but the importance of the increased transmitter release to reversal of **neuromuscular** blockade is not clear. Continuous exposure to the carbamate- or organophosphate-containing inhibitors causes degeneration of prejunctional and postjunctional structures, apparently because these structures accumulate toxic amounts of calcium. Calcium channel blockers such as verapamil prevent the neural actions of these drugs. All the drugs of this class also act in or on receptors to influence the kinetics of the open-close cycle and to block the ion channel.[57][58] The clinical significance of the drugs on reversal of nondepolarizers is not known.

A new approach to reversing residual **neuromuscular** block is by direct binding of the relaxant by means of chemical interaction. Antidote drugs that work by binding other drugs include protamine, citrate anticoagulation, lead or copper chelators, and RNA molecules recombinantly engineered to bind drugs.

Cyclodextrins (i.e., small, cyclic polysaccharides) are one such compound synthesized from starch by bacteria as early as 1891. The γ-cyclodextrin derivative ORG25969 binds steroidal relaxants with very high affinity, resulting in inactive muscle relaxants. The kidney then removes these complexes. Preliminary studies show promising results.[83]

KEY POINTS

1. The **neuromuscular** junction provides a rich array of receptors and substrates for drug action. Several drugs used clinically have multiple sites of action. The muscle relaxants are not exceptions to the rule that most drugs have more than one site or mechanism of action. The major actions seem to occur by the mechanisms and at the sites described for decades: agonistic and antagonistic actions at postjunctional receptors for depolarizing and nondepolarizing relaxants, respectively. This description of **neuromuscular** drug action is a simplistic one. **Neuromuscular** transmission is impeded by nondepolarizers because they prevent access of acetylcholine to its recognition site on the postjunctional receptor.
2. If the concentration of nondepolarizer is increased, another, noncompetitive action—block of the ion channel—is superimposed. The paralysis is also potentiated by the prejunctional actions of the relaxant, preventing the release of acetylcholine. The latter can be documented as fade that occurs with increased frequency of stimulation. A more accurate description of the relaxant effects recognizes that the **neuromuscular** junction is a complex and dynamic system, in which the phenomena produced by drugs are composites of actions that vary with drug, dose, activity in the junction and muscle, time after administration, the presence of anesthetics or other drugs, and the age and condition of the patient.
3. Inhibition of the postjunctional acetylcholinesterase by anticholinesterases increases concentration of acetylcholine, which can compete and displace the nondepolarizer-reversing paralysis. These anticholinesterases also have other effects, including those on nerve terminals and on the receptor, by means of an allosteric mechanism. Cyclodextrins are reversal compounds that detoxify the effects of steroidal muscle relaxants only by directly binding to them.
4. Depolarizing compounds initially react with the acetylcholine recognition site and, like the transmitter, open ion channels and depolarize the end-plate membrane. Unlike the transmitter, they are not subject to hydrolysis by acetylcholinesterase and so remain in the junction. Soon after administration of the drug, some receptors are desensitized and, although occupied by an agonist, do not open to allow

current to flow to depolarize the area.

5. If the depolarizing relaxant is applied in high concentration and allowed to remain at the junction for a long time, other effects occur. These include entry of the drug into the channel to obstruct it or to pass through it into the cytoplasm. Depolarizing relaxants also have effects on prejunctional structures, and the combination of prejunctional and postjunctional effects plus secondary ones on muscle and nerve homeostasis results in the complicated phenomenon known as *phase II blockade*.

6. Intense research in the area of **neuromuscular** transmission continues at a rapid pace. The newer observations on receptors, ion channels, membranes, and prejunctional functions reveal a much broader range of sites and mechanisms of action for agonists and antagonists.

7. Some of the other drugs used clinically (e.g., botulinum toxin) have effects on the nerve and therefore indirectly on muscle.[35] Nondepolarizing relaxants administered even for 12 hours or prolonged periods can have effects on postsynaptic receptor simulating denervation.[85][86] In recognizing these sites and mechanisms, we begin to bring our theoretical knowledge closer to explaining the phenomena observed when these drugs are administered to living humans.

8. Most recent work seems to be focused on the postjunctional membrane and the control of acetylcholine receptor expression in normal and diseased states. The presence or absence of the mature and immature isoforms seems to complicate matters further. In certain pathologic states (e.g., sepsis, burns, immobilization, chronic use of relaxants), upregulation of acetylcholine receptors occurs, usually with expression of the immature isoform. The altered functional and pharmacologic characteristics of these receptors results in increased sensitivity with hyperkalemia to succinylcholine and resistance to nondepolarizers.

9. An area of increasing attention is the control of the expression of mature versus immature receptors and the role of the immature isoform of the receptor in the muscle weakness associated with the diseases enumerated. The immature isoform expression is probably related to aberrant growth factor signaling. Mutations in the acetylcholine receptor, which result in prolonged open-channel time, similar to that seen with the immature receptor, even the presence of normal receptor numbers, can lead to a myasthenia-like state.[37] The weakness is usually related to the prolonged open-channel time. It is possible that the synaptic area expression of the immature receptor, which has a prolonged open-channel time, may simulate a myasthenia-like state.

10. Attenuated growth factor (e.g., insulin) signaling may also cause apoptosis in muscle. Loss of muscle mass due to apoptosis may compound the muscle weakness of critical illness related to expression of immature isoform receptors. In the future, it may be possible to manipulate the signaling mechanism to alter the expression of the receptor isoforms, attenuate apoptosis, and improve muscle function. Alternatively, these goals could be achieved by gene therapy.

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