Physiology of the neuromuscular junction

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The neuromuscular junction consists of a motor neurone and a muscle cell separated by a narrow synaptic cleft. The nerve and muscle do not come into direct contact. Transmission of the action potential from the nerve to the muscle occurs by release of acetylcholine from the presynaptic nerve terminal.

Motor neurone

The motor neurones which control skeletal muscle are long cells which have their origin in the ventral horn of the spinal cord. They extend to muscle cells in the periphery over a distance of up to 1 m. The metabolic centre of the nerve cell is its cell body, which lies near its origin. Information from the cell body is transmitted down a long cylindrical structure known as the axon. Axons are typically 10–20 µm in diameter and are surrounded by a myelin sheath. This sheath serves to increase the speed of transmission of the action potential to the neuromuscular junction. It consists of many layers of cell membrane tightly wrapped around each other acting as an insulator. The myelin sheath is interrupted by gaps (nodes of Ranvier) which participate in the propagation of the action potential along the nerve, speeding up nerve conduction.

Before the nerve reaches the neuromuscular junction, the axon branches into several terminals to innervate many muscle cells. A muscle cell has only one neuromuscular junction and is innervated by only one nerve. A nerve, and the muscle cells which it innervates, comprise a motor unit. The number of muscle cells per motor unit varies from a few to several thousand, depending on the function of the muscle. The largest number is seen in strong bulky muscles concerned with coarse movement; the smallest number are in muscles which perform delicate movements (e.g. the eye).

The synapse is the area of the nerve lying closest to the muscle cell; it is situated opposite a specialised area of the muscle cell called the end plate. The synapse and the end plate are separated by a gap (approximately 20 nm) called the synaptic or junctional cleft which is filled with extracellular fluid. It is at the synapse that the action potential, which has travelled along the nerve, causes the release of the neurotransmitter, acetylcholine. Acetylcholine travels across the synaptic cleft and binds selectively to the post-synaptic motor end plate of the muscle causing an action potential to travel through it.

Motor end plate

The motor end plate is a small, specialised area of the muscle which is very rich in acetylcholine receptors. It is oval in shape, measuring 15–30 µm by 20–50 µm. The surface of the muscle at the end plate is deeply folded with many ridges and secondary clefts. The ridges have a high concentration of acetylcholine receptors on the crest of their folds (Fig. 1). There are 1–10 million receptors at each end plate and the receptor density is high (10,000–20,000 µm⁻²).

Synthesis and storage of acetylcholine

Acetylcholine is synthesised from choline and acetylcoenzyme A in the axoplasm of the cholinergic nerve terminals. The reaction is catalysed by the enzyme choline acetyltransferase. Choline acetyltransferase is synthesised in the ribosomes in the cell body of the motor neurone and passes along the axon to its terminal where its concentration is highest.

The majority of the choline used in acetylcholine synthesis is derived from the extracellular fluid. Most of this choline comes from the diet, although a small amount is synthesised in the liver. About half of the choline formed by the breakdown of acetylcholine at the neuromuscular junction is taken up again into the nerve terminals.

Key points

The neuromuscular junction consists of a motor neurone and a muscle cell separated by a narrow synaptic cleft.

Acetylcholine is released from the nerve ending in response to a nerve stimulus, crosses the synaptic cleft and binds to the post-synaptic nicotinic receptor on the surface of the muscle cell.

The nicotinic receptor consists of five subunits (pentameric complex) which are joined to form a cylindrical ring that passes through the muscle cell membrane into its cytoplasm.

Two acetylcholine molecules bind to the two α-subunits of the pentameric complex, inducing a conformational change which opens the channel to the open conformation. Consequently, sodium ions enter the cell in large numbers and, to a lesser degree, potassium ions leave it.

DOI 10.1093/bjacepd/02.05.129
by a carrier-facilitated transport mechanism before being converted back into acetylcholine.

Acetylcholine can be found throughout the axoplasm and cytosol of the cell body. Its highest concentration is within the axon terminal. There are different pools or vesicles of acetylcholine in the terminal that have variable availability for release. About 1% of the vesicles form the immediately releasable store which is responsible for the maintenance of transmitter release under conditions of low nerve activity. Nearly 80% of acetylcholine is in a reserve pool which is released in response to nerve impulses. The remainder is termed the stationary store (Fig. 1).

Most acetylcholine is stored in vesicles which are synthesised in the cell body and pass down the axon to the nerve terminal by axoplasmic flow. Only about 20% is dissolved in the cytosol. Each vesicle contains approximately 12,000 molecules of acetylcholine. The acetylcholine is loaded into the vesicles by an active transport process in the vesicle membrane involving an Mg2+-dependent proton pumping ATPase. The acetylcholine molecules in the axoplasm combine with a transport protein which shuttles across the vesicle membrane and exchanges each acetylcholine molecule for a H+ ion.

**Release of acetylcholine**

The release of acetylcholine may be spontaneous or in response to a nerve impulse. There are four modes of spontaneous release – quantal, subquantal, giant and molecular leakage. Random miniature end plate potentials (mepps) of 0.5–1 mV may be detected by an intracellular electrode in the absence of an action potential. These are thought to be caused by quantal release of acetylcholine. The mechanisms of the four modes of release are: (i) quantal release due to exocytosis of one synaptic vesicle; (ii) subquantal release from exocytosis of an incompletely filled vesicle; (iii) giant release where a quantity of axoplasm containing acetylcholine is ejected through the membrane; and (iv) non-quantal leakage by diffusion of acetylcholine through the membrane.

When the nerve terminal is invaded by a nerve impulse, Ca2+ channels (P type) in the terminal membrane open, Ca2+ enters the nerve terminal and there is a Ca2+-dependent, synchronous release of the contents of 50–100 vesicles (Fig. 2). To enable the contents of the vesicle to be released, the vesicles must be docked at special release sites (active zones) in that part of the terminal axonal membrane which faces the post-junctional acetylcholine receptors. These are the vesicles which form the immediately available store. Once the vesicle contents have been discharged, they are rapidly refilled from the reserve store. The reserve vesicles are anchored to actin fibrils in the cytoskeleton by vesicular proteins called synapsins. Some of the Ca2+ ions that enter the axoplasm on arrival of the nerve impulse bind to calmodulin which then activates an enzyme, protein kinase II. Protein kinase II phosphorylates synapsin which then dissociates from the vesicle, allowing the vesicle to move forward to the release site (Fig. 2).

The docking of the vesicle and subsequent discharge of the vesicular contents involves several proteins. These include the...
Ca^{2+} binding proteins synaptotagmin and synaptobrevin (integral vesicle associated membrane proteins (VAMP)), synaptosomal associated protein of 25 kDa (SNAP-25) and syntaxin which are integral terminal axonal membrane proteins present at the active site or release zones. Docking involves an interaction between synaptobrevin, SNAP-25 and syntaxin. In the absence of Ca^{2+}, synaptotagmin functions as a vesicle clamp holding the vesicles in a fusion-ready state but blocking release of their contents. Calcium entry and binding to synaptotagmin activates an ATPase that becomes part of the vesicle active zone complex. Hydrolysis of ATP, together with synaptotagmin binding, leads to exocytosis.

The acetylcholine receptor

Acetylcholine receptors in the post-junctional membrane of the motor end plate are of the nicotinic type. The density of acetylcholine receptors at the end plate has been estimated as 10,000–20,000 µm^{-2}. The high density is facilitated by the presence of junctional folds (Fig. 1). Nicotinic receptors are made up of five protein subunits joined together to form a ring that penetrates through and projects on each side of the membrane (Fig. 3). Each type of protein subunit is specified by a different gene. The subunits have varying molecular weights and different properties. They are denoted by the Greek letters α, β, γ, δ and ε. There are 9 different α-type subunits (α1–α9), 4 β-subunits and 1 each of the γ, δ and ε units. Therefore, the theoretical number of different nicotinic receptor subtypes is large, but the number is limited in that all receptors contain 2 α-subunits and 1 δ, β and ε unit. (In the fetus, γ replaces ε, see below.) In addition, there are restrictions on the ability of some α-subunits to pair with others.

The receptors are synthesised in muscle cells which make a series of protein subunits and assemble them like barrel staves into cylindrical receptors. Each cylinder crosses from one side of the cell membrane to the other and has a central funnel-shaped pore which is an ion channel. The ion channel is 4 nm in diameter at the entrance and narrows to < 0.7 nm within the membrane. The receptor complex is 11 nm in length, half of which protrudes from the extracellular surface of the membrane. The protein passes through the membrane but only extends 2 nm into the cytoplasm of the muscle cell. Several proteins have been identified which appear to aggregate the receptors and anchor them to the cytoskeleton. It is thought that agrin is secreted by the motor nerve and triggers localised clustering of the receptors in the end plate. Rapsyn, which is associated with the inner surface of the postjunctional membrane, links the receptors to the underlying cytoskeleton.

When two acetylcholine receptors bind to the pentameric complex, they induce a conformational change in the proteins of the α-subunits which opens the channel. At its narrowest, the channel is large enough to let all cations pass through indiscriminately. Potassium ions leak from the inside of the
cell to the outside but this movement is minor compared with the movement of Na⁺ ions from outside to the inside. The inside of the cell is negative with respect to the outside and has a resting membrane potential of –80 mV. Thus, the Na⁺ ions are attracted to the inside of the cell and make it more positive. This induces a depolarisation or change toward a less negative charge and, once a threshold of –50 mV is reached, voltage-gated sodium channels on the sarcolemma open and allow the flow of Na⁺ ions into the muscle. This increases the rate of depolarisation, forming an action potential that passes around the whole sarcolemma, causing the muscle to contract.

The amount of acetylcholine released following a nerve action potential is far in excess of what is needed to reach the threshold at the end plate. It is estimated that only 6–25% of the acetylcholine normally released is required to reach the threshold potential. The activated acetylcholine receptor stays open for 1 msec, during which time 10⁵ Na⁺ ions enter the cell. The receptor and its channel make a powerful amplifier; the current produced by 2 molecules of acetylcholine is converted by a receptor-channel into a current carried by thousands of ions of Na⁺, K⁺ and Ca²⁺. The receptor is also a switch. It is closed until the acetylcholine binds to it and then it snaps open and passes current. When the acetylcholine leaves, the channel shuts and the current is cut off.

**Acetylcholinesterase**

Acetylcholine molecules that do not react with a receptor or are released from the binding site are destroyed almost immediately by the enzyme acetylcholinesterase in the junctional cleft. Acetylcholinesterase is an asymmetric protein which is made in the muscle under the end plate. It is secreted from the muscle but remains attached to it by thin stalks of collagen attached to the basement membrane (Fig. 3). Most of the molecules of acetylcholine released from the nerve initially pass between the strands of enzymes to reach the post-junctional receptors but, as they are released from the receptors, they meet acetylcholinesterase molecules and are broken down. Acetylcholine is a potent messenger but it is destroyed < 1 msec after it has been released.

**Perijunctional zone**

Surrounding the end plate is an area of muscle called the perijunctional zone which is important to the function of the neuromuscular junction. It is in this region that the potential developed at the end plate is converted to an action potential which then passes through the muscle to initiate contraction. It contains a mixture of the receptors found in the neuromuscular junction and the Na⁺ channels which are ordinarily found on the muscle membrane. This mixture enhances the ability of the perijunctional zone to respond to the depolarisation produced by the postsynaptic receptors and transduces it into the wave of depolarisation that travels along the muscle causing contraction.
### Extrajunctional receptors

Apart from the receptors found at the neuromuscular junction of normal healthy muscle, there is another type of receptor found in muscle cells that has little or no activity. These are termed extrajunctional receptors. They increase in number following events such as denervation injury, burn or cerebrovascular accident.

Junctional and extrajunctional receptors differ in several aspects. Junctional receptors are found at the end plate of the muscle membrane. Extrajunctional receptors tend to be concentrated around the end plate where they mix with junctional receptors. They can also be found anywhere on the muscle membrane. The structure of the extrajunctional receptor is different from that of the junctional receptor in that, although it has a similar pentameric structure with 5 subunits, the adult ε-subunit is replaced by the fetal γ-subunit. The ε- and γ-subunits do not differ significantly in amino acid structure but the differences are sufficient to affect the physiology and pharmacology of the receptor and its ion channel.

Extrajunctional receptors are not found in normal active muscle but appear very rapidly whenever muscle activity has ended or after injury has been sustained. They can appear within 18 h of injury and an altered response to neuromuscular blocking drugs can be detected within 24 h of the insult. They disappear when muscle activity returns to normal.

Extrajunctional receptors have a shorter metabolic half-life than junctional receptors. The metabolic half-life of extrajunctional receptors is under 24 h, whereas that of mature junctional receptors is about 14 days. Ion conductance and channel opening times also vary between the 2 types of receptor. Extrajunctional receptors have a 2–10-fold longer opening time than mature junctional receptors and ion conductance through the channel is smaller. There is also a difference in the response of the receptors to depolarising and non-depolarising neuromuscular blocking agents. When there are large numbers of extrajunctional receptors present, resistance to non-depolarising muscle relaxants develops with an increased sensitivity to depolarising muscle relaxants such as succinylcholine. In its most extreme form, the increased sensitivity to succinylcholine results in a lethal hyperkalaemic response. Succinylcholine depolarises both the junctional and extrajunctional receptors with the exaggerated efflux of intracellular K⁺ resulting in hyperkalaemia. The longer opening time of the ion channel on the extrajunctional receptor also results in a larger efflux of ions from each receptor.

### Synaptic maturation

At birth, the synapse is relatively immature but it undergoes dramatic structural and functional changes during the first few weeks of life. In the fetal neuromuscular junction, the acetylcholine receptors contain the γ-subunit. At birth, a mixture of both γ- and ε-subunits are present. Synaptic nuclei in the nerve cell down regulate the γ gene expression and activate the ε gene shortly after birth.

Each muscle fibre is multiply innervated at birth and all the inputs into each muscle fibre are shared by a common plaque on the post-synaptic membrane. Shortly after birth, all but one of the inputs are withdrawn in a process called synapse elimination. Synaptomедин mediates the multineuronal innervation, while synaptotoxin promotes its withdrawal. However, shortly after birth, all muscle fibres become innervated by a single nerve fibre and the post-synaptic apparatus becomes a convoluted branching process which corresponds to the branches of the single nerve terminal. Subsequent expansion of the nerve terminal and growth of the muscle fibres changes the neuromuscular junction to the adult configuration.

The adult neuromuscular junction persists throughout life but it is in a state of dynamic equilibrium with branches of nerves sprouting and retracting. However, with increasing age, the efficacy of the factors that maintain the synapse declines and the number of acetylcholine receptors at each junction starts to decline; extrajunctional receptors appear and terminal sprouting becomes more prevalent. Some portions of the neuromuscular junction are lost. It is thought that physical exercise can retard this sprouting and degeneration, whereas it is stimulated by inactivity.

### Key references


Mirakhur RK, McCourt KC, Carroll MT. The physiology of neuromuscular transmission, RCA Newsletter 1999; 45: 54–8


See multiple choice questions 85–88.