

Experiment AN-3: Neuromuscular Studies

Background

The purpose of this experiment is to demonstrate some of the electrical properties of a muscle and its motor neurons. Nerve and muscle action potentials will be recorded from a nerve-muscle preparation. The conduction time and synaptic delay of the neuromuscular unit will be determined. The effect of stimulus frequency, as well as the effects of specific chemical agents upon the muscle and nerve and the neuromuscular junction, will also be measured.

The Motor Unit

Skeletal muscles are innervated by motor neurons whose cell bodies are located in the ventral horn of the gray matter of the spinal cord. Although each skeletal muscle fiber is innervated by only one motor neuron, each motor neuron innervates more than one muscle fiber. A motor neuron and the population of muscle fibers it innervates form the basic functional unit of the neuromuscular system, the motor unit. Each motor unit in a vertebrate behaves in an all or none fashion; when the motor neuron fires, the innervated muscle fibers contract maximally and in unison. The number of muscle fibers innervated by a single neuron varies from three to several hundred depending on the function of the muscle. Motor units that control fine movement, such as those of the ocular muscles, are innervated by a large number of neurons and contain very few muscle fibers. On the other hand, large muscles that do not require a fine degree of control, such as the gastrocnemius, have several hundred muscle fibers in a motor unit.

The junction of a nerve axon terminal and another cell (neuron or muscle) is known as a synapse. The synapse between a motor neuron and a muscle fiber is known as the neuromuscular junction. In 98% of all muscle fibers, there is only one junction, and this synapse is located in the center of the muscle fiber. The muscle action potential begins at this point and spreads to either end of the muscle fiber. This spreading of the potential from the center allows for the nearly coincidental contraction of all sarcomeres in the muscle. Since the units are contracting together, and not separately, the development of muscle tension is more efficient. Usually muscle fibers from adjacent motor units interlock, with small bundles of one motor unit lying among similar bundles in a second unit. This interaction also increases the amount of coincident contraction.

The degree of muscle contraction is primarily a function of the number of motor units that are activated. Motor units have different stimulus threshold values depending on the cell and fiber composition of each unit. As the strength of the stimulus is increased, it surpasses the threshold voltages of a greater number of motor units in the muscle. As more motor units are excited, the strength of the muscle contraction increases to a maximum level, where all fibers are excited.

The Neuromuscular Junction

The neuromuscular junction of vertebrates has been intensely studied as a model of general synaptic function because its size and accessibility are greater than synapses within the central nervous system ([Figure AN-3-B1](#)). This synapse is a critical point in communication between the neural and muscular systems.

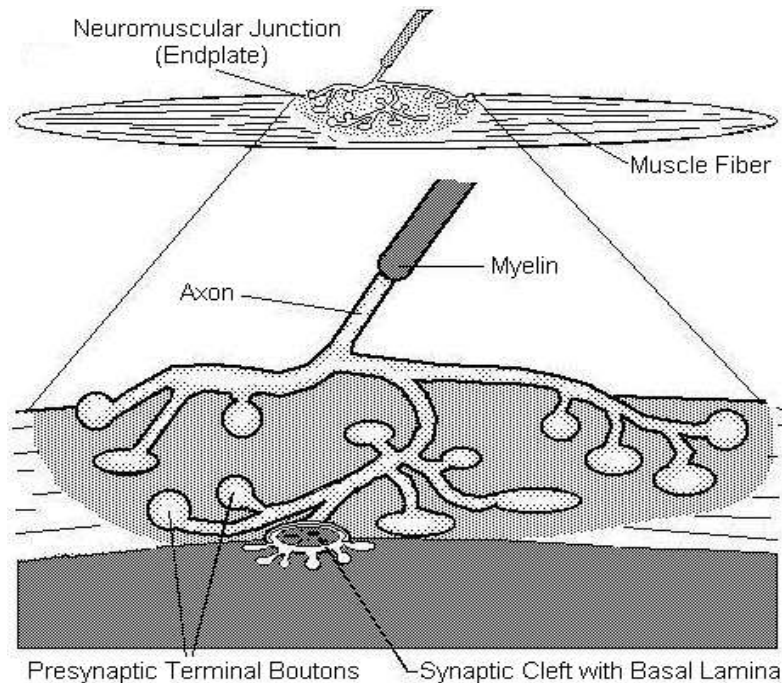


Figure AN-3-B1: The neuromuscular junction.

The distal end of the axon branches into many presynaptic terminals so that each muscle fiber has at least one junction with the motor neuron. The terminals innervate the muscle at a specialized region of the muscle (or postsynaptic) membrane known as the endplate. The space between the terminal and the endplate is called the synaptic cleft. It is 20 to 30 nanometers wide and contains the basal lamina, which is a thin layer of connective tissue through which extracellular fluids diffuse.

Synaptic Conduction

Motor neurons use acetylcholine (ACh) to transmit nerve impulses across the synapse to the muscle fibers. The presynaptic terminals have a high concentration of membrane-bound synaptic vesicles containing ACh. These vesicles fuse with the terminal membrane to release ACh into the synaptic cleft ([Figure AN-3-B2](#)). Although the mechanism is not completely understood, calcium (Ca^{++}) is known to be necessary in this process. When a nerve action potential depolarizes the presynaptic terminal, Ca^{++} channels are opened causing an influx of Ca^{++} into the terminal. Once inside the terminal, Ca^{++} aids with the fusion of synaptic vesicles to the terminal membrane. The time consumed during the processes which occur after the arrival of the presynaptic action potential and before the elicitation of the muscle action potential is known as the synaptic delay. The fusion of vesicles to the membrane and the subsequent release of ACh occur about 0.3 msec after the arrival of the nerve action potential, which accounts for most of the synaptic delay.

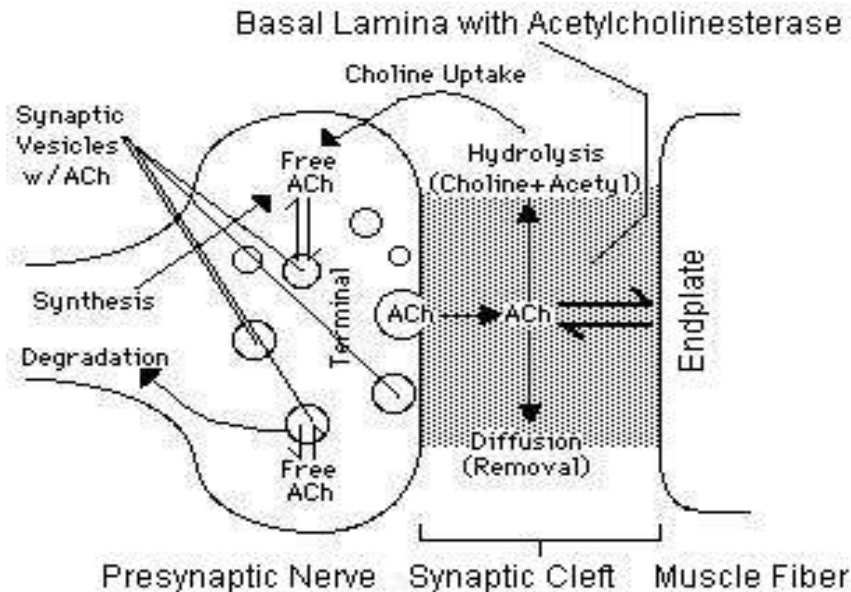


Figure AN-3-B2: Disposition of acetylcholine at the neuromuscular junction.

Once released, acetylcholine rapidly diffuses (in 0.01 msec) across the synaptic cleft and binds with specific receptors on the muscle endplate. This process greatly increases the permeability of the muscle membrane to positive ions. The concentration and potential gradients of this membrane permit Na^+ to enter the muscle fiber, depolarizing the membrane. The muscle membrane potential rises in the area of the endplate, creating a local endplate potential, which further increases the membrane's permeability to Na^+ . When the endplate potential reaches its threshold value, a muscle action potential is generated. If the muscle action potential is large enough, it will cause muscle fibers to contract.

Within 1 msec of its release from the presynaptic terminal, much of the excessive amount of acetylcholine has already diffused out of the synaptic cleft and no longer has a chance of acting on the postsynaptic membrane. The remaining molecules are hydrolyzed to choline and acetate by the enzyme acetylcholinesterase, which is in the matrix of the basal lamina. The hydrolysis can occur before or after acetylcholine binds to the receptor sites since the binding is freely reversible. The short period of time that some acetylcholine molecules remain in contact with the muscle fiber membrane is almost always sufficient to excite a muscle action potential.

After the hydrolysis of acetylcholine, choline is actively transported back into the axon terminal by a pump in the presynaptic membrane. Inside the terminal, choline is acetylated by choline acetyl transferase, and the resynthesized acetylcholine is stored in presynaptic vesicles to be used again.

Excitation-Contraction Coupling

Once a nerve action potential has been transmitted across the synaptic cleft, and the endplate potential has risen above its threshold value, a muscle action potential is initiated. This action potential elicits an electrical current which spreads along the muscle fiber and its transverse tubules (T tubules), which penetrate all the way through the muscle fiber. The action potentials in the T tubules release calcium from the adjacent sarcoplasmic reticulum. When the calcium diffuses to the myofibrils, chemical changes occur in the muscle fiber and contraction begins. This overall process for controlling muscle contraction is called excitation-contraction coupling.

Muscle Action Potentials

The initiation and conduction properties of muscle action potentials are almost the same as those of nerve action potentials, except for some quantitative differences:

- The resting membrane potential of skeletal muscle fibers is approximately -90 mV, which is the same as in large myelinated nerve fibers, but more negative than in most neurons.
- The duration of skeletal muscle action potentials is up to 5 times longer than the duration of those from large myelinated nerve fibers.
- The velocity of conduction in skeletal muscle fibers is significantly lower than the velocity of conduction in the nerve fibers that innervate these muscle fibers.

Pharmacology

The neuromuscular junction is susceptible to many drugs which may have one of several effects:

Acetylcholinesterase Inhibition

Physostigmine (eserine) and diisopropyl fluorophosphate (DFP) inhibit the hydrolytic enzyme, acetylcholinesterase. Inhibition of acetylcholinesterase prevents degradation of acetylcholine. Due to the continued presence of acetylcholine, the muscle fiber contracts and then becomes unresponsive to further stimulation (contracted paralysis). For example, small quantities of DFP induce twitching of the facial muscles and tongue, but larger amounts lead to death from suffocation (breathing muscles are continuously depolarized).

Receptor Blockage

Curare and its synthetic analog gallamine triethiodide bind to the postsynaptic receptor site and make it unavailable to acetylcholine. These two drugs are inhibitory because they have molecular structures which mimic acetylcholine and are therefore capable of binding at the receptor sites. However, these drugs are not capable of stimulating muscle fibers. An injection of curare causes progressive flaccid paralysis of the skeletal musculature, starting with the muscles of the eyes, moving to the head and neck, and finally to the limbs and breathing.

The effects of many drugs which inhibit acetylcholinesterase or block ACh receptors are reversible with time.

Other Effects

Neuromuscular transmission can be prevented by blocking Na⁺ channels and thereby inhibiting nerve action potentials (tetrodotoxin), inhibiting acetylcholine synthesis (hemicholinium), inhibiting acetylcholine release (procaine, botulinus toxin), mimicking acetylcholine by depolarization of postsynaptic membrane (succinylcholine, decamethonium), or blocking metabolic sources of energy (cyanide).

Neuromuscular transmission is also affected by altering ionic concentrations. Synaptic blockage can be caused by permanently depolarizing the postsynaptic membrane with a high concentration of K⁺. Due to its regulatory function, a low concentration of extracellular Ca⁺⁺ will inhibit ACh release. Since magnesium (Mg⁺⁺) is also a divalent ion, it is capable of competing with Ca⁺⁺. Therefore, a high concentration of Mg⁺⁺ blocks Ca⁺⁺ from doing its job and will inhibit ACh release.