What Makes Neuromuscular Junctions and Muscles Work and Not Work

Ronald D. Miller, MD San Francisco, California

I. Introduction

The development of neuromuscular blocking drugs (i.e., muscle relaxants) has dictated the need for anesthesiologists to understand the anatomy, physiology, and pharmacology of neuromuscular function. This knowledge is necessary in order to predict the outcome of multiple drug interactions that may occur in the perioperative period, in addition to their actions being altered by changes in physiology and pathopharmacology.

II. Basic Anatomy

The basic function of the neuromuscular junction is to transmit and receive chemical messages. Each motor neuron runs from the ventral horn of the spinal cord to the neuromuscular junction in the form of a large myelinated axon. This axon carries not only electrical signals from the spinal cord to the muscles, but also all of the biochemical apparatus needed to transform the electrical signal into a chemical one. All of the ion channels, enzymes, and other components needed by the nerve ending to synthesize, store, and release acetylcholine are made in the cell body and transmitted to the nerve ending. Only small molecules, such as the choline and acetate, used to synthesize acetylcholine are obtained locally by the nerve ending. Acetylcholine is transmitted to the post-junctional area, causing depolarization and a subsequent action potential, which sweeps the muscle to initiate contraction. A description of each one of these areas is found below.

III. Dissection of Each Component of Neuromuscular Transmission

A. Motor Nerve Endings

A nerve action potential is the normal activator of the system that releases acetylcholine, but is not the trigger per se (i.e., that function belongs to a calcium influx initiated by an action potential from the spinal cord). It is not only the concentration of calcium that influences the number of quanta of acetylcholine released, but also the length of time during which the calcium flows into the nerve ending. The exact mechanism by which calcium causes release of acetylcholine is not yet known, but its entry into the nerve seems to trigger a series of phosphorylation reactions, which disrupt the resting state of the nerve. Specifically, phosphorylation of synapsin causes mobilization of synaptic vesicles at the presynaptic plasma membrane. Vesicles are then released which are full of acetylcholine. There seems to be two

pools of vesicles, a ready releasable store and a reserve store. The ready releasable vesicles are a bit smaller, and they are limited to an area very close to the nerve membrane where they are probably bound to the active zones. These vesicles are the only ones that are ordinarily released. The membrane of the vessel fuses with that of the nerve and becomes incorporated into it, but only temporarily. The patch of membrane that originated from a vesicle contains special proteins, which allow the vesicular membrane to be recovered and return into the cytoplasm as a rudimentary vesicle. In the end, the vesicle is filled with acetylcholine transported into it from the cytoplasm and is moved into the position for release. These vesicles are used again and again, until worn out and transported back to the nerve's cell body for destruction.

The majority of the vesicles in the nerve ending are the larger "reserve" vesicles. Some of these vesicles have been recycled after use and transmission, but are mostly new ones made in the cell body and transported to the nerve ending. From their position on the cytoskeleton, they made be moved to the readily releasable store to replace worn out vesicles and/or to participate in transmission when the nerve is called upon to work especially hard (e.g.., when it is over stimulated at very high frequencies, such as a tetanic stimulus). An effect of increasing calcium in the nerve ending is seen clinically as the so called "post-tetanic potentiation" which occurs after a nerve of a patient paralyzed with a non-depolarizing muscle relaxant is stimulated at high tetanic frequencies. Calcium enters the nerve with every stimulus, but it cannot be excreted as quickly as the nerve is stimulated, and therefore accumulates during the tetanic period.

Sodium flux seems to be an important factor in the overall process and in many of the intermediate steps. It is the sodium ion that causes the depolarization of the nerve ending and the voltage change per se of ultimately releasing acetylcholine.

B. Acetylcholinesterase

The acetylcholine release from the nerve diffuses across the junctional cleft and reacts with specialized receptor proteins in the endplate to initiate muscle contraction. Acetylcholinesterase is an asymmetric protein made in the muscle under the endplate. It is secreted from the muscle, but remains attach to it via thin stalks of collagen fastened to the basement membrane. Schematically it is similar to bundles of balloons attached by strings to

muscle. Approximately 50% of the acetylcholine released from the motor nerve terminal is hydrolyzed by acetylcholinesterase. The remaining 50% passes between the enzymes to reach the post-junctional receptors. But as they are released from receptors, they invariably encounter acetylcholinesterase and are destroyed. Of course, neostigmine and other antagonists of non-depolarizing muscle relaxants act by increasing the amount of this enzyme in order to increase the amount of acetylcholine to competitively overcome the paralyzing effect of muscle relaxants. Drugs, such as neostigmine, also have other actions such as a weak agonist action at the junction.

C. Postjunctional Receptors

Receptors, which are cholinergic, are synthesized in muscle cells. The receptors are barrel-like which are inserted into the membrane and held rigidly in place in such a way that each cylinder crosses from one side of the muscle cell membrane to the other. During my presentations, illustrations of this will be shown. Normally, the cylinders are closed, but if acetylcholine reacts with specific sites on the extracellular end, the proteins undergo a change and confirmation that opens a channel in the center of the cylinder, allowing cations to move along their concentration gradients. When the channel is open, sodium and calcium flow from the outside of the cell to the inside and potassium flows from the inside to the outside. The net current is depolarizing and creates the endplate potential that stimulates the muscle to contract.

Each receptor has five subunits, which are designated α, α, β, δ, $\tilde{\varepsilon}$ When both alpha subunit sites are occupied by acetylcholine, the protein molecule undergoes the confirmation change that forms a channel in which ions can flow. The current carried by the ion depolarizes the adjacent membrane. Both alpha subunits must be occupied simultaneously by acetylcholine. If only one alpha site is occupied, the channel remains closed. This is the basis for prevention of depolarization by muscle relaxants, such as vecuronium, atracurium, etc. By doing so, they prevent acetylcholine from binding to the alpha subunits and opening the channel. This is a competitive interaction, which is why neostigmine works to reverse the neuromuscular blockade.

IV. Mechanisms of Drug Action

A. Nondepolarizing Muscle Relaxants

As indicated above, the non-depolarizing muscle relaxants prevent depolarization of the endplate because they are attracted to the acetylcholine recognition sites of the alpha subunits, and while there they may prevent acetylcholine from binding and causing the ion channel to open. This is a competitive interaction, the outcome of which is dependent on the relative concentration of the chemicals and their comparative affinity for the receptors. Differences in onset and duration of muscle relaxants are probably, in part, dependent on their affinity with the receptor.

B. Depolarizing Muscle Relaxants

At the molecular level, the depolarizing muscle relaxants, such as succinylcholine, mimic the effect of acetylcholine. If two molecules of agonists (possibly acetylcholine and/or succinylcholine) attach to the receptor, the channel will open and pass current, which causes the endplate to depolarize. When the agonists attach only briefly, the opening of the channel is for a very short duration. In this sense, there is little difference between acetylcholine and succinylcholine either in effect or duration of action.

However, succinylcholine is not susceptible to hydrolysis by acetylcholinesterase as is acetylcholine. The drug is not eliminated from the junctional cleft until after it is eliminated from plasma. Therefore, the time required to clear it from the body as a whole is the principle determinant on how long the drug effect lasts. Whole body clearance of succinylcholine is very slow as compared to the destruction of acetylcholine by junctional acetylcholinesterase, even when the plasma cholinesterase is normal. Because the succinylcholine molecules are not cleared from the cleft quickly, they react repeatedly with receptors attaching to one almost immediately after separating from another. In this way, they repeatedly open channels and continually depolarize the endplate. Very quickly there are so many receptors in a depolarized state that additional action potentials cannot occur.

C. Desensitization

Receptors, which bind agonists such as succinylcholine, but do not result in the opening of the channel, are called "desensitized". Receptor molecules constantly undergo spontaneous change and confirmation, including transformation into and out of desensitized states.

If receptors are desensitized, neuromuscular transmission will be impaired and the system will be more susceptible to blockade by neuromuscular blocking drugs. Many drugs used by anesthesiologists may promote the shift of receptors from a normal state to a desensitized state, and may react with desensitized molecules to prevent them from returning to normal. These drugs include volatile anesthetics, neostigmine, local anesthetics, and even barbiturates.

A desensitized block is frequently called a Phase II block inappropriately. A Phase II block is something quite different and will be discussed below.

D. Channel Blockade

Two major types of channel blockade can occur -- open channel and closed channel blockade. Certain drugs can react around the mouth of the channel and by their presence prevent physiologic ions from passing through the channel and depolarizing the endplate. Some antibiotics such as cocaine and naloxone are examples.

The much more common blockade is called an open channel blockade in which a drug molecule enters a channel that has been opened by a reaction with acetylcholine, but does not penetrate all the way through. If an open channel block occurs then it cannot be closed in order to be depolarized again. In essence, there is a block of neuromuscular transmission when an open channel blockade occurs. Nondepolarizing muscle relaxants have a preferential affinity for the receptors that are at the entrance of the channel. However, when large doses of muscle relaxants are used, they enter the channel where acetylcholine cannot gain access. As a result, when a channel blockade occurs with large doses of muscle relaxants, neostigmine and other acetylcholinesterase inhibitors cannot reverse a nondepolarizing blockade.

Succinylcholine is particularly interesting because as agonists, they open channels, but as slender molecules they can also enter and block them. When one gets a prolonged neuromuscular blockade, it is sometimes because succinylcholine has entered the channel itself.

E. Phase II Block

Phase II block results from continuous exposure of the neuromuscular junction to depolarizing drugs such as succinylcholine or neostigmine. With succinylcholine, the junction is depolarized by the initial application of that drug, but the membrane potential gradually recovers to normal even though the junction is still exposed to the drug. Some call this a desensitization neuromuscular blockade, which, as indicated above, is incorrect. The neuromuscular transmission usually remains blocked throughout the exposure. Ironically, administration of neostigmine will augment a depolarizing block, but antagonize a Phase II block. More specifically, the blockade may be refractory to reversal by neostigmine early and late, but it may be reversible during the mid period. The reason is not known, but one possibility may be that the activity of the sodium-potassium ATPase pump restores the junctional membrane potential towards normal, the electrical influence on the sodium channels in the perijunctional zone wanes, and the sodium channels complete their cycle and return to a resting state. In this respect, the response of a Phase II block and a nondepolarizing block are very similar. Including their response to peripheral nerve stimulation, as used during surgery.

V. Antagonism of Neuromuscular Blockade

Since the nondepolarizing muscle relaxants block 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 by giving acetylcholinesterase inhibitors, such as neostigmine or edrophonium. Two factors are involved, the first of which is the concentration of acetylcholine. By increasing the number of molecules of acetylcholine at the receptor site, the probability that acetylcholine molecules will occupy the recognition sites of the receptor increases. It also increases the probability that an unoccupied receptor will become occupied.

The second factor important to the competitive position of acetylcholine is the length of time acetylcholine is in the cleft. Acetylcholine cannot displace a molecule of muscle relaxant from a receptor. It must wait for the muscle relaxant to dissipate spontaneously before it can compete for the freed site. The nondepolarizing muscle relaxants bind to the receptor for slightly less than 1 millisecond, which is longer than the normal lifetime of acetylcholine. Thus, the destruction of acetylcholine normally takes place so quickly that most of it is destroyed before any significant

number of antagonist molecules have dissociated. Prolonging the time which acetylcholine is in the junction allows time for dissociation of the muscle relaxant and for receptors to be freed and made available to acetylcholine.

As indicated above, if overdoses of muscle relaxants have given such, that they produce "channel blockade" the antagonists (neostigmine and edrophonium) have difficulty reversing the block.

VI. Unusual Associations

The answers to the below questions will facilitate one's understanding of the relationship between the many variables that influence neuromuscular transmission. The answers to these questions will be discussed during my lecture:

A. What is the difference between a Phase II block and a nondepolarizing block?
B. What is the difference between a nondepolarizing block and myasthenia gravis?
C. What is the difference between acetylcholine and succinylcholine?

References*

1. Standaert, FG: Neuromuscular physiology and pharmacology in Anesthesia. Edited by Miler RD. Churchill Livingstone, New York, 1990, pp. 731
2. Taylor P, Schumacher M, MacPhee-Quingley K et al: The structure of acetylcholinesterase: relationship to its function and cellular disposition. Trends Neurosci 10:93, 1987
3. Kelly RB: The cell biology of the nerve terminal. Neuron 1:431, 1988
4. Miles K, Huganir RL: Regulation of nicotinic acetylcholine receptors by protein phosphorylation. Mol Neurobiol 2:91, 1988
5. Standaert FG: Basic chemistry of acetylcholine receptors. Anesthesiol Clin North Am 11:205, 1993
6. Naquib M, flood P, McArdle JJ, Brenner HR: Advances in the neurobiology of the neuro-muscular junction. Anesthesiology 2002; 96:202-31
7. Theroux MC, Akins R, Barone C, Boyce B, Miller F, Dabney KW: Neuromuscular junction in cerebral palsy. Anesthesiology 2002; 96:330-5
8. Lee C: Structure, conformation, and action of neuromuscular blocking drugs. Br J Anaesth 2001; 87:775-769

* These are general review articles.