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Physiology of Neuromuscular Transmission

Réka Nemes MD, J. Ross Renew MD, Sorin J. Brull MD, FCARCSI (Hon)

Neuromuscular Junction

The neuromuscular junction is a synapse between the tightly apposed presynaptic motor neuron terminal and the postsynaptic muscle fiber. This is where a chemical process (release of acetylcholine [ACh] from the nerve ending) leads to an electrical event (muscle membrane depolarization) that results in a mechanical effect (muscle contraction) (Fig. 31.1). Large motor nerve axons branch as they course distally within skeletal muscle. Ultimately, the axons divide into 10 to 100 smaller terminal nerve fibers and lose their myelin sheath, each innervating a single muscle fiber. The combination of the terminal neural fibers that originate from one axon and the muscle fibers they innervate forms a motor unit. The average number of muscle fibers innervated by a single motor neuron defines the innervation ratio, which in humans varies from 1 : 5 to 1 : 2000. For smaller muscles that are specialized for fine and precise movement (e.g., hand muscles, ocular muscles), the innervation ratio is low (1 : 5, or five muscle fibers per neuron), whereas large antigravity back muscles have very high innervation ratios (1 : 2000). Transmission from nerve to muscle is mediated by ACh, which is synthesized in the nerve terminal and stored in specialized vesicles. Each nerve terminal contains approximately 500,000 vesicles (also called *quanta*, and each containing up to 10,000 ACh molecules) arranged in a specialized region of the membrane where the synaptic vesicles are stored, called the *active zone*. ACh is released by exocytosis into the junctional cleft after an appropriate nerve impulse reaches the nerve terminal. ACh diffuses across the 50- to 70-nm cleft to bind the nicotinic cholinoceptors on the postjunctional muscle membrane, initiating muscle contraction.

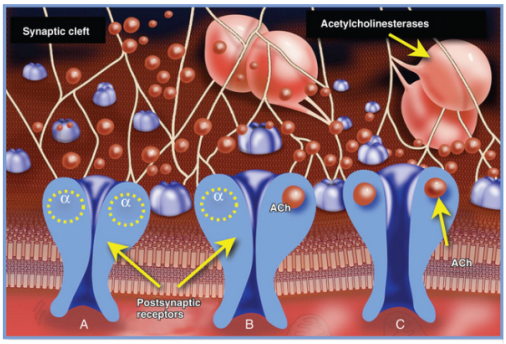

FIG. 31.1 Normal neuromuscular junction. The synaptic cleft contains an acetylcholinesterase enzyme that hydrolyzes acetylcholine (ACh). The receptors contain the ACh recognition site on the α subunits. Once both subunits are bound by ACh, the inactive (closed) receptors A and B undergo a conformational change and become active (open) by developing a central channel for cation exchange (receptor C). (Illustration courtesy of Dr. Frank G. Standaert.)

Function of the Neuromuscular Junction

Acetylcholine Synthesis

ACh is synthesized from acetyl coenzyme A and choline under the catalytic influence of choline *O*-acetyltransferase enzyme in the axoplasm (Fig. 31.2). The ACh is transported into vesicles by a specific carrier-mediated system. Approximately 80% of the ACh present in the nerve terminal is located in the vesicles, with the remainder dissolved in the axoplasm.

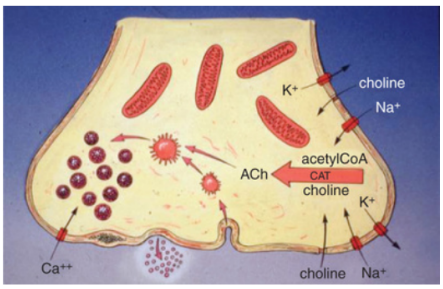

FIG. 31.2 The nerve (presynaptic) terminal. There is no myelin sheath; acetylcholine (ACh) is synthesized from acetyl coenzyme A (acetylCoA) and choline under the catalytic influence of choline *O*-acetyltransferase (CAT). Once formed, ACh is packaged into vesicles that are available for release into the cleft via exocytosis. (Illustration courtesy Dr. Frank G. Standaert.)

Nerve Terminal Depolarization

Depolarization of the nerve terminal follows the arrival of the nerve action potential and results from sodium influx through membrane sodium channels. The influx of sodium alters the membrane potential from −90 mV toward the membrane potential of sodium (+50 mV). However, at a membrane potential near 0 mV, potassium channels open and sodium channels begin to close, and the membrane potential reaches +10 mV. During depolarization, calcium ions also enter the nerve terminal, where they are sequestered in the sarcoplasmic reticulum and mitochondria.

The calcium influx into the axon lasts as long as the resting membrane potential is not restored. High-frequency tetanic stimulation of the nerve results in the intracellular accumulation of Ca^{2+} ions, which enhances the release of larger than normal amounts of ACh. This phenomenon is called *posttetanic potentiation*.

Acetylcholine Release

ACh is released spontaneously and tonically from the vesicles into the synaptic cleft, leading to small depolarizations (5 mV) at a frequency of 1 to 3 Hz, known as *miniature endplate potentials*. Each miniature endplate potential is believed to represent the effect of the contents of a single vesicle containing 6000 to 10,000 ACh molecules, or 1 quantum. These miniature endplate potentials do not result in muscle contraction. However, a threshold action potential causes accelerated ACh release of 50 to 400 quanta by a voltage-gated calcium-dependent exocytosis process from the active zone into the synaptic cleft. The extent of calcium influx into the presynaptic neuron determines the number of ACh quanta released into the cleft and is a function of the duration of nerve depolarization. Only approximately 50% of the ACh released into the cleft reaches the postsynaptic receptors; the rest is hydrolyzed by acetylcholinesterases contained within the cleft, is reuptaken into the presynaptic terminal, or diffuses out of the cleft. However, this amount of ACh is still 10 times greater than the minimum required to achieve postsynaptic ACh receptor threshold, and sufficient postjunctional membrane depolarization occurs to produce a threshold endplate potential and activation of the excitation-contraction sequence that results in muscle contraction.

The system of neuromuscular transmission is so effective that only 25% of the postjunctional nicotinic ACh receptors (nAChR) need to be activated to produce muscle membrane depolarization and fiber contraction. This constitutes the so-called margin-of-safety. On the other hand, this also implies that up to 75% of the nAChRs may still be occupied by neuromuscular blocking agent (NMBA) molecules when muscle fatigue is no longer detectable, either clinically or electrophysiologically.

Transmitter Mobilization

The rate at which available ACh stores are replaced is termed *transmitter mobilization*. Evidence suggests that there is a positive feedback loop for ACh.

Postjunctional Events

Released ACh diffuses across the synaptic cleft and binds to a receptor on the postjunctional membrane (see later). This receptor forms a membrane ion channel. Two molecules of ACh must bind the receptor (one molecule to each of the two recognition sites; see later) before the receptor undergoes the conformational change necessary to open the receptor channel to ion flow. These channels are chemically sensitive but cannot discriminate between sodium and potassium ions. Once the channels are opened, ion flow makes the immediate area more positive. Each elementary current pulse is additive and summates to produce an endplate current. The endplate current depolarizes the endplate membrane to produce the endplate potential. Once the endplate potential reaches the critical threshold, a propagating action potential is

triggered that is directed away from the endplate and results in activation of a muscle fiber contraction.

Junctional Cholinesterase

The neuromuscular junction contains two forms of acetylcholinesterase: a dissolved form in the nerve terminal axoplasm and a membrane-bound form anchored to the basement membrane of the junctional cleft. The enzyme acts to rapidly hydrolyze released ACh to choline and acetate. The kinetics of this enzyme in the neuromuscular junction cause a single ACh molecule to react with a single cholinoceptor before it is inactivated by the AChE.

Postsynaptic Receptors

The postsynaptic muscle membrane at the cleft contains multiple invaginations that markedly increase the membrane surface area. At the top of these folds are high concentrations (up to 10,000–20,000 receptors/μm^2) of nicotinic acetylcholine receptors. Outside the synaptic cleft area, the concentration of receptors is at least 1000 times lower. The nAChRs are pentameric proteins consisting of two α subunits (protomers) and one β, δ, and ε subunit each; the receptors are anchored to the postsynaptic muscle membrane by proteins such as agrin and rapsyn. In the adult mammal, the receptors are designated as α_2 βδε (Fig. 31.3). Stereochemically, they are arranged in a counterclockwise order as α, ε, α, δ, β. Fetal nAChR is similar to that of the adult, except that fetal nAChR has a γ protomer that is replaced in the adult by the ε protomer. The five subunits of nAChR form a rosette surrounding a central transmembrane pore with a diameter of approximately 0.7 nm. Each α subunit possesses a recognition site for ACh at the αε and αδ subunit interfaces. When ACh binds to both α recognition sites, the receptor undergoes a conformational change and the central pore opens, allowing sodium flux that produces a brief (6.5 ms) current.

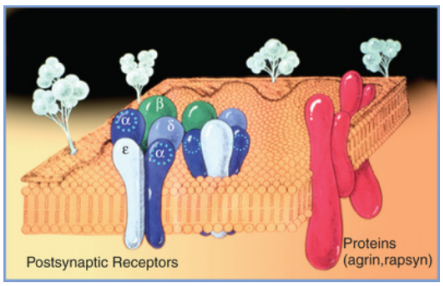

FIG. 31.3 The postsynaptic receptors are pentameric proteins consisting of two α subunits and one β, δ, and ε subunit each. They are anchored to the postsynaptic membrane by agrin and rapsyn proteins. Anticholinesterases are tethered to the basement membrane. (Illustration courtesy Dr. Frank G. Standaert.)

Presynaptic Receptors

A second type of nicotinic receptor is found on the nerve terminal. The prejunctional nAChRs have three α subunits and two β subunits. Similar to the postsynaptic receptors, they are also blocked by neuromuscular blocking agents but are relatively selective for calcium fluxes. They are thought to help mobilize ACh during periods of high ACh demand, such as high-frequency (tetanic) stimulation. Blockade of these receptors is thought to account for the tetanic fade produced by partial nondepolarizing block.

In contradistinction, succinylcholine does not bind to prejunctional nAChRs, which explains why no fade is seen during depolarizing neuromuscular blockade.

Upregulation and Downregulation

Clinical hypersensitivity and hyposensitivity (resistance) to NMBAs are observed in a number of pathologic states. The concepts of upregulation and downregulation of receptor sites have been introduced to provide a cohesive theory of receptor-drug interaction that can explain a mechanism for abnormal effects of NMBAs in the clinical setting.

Upregulation

An increase in the number of nAChRs develops on the postjunctional membrane in conditions involving decreased stimulation of the neuromuscular junction over time (Box 31.1). Upregulation leads to hypersensitivity to the agonists ACh and succinylcholine (SCh) and decreased sensitivity to antagonists such as nondepolarizing NMBAs. Upregulation can lead to lethal potassium release from cells after SCh administration in patients with motor neuron lesions, burns, muscle atrophy from disuse, and severe trauma and infections, as well as in those who have received NMBAs over a prolonged period in the intensive care unit. The phenomenon can develop in 3 to 5 days when there is total loss of ACh activity at the endplate. Pretreatment with NMBAs does not predictably prevent SCh-induced hyperkalemia. In other conditions for which chronic anticonvulsant therapy is prescribed, such as cerebral palsy and epilepsy, resistance to NMBAs is seen without potassium release after SCh use.

Box 31.1

Conditions Associated With Acetylcholine Upregulation and Downregulation

Upregulation: ↑ Agonist Sensitivity, ↓ Antagonist Sensitivity

- Upper and lower motor neuron lesions
- Burns
- Severe infection
- Prolonged use of neuromuscular blocking agents
- Muscle trauma
- Cerebral palsy
- Long-term use of anticonvulsant agents

Downregulation: ↓ Agonist Sensitivity, ↑ Antagonist Sensitivity

- Myasthenia gravis
- Organophosphate poisoning
- Exercise conditioning

Downregulation

Increased sensitivity to antagonists (e.g., NMBAs) and decreased sensitivity to agonists (e.g., SCh) develop in conditions of chronic agonist stimulation of receptors. These effects can occur with chronic reversible (e.g., neostigmine) or irreversible (e.g., organophosphate) cholinesterase inhibitor use. Most patients with myasthenia gravis have antibodies to ACh receptors that cause the neuromuscular junction to function as if it had fewer receptors. These patients are relatively resistant to SCh but extremely

sensitive to NMBAs. Downregulation is also thought to occur in muscle groups that show a greater degree of paralysis after exercise conditioning.

Acknowledgment

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Suggested Readings

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Acetylcholine receptor anatomy

The acetylcholine receptor (AChR) is a membrane protein that binds to the neurotransmitter acetylcholine (Ach). These receptors can be divided into two main types of distinct receptors, nicotinic and muscarinic. Nicotinic acetylcholine receptors (nAChR) are pentameric ligand-gated ion channels, whereas muscarinic acetylcholine receptors (mAChR) are seven-helix G-protein coupled membrane proteins.

Nicotinic acetylcholine receptors (nAChR) These ligand-gated ion channels are present at the neuromuscular junction and signal muscular contraction with stimulation. The mature nicotinic acetylcholine receptor at the postsynaptic (muscular) membrane is composed of 5 subunits (two α, and one each of β, δ, and ε subunits). These subunits are arranged in a barrel or cylindrical shape around a central pore. Each of the two α subunits has an acetylcholine-binding site. The protein-receptor complex spans the entire membrane and extends from cytoplasm to beyond the extracellular membrane. Acetylcholine binds to the α subunit; both α subunits must be bound to an acetylcholine molecule in order to trigger conformational change of ion channel to allow influx of calcium and sodium ions intracellularly, and to allow efflux of potassium out of cell.

The fetal or immature receptor is also referred to as “extrajunctional” because it can be located anywhere in the muscle membrane, inside or outside the neuromuscular junction. It consists of α, β, δ, and γ subunits; there are two subunits of α and one each of the others.

The α subunit is also the site of action of acetylcholine receptor agonists and antagonists.

Finally, the ganglion type nicotinic receptor is a type of nicotinic acetylcholine receptor that is located in the autonomic ganglia.

Muscarinic acetylcholine receptors (mAChR) These receptors are seven transmembrane G-protein coupled receptors. The structure of the receptors varies between tissues and different times in development. There are five described subtypes of muscarinic receptors (M1-M5). mAChR play major role in the parasympathetic nervous system for diverse functions, including regulation of smooth muscle activity, wakefulness, hormone secretion, heart rate.

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Succinylcholine

Sarah E. Dodd MD

Clinical Use

Succinylcholine has been the longstanding muscle relaxant of choice for laryngospasm and rapid-sequence intubation. Although higher-dose rocuronium (1.2 mg/kg) also provides rapid intubating conditions, succinylcholine was found to be clinically superior in a Cochrane Review when also considering the duration of action. Unfortunately, there are a number of considerations and side effects that are frequently encountered following succinylcholine administration. These include anaphylaxis, hyperkalemia, malignant hyperthermia, cardiac arrhythmia, prolonged apnea, phase II blockade, and postoperative myalgia, in addition to increases in intraocular, intragastric, and intracranial pressures.

Pharmacology

Succinylcholine is a neuromuscular blocking medication that depolarizes the postjunctional membrane by interacting with the alpha subunits of nicotinic acetylcholine receptors (Fig. 58.1), causing skeletal muscle contraction. This depolarization leads to variable muscle fasciculation followed by flaccid paralysis. Acetylcholine molecules are usually metabolized quickly by acetylcholinesterase molecules, but hydrolysis of succinylcholine is comparatively slow, resulting in sustained depolarization and muscle relaxation. The rapid breakdown of succinylcholine by butyrylcholinesterase (BChE) (also known as *plasma cholinesterase* or *pseudocholinesterase*) in plasma allows only 5% to 10% of the drug to reach the neuromuscular junction and hydrolyzes it after it diffuses away from the junction.

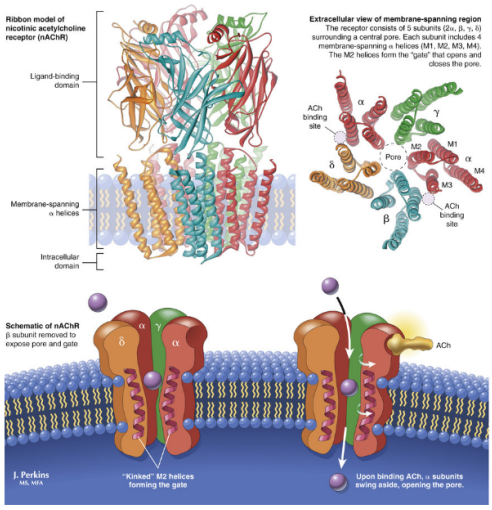

FIG. 58.1 Nicotinic acetylcholine receptor (nAChR). *ACh*, Acetylcholine. (Netter illustration from http://www.netterimages.com . © Elsevier Inc. All rights reserved.)

Phase II Blockade

Repeated dosing or continuous infusion of succinylcholine may produce a prolonged neuromuscular blockade, and the train-of-four pattern is one of fade. This is called *phase II blockade*, in contrast to *phase I blockade*, which is the typical succinylcholine pattern (Box 58.1). The mechanism is not completely known, but it is likely related to electrolyte shifts at the neuromuscular junction and desensitization of acetylcholine receptors, resulting in tachyphylaxis. Neostigmine may reverse phase II blockade, but this reversal is unreliable and should only be attempted if the patient has recovered a twitch after a peripheral nerve stimulator is applied.

Box 58.1

Causes of Changes in Butyrylcholinesterase Activity

Inherited Causes

Genetic variants that lead to decreased or increased activity

Physiologic Causes

Decreases in the last trimester of pregnancy
Reduced activity in the newborn

Acquired Decreases

Liver diseases
Cancer
Debilitating diseases
Collagen diseases
Uremia
Malnutrition
Hypothyroidism

Acquired Increases

Obesity
Alcoholism
Hyperthyroidism
Nephropathy
Psoriasis
Electroconvulsive therapy

Drug-Related Causes

Neostigmine
Pyridostigmine
Chlorpromazine
Echothiophate iodide
Cyclophosphamide
Monoamine oxidase inhibitors
Pancuronium
Oral contraceptives
Organophosphates
Hexafluorenium
Bambuterol
Esmolol

Other Causes of Decreased Activity

- Plasmapheresis
- Extracorporeal circulation
- Tetanus
- Radiation therapy
- Burns

Adapted, with permission, from Whittaker M. Plasma cholinesterase variants and the anaesthetist. *Anaesthesia.* 1980;35:174–197.

Succinylcholine-Associated Apnea

The effects of succinylcholine are terminated by its metabolism by BChE. However, some patients have atypical BChE enzymes and such patients who receive a conventional dose of succinylcholine can experience a prolonged neuromuscular block. More than 30 different variants of BChE have been described, although not all of them are associated with prolonged apnea after administration of succinylcholine. Homozygotes of atypical enzymes (approximately 1 in every 3200 patients) can have a greatly prolonged duration of succinylcholine-induced neuromuscular blockade, whereas heterozygotes (approximately 1 in every 480 patients) experience only a modest prolongation. A patient with two different atypical enzymes may also have prolonged blockade. The most common atypical BChE can be detected with dibucaine, an amide-linked local anesthetic agent that inhibits 80% of the activity of normal BChE, compared with only 20% inhibition of the homozygote atypical enzyme. A dibucaine number of 80 (i.e., percentage of inhibition) confirms the presence of normal BChE. However, the dibucaine number does not reflect the quantity of BChE present, but rather the quality of the enzyme and its ability to hydrolyze succinylcholine. The activity of BChE refers to the number of succinylcholine molecules hydrolyzed per unit of time, expressed in international units. Fig. 58.2 illustrates the correlation between the duration of succinylcholine action and BChE activity.

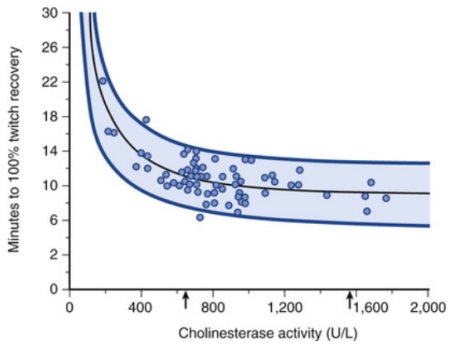

FIG. 58.2 Correlation between the duration of succinylcholine neuromuscular blockade and butyrylcholinesterase activity. The normal range of activity lies between the *arrows*. (Modified from Viby-Mogensen J. Correlation of succinylcholine duration of action with plasma cholinesterase activity in subjects with the genotypically normal enzyme. *Anesthesiology.* 1980;53:517–520.)

Succinylcholine-related apnea from the various abnormal BChE phenotypes is usually of shorter duration than the surgical procedure. Skeletal muscle paralysis of excessive duration requires maintenance of mechanical ventilatory support and continuation of anesthesia or sedation, typically in the postanesthesia care unit or the intensive care unit, until neuromuscular function returns. Some have advocated transfusion of fresh frozen plasma to replace butyrylcholinesterase, but the risks of transfusion are far higher than those associated with a few hours of mechanical ventilation. Neostigmine administration is not appropriate in these circumstances because it inhibits the degradation of succinylcholine by BChE. Succinylcholine interferes with both quantitative and qualitative assays; therefore it is preferable to postpone testing until the day after an episode of prolonged neuromuscular blockade associated with the use of succinylcholine to ensure accurate results.

Variances in Butyrylcholinesterase Activity

From birth to age 6 months, the activity of BChE is 50% of that in nonpregnant adults. Activity reaches 70% of adult activity by age 6 years and normal adult levels at puberty. Pregnancy is associated with a 25% to 30% decrease in BChE activity from week 10 to postpartum week 6, and this finding is clinically insignificant. Decreased BChE

activity can also be seen in a number of disease states and with administration of various drugs. Hepatitis, cirrhosis, malnutrition, cancer, and hypothyroidism are associated with decreased BChE activity in plasma. The alteration in BChE activity may be useful as a marker of hepatic synthetic function. Certain drugs, including acetylcholinesterase inhibitors, pancuronium, procaine, hexafluorenium, and organophosphate insecticides, inhibit BChE, whereas other drugs, including chemotherapeutic agents, can cause decreased BChE synthesis. BChE measurements can be used as a marker of occupational exposure to insecticides. Decreasing BChE activity to 25% of the control level, as seen in severe liver disease, prolongs succinylcholine duration of action from 3.0 ± 0.15 min to 8.6 ± 0.7 min, an increase that is usually undetectable in the clinical setting. Other diseases, such as thyrotoxicosis and nephrotic syndrome, are associated with increased BChE activity that probably has no clinical significance (Box 58.1).