Physiology and pharmacology of neuromuscular block.

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Neuro muscular transmition fascinate anesthesiologist ever since the usage of curare as a muscle relaxant in anesthetic practice. Claude Bernard in his series of studies of the effects of curare on nerve muscle preparations suggested the electrical transmission in the nerve and the presence of chemical compounds with a critical function for the transmission of information from nerve to muscle. Vulpian later proposed that there must be a distinct junction between the nerve and the muscle and his suggestions were confirmed and further advanced by Langley who in 1905 demonstrated the role for a chemical compound released by the nerve and transmitted across the NMJ into the muscle to initiate a muscle contraction.

Morphology of neuromuscular junction

The neuromuscular junction is a specialized structure both on the nerve side and on the muscle side to transmit and receive chemical messages. From the ventral horn of the spinal cord and medulla each motor neuron s large, myelinated axon branches near a muscle and innervate a group of muscle fibers which will be scattered all over the muscle and it function as an unit known as motor unit. As the axon terminal reaches the muscle fiber, it loses its myelin and forms a spray of terminal branches against the muscle surface, and is covered only by Schwann cells.

The neuro muscular junction consists of three distinct parts

1. Presynaptic Zone (The distal motor nerve ending)

2.The synaptic cleft

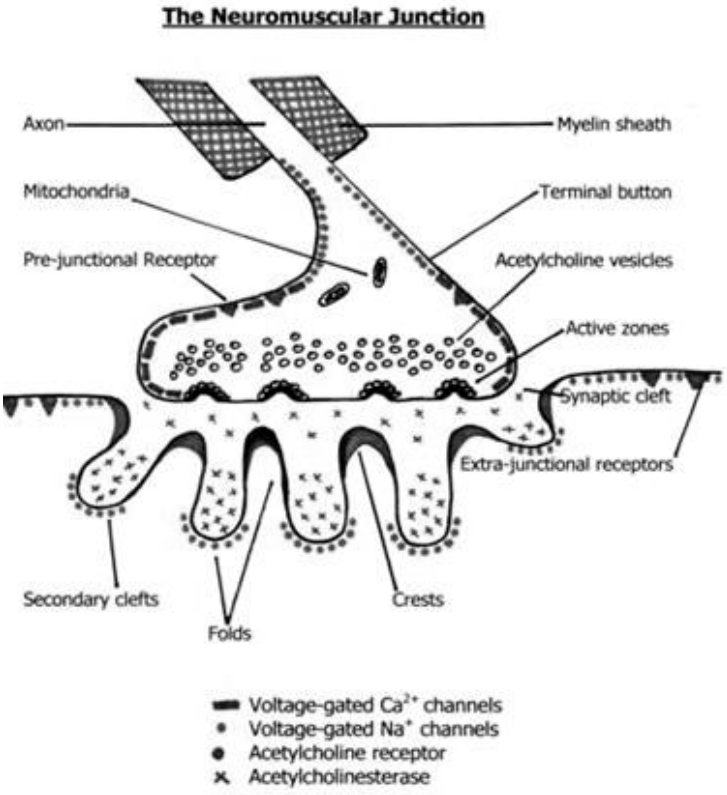
3.Postsynaptic Zone

These elements together form the NMJ, where the information from nerve to muscle is transmitted via the instantaneous release of acetylcholine (ACh) and activate ligand-gated, fast-acting nicotinic acetylcholine receptors (nAChRs). The nerve is separated from muscle by a

distance of 20nm and they are held together by a protein filament called basal lamina. The muscle membrane is deeply corrugated and at entrance of the cleft as much as 5 millions AChR are densely packed

The presynaptic zone

The presynaptic part consists of the distal demyelinated part of the motor nerve axon and it is encapsulated by a terminal Schwann cell surrounding and anchoring the nerve ending into the muscle membrane. Apart from supporting, Schwann cell plays an important role in regeneration of nerve terminal by releasing nerve growth factor and neuregulin and clean the presynaptic zone by phagocytosis. The bulk of the motor nerve cytoplasm filled with numerous synaptic vesicles filled with acetyl choline and mitochondria.



Acetylcholine synthesis and metabolism

Nervous system of the higher mammals has to depend upon the external source for Choline since they cannot synhesis choline. It is supplied through diet and synhesised in the liver. Choline from the junctional cleft is actively transported across the plasma membrane in to

the cytoplasm of the nerve terminal by sodium-dependent high affinity choline uptake system (SADACU). In the cytoplasm choline is acetylated by choline acetyl-O-methyl tranferase to form Acetylcholine.

Vesicular storage of acetylcholine`

Acetylcholine is stored in the synaptic vesicles and can be released by exocytosis. The acetylcholine is actively transported in to the vesicles against concentration and proton gradient by a system distinct from SADACU. Apart from acetylcholine high concentration of Ca++ ion and ATP are also stored in the vesicles

Acytylcholine release

The Acetylcholine is released in the form of Vesicles (qunta)when an action potential arrives. The vesicles are of two types. One is readily available pool called VP2 close to active zone another reserve pool called VP1 tethered to the cytoskeleton filaments actin, synapsin, synaptotagmin and spectrin. When action potential arrives Ca++ enter these vesicles are detached from their attachment and mobilized towards the active zone and get attached to the nerve membrane by SNSRE protein known as docking. Then a fusion pore develop releasing the acetylcholine molecules. Each vesicles estimated to carry nearly 10,000 molecules and each action potential makes 50 to 100 vesicles to discharge. Later the vesicles are retrieved and filled up known as recycling

When an action potential arrives to the nerve terminal, the P-type voltage gated Ca++ channels opens up and a large influx of Ca++ ion takes place from the extra cellular space and trigger the exocytosis of the acetylcholine vesicles. Reducing extracellula Ca++ reduces the quntal release of Acetylcholine, similarly increasing the concentration of Ca+ increase the Acetyl choline release four fold. Through K channels K+ efflux out and check Ca++ influx there by acetylcholine release.

Clinical Significance of Calcium and Potassium Channels

Hypocalcemia interfere with reversal of muscle relaxants.

Post tetanic facilitation is due to accumulation of Ca++ in the cytoplasm of motor end plate which increases the release of acetyl choline.

Aminoglycoside antibiotics block voltage-dependent calcium channels in intact.

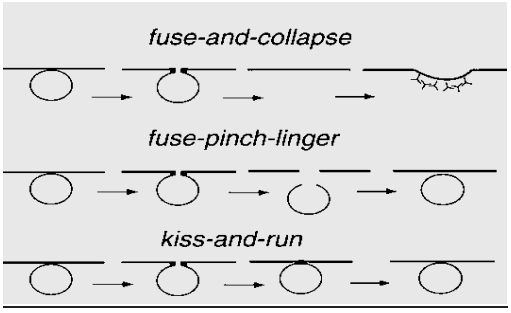
Eaton-Lambert myasthenic syndrome the antibodies are produced against the voltage gated Ca+ chanels leading to mucscle weakness and inadequate response to depolarizers and prolonged response to non depolarizers.

Voltage gated Ca+ P-type channels are not blocked by the Ca+ channel blockers like verapamil,diltiazem and nifedipine since they act by blocking the L type Ca+ channels there fore their interaction with NDMRs are clinically insignificant.

Higher magnesium concentration block the Ca+ through the P channels and this is the cause for maternal and foetal muscle weakness in pre-eclampsia.

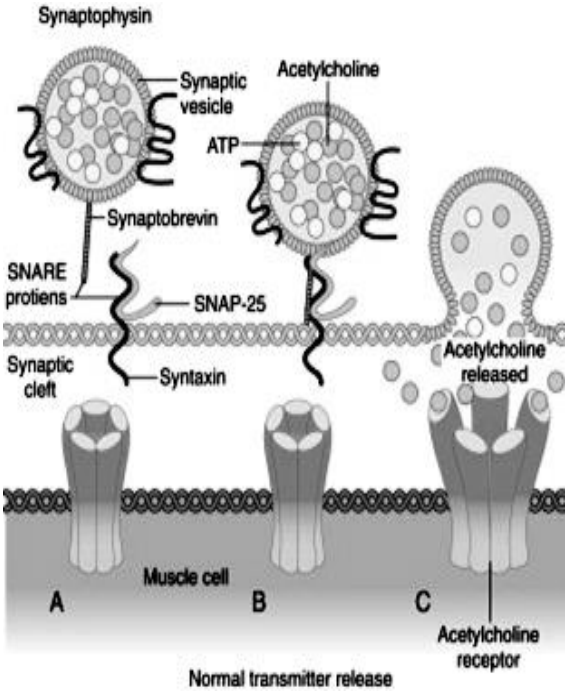
4 aminopyridine is a selective K+ channel blocker there by it increases the Ca+ influx which has been successfully demonstrated as an useful in Eton-Lambert myasthenic syndrome and multiple sclerosis.

Molecular mechanism of Exocytosis and recycling of Ach vesicles



The acetylcholine vesicles release their content through membrane by three mechanism. “Fuse and collapse” where the vesicular membrane totally fuse with the nerve membrane. “Kiss and run” where vesicles just touch the membrane and through a small hole the content is emptied. “Fuse –Pinch-Linger” where vesicle fuse with membrane later after emptying with the help of protein dynamin vesicles are reformed in to the plasma.

SNARE hypothesis



The current understanding is a Protein Complex mechanism known as "SNARE hypothesis" proved to play a major role in exocytosis of the Acetylcholine vesicles. Four proteins plays a major role in this SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor (SNARE) proteins hypothesis. Synaptotagmin and Synaptobrevin are integral to the vesicular membrane and Syntaxin and SNAP-25 are integral part of the nerve membrane. As the vesicles contact the nerve membrane synaptobrevin in the vesicular side forms a complex with Syntaxin and SNAP-25 . Assembly of this complex guide the vesicles to the active zone. The Ca++ influx is sensed by the Synaptotagmin in the vesicles leads to burst release of Acetylcholine. Further research and understanding is required to fulfill many lacunae in this SNARE protein mechanism.

Clinical application SNARE hypothesis and Exocytosis

Boltulinum toxin cleaves the snare protein there by inhibiting the release of acetylcholine from the vesicles and leads to paralysis.

α-latrotoxin a powerful neurotoxin from black widow spider act by triggering massive exocytosis probably by enhancing Ca+ entry.

The synaptic cleft

The synaptic cleft spans 50 nm from the nerve ending to the muscle membrane. The cleft contains a basal lamina made up of a multitude of large molecules forming an extracellular matrix that aids cell adhesion and appropriate neuromuscular signalling processes. Here, molecules suchas acetylcholinesterase, various isoforms of the alpha- and beta-laminin, agrin, and collagen interact and modulate conditions for appropriate neurotransmission. ACh is degraded by acetylcholinesterase in the synaptic cleft. Here, acetycholinesterase which are secreated from muscles is anchored to the lamina from where it rapidly degrades acetylcholine

Clinical relevance

In synaptic form of congenital myasthenic syndrome the cholinestrase is low. Denervation decreases acetylcholinesterase at the junctional and extrajunctional areas. Organophosphate pesticides or nerve gas (e.g., sarin) inactivate AChE by phosphorylating the serine hydroxyl group located at the active site of AChE.

The postsynaptic membrane

The postsynaptic membrane consists of multiple folds located opposite the presynaptic nerve terminal. These primary (shallower) and secondary (deeper) folds of the postsynaptic membrane expand its surface area many-fold. On the shoulder of these folds, nAChRs cluster at high density and are anchored into the cell membrane by a complex system of cytoskeletal proteins, for example, dystroglycans. In close proximity to the highly specialized postsynaptic membrane is the perijunctional zone. The perijunctional zone has a higher density of sodium channels than in other parts of the cell membrane, making this part of the muscle membrane more capable of amplifying the responses to depolarization and thus to promote the transduction process that finally leads to muscle contraction

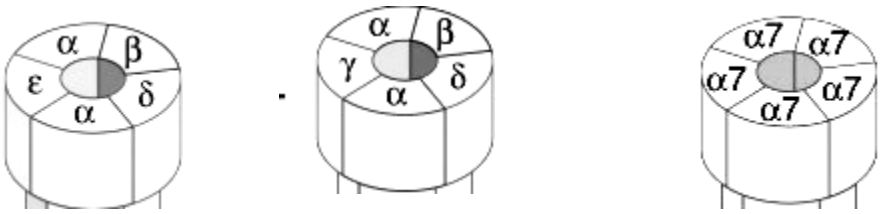
Nicotinic Acetyl choline recptor –nAChR

The nAChR is the prototype for the cys-loop superfamily of ligand-gated ion channels which also includes GABA, glycine and 5-HT3 receptors. All members of the cys-loop ligand-gated ion channel superfamily share a common structure and function. They have a common architecture with five subunits surrounding a central pore. Activation of the nAChR by ACh leads to an influx of cat ions (Na+ and Ca++) and does not allow anions. The current that passes through each open channel is 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 nAChRs have been subdivided into muscle , neuronal and non neuronal subtypes based on their classical major site of expression;

Three isoforms of postjunctional nicotinic AChRs exist, a junctional or mature receptor, an extrajunctional or immature (fetal) receptor, and the neuronal α7 receptor



mature fetal neuronal α7

Each receptor has five subunits. The mature receptor consists of α, β, δ, and ε and the fetal receptor, α, β, δ, and γ. there are two subunits of α and one of each of the others. The neuronal α7 AChR consists of five α7-subunits.

Non depolalarizing Block

Both α-subunits must be occupied simultaneously by Acetylcholine for opening the channel. If only one of them is occupied, the channel remains closed. But NDMRs like tubocurarine it is enough if it act by binding to one α-subunits and thus preventing acetylcholine from binding and opening the channel. If the concentration of NDMR is high and both the α-subunits are blocked then four fold increase of Ach is required to competitively replace NDMR.

Clinically paralysis produced by high concentrations of antagonist is more difficult to reverse than that produced by low concentrations. After large doses of NDMRs, cholinesterase inhibitors may be ineffective until the concentration of relaxant in the perijunctional area decreases to a lower level by redistribution or elimination of the drug. For this reason one has to reverse only residual NDMR block identified clinically or by using nerve stimulator.

Depolalarizing Block

By virtue of the structural similarity succinylcholine act on the Ach receptor in the motor end plate. Unlike Ach, succinylcholine is not hydrolyzed immediately the depolarization continues. When depolarization continues the perijunctional Na+ channels remain closed where as the Na+ channels away from the motor end plate on the muscle membrane remain in resting state. So impulses cannot travel from motor end plate but the muscle is amenable for direct electrical stimulation. This phenomenon of differential behaviour of Na+ channel is known as "Accommodation"

The extraocular muscles contain tonic muscle, which has multiple motor end plates from different axons. Despite its innervations, the ocular muscles express both mature and fetal receptors. Accommodation does not occur, and these muscles can undergo a sustained contracture in the presence of Succinylcholine. The tension thus developed forces the eye against the orbit and accounts for part of the increase in intraocular pressure produced by depolarizing relaxants. Still clinician and researchers are divided in their opinion regarding the Succinylcholine induced raise in intraocular pressure and extrusio of the intraocular content is due to the tonic contraction of the extraocular muscle

Noncompetitive Actions of Neuromuscular Drugs

. Drugs like procaine, ketamine, inhaled anesthetics, or other drugs that dissolve in the membrane lipid may change the opening or closing characteristics of the channel either by desensitize the receptors or by blocking the Na++ and Ca++ channels directly. These blocks are independent of the classic effects based on competitive inhibition of acetylcholine. 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.

Clinical not only NMBD act on the ACh receptor, snake poisons like α bungarotoxin irreversibly bind with post synaptic ACh receptors and paralyses skeletal muscles where as the β bugarotoxin binds to the presynaptic ACh receptors and prevent the ACh release. And in Myasthenia gravis autoantibodies against muscle nAChRs leading to reduction in number of the receptors in turn leads to muscle weakness. Increase in ACh concentration by anticholinestrase partially reverse the weakness of these patients. And they are sensitive to nondepolarizer and resistant to depolarizers.

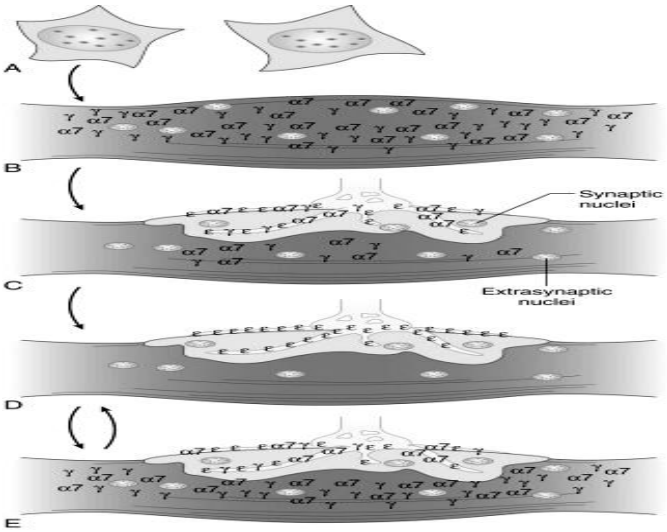
Desensitization Block

Some receptors that bind to agonists, however, do not undergo the conformation change to open the channel. Receptors in these states are termed *desensitized* (i.e., they are not sensitive to the channel-opening actions of agonists) The mechanisms by which desensitization occurs are not known.

Phase II Block

A 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. 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 the sub–end-plate elements themselves. The activity of the 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. Fade and post titanic facilitation will be present as in nondepolarizing block. Reversal with cholinesterase is unpredictable.

Maturation Of NMJ and Ach Receptors



Before birth, each muscle cell commonly has contacts with several nerves and has several neuromuscular junctions. At birth, only one end plate remains. Once formed, the nerve-muscle contact is permanent. In adult one muscle fiber contain only one end plate with exception to Ocular muscles where they are multiple innervation with several neuromuscular junctions along the surface of each muscle cell. As motor nerve axons grow into the developing muscle, and these axons bring in nerve-derived signals (i.e., growth factors), including agrin and neuregulins (NRβ-1 and NRβ-2), essential for maturation of myotubules. Agrin is a protein from the nerve that stimulates postsynaptic differentiation by activating muscle-specific kinase (MuSK), Agrin, MuSK and ErbB proteins are responsible for the Ach receptors scattered all over the muscles to cluster at the area immediately beneath the nerve and also for its maturation and stabilization

Extra Junctional receptor.

Just before and shortly after birth and after denervation the immature, γ-subunit–containing AChRs and α7AChR are distributed all over the muscle membrane including the motor end plate and they are known as extrajunctional receptors.

In the early fetal stage, mononucleated myoblasts, derived from the mesoderm, fuse with each other to form multinucleated myotubes. The γ-subunit–containing immature acetylcholine membrane before innervation. As the nerve makes contact with muscle, clustering of the receptors occurs at the synapse and is associated with loss of extrasynaptic receptors. Maturation of the junction is said to occur when ε-subunit–containing receptors replace the γ-subunit and α7-containing acetylcholine receptors at the neuromuscular junction. A child is usually about 2 years old before nerve-muscle contacts are mature. These fetal type of receptors open up for a longer duration for efflux of K+ ion and they are more sensitive to Depolarizer and resistant to NDMB.

The α7 AChRs in muscle are different from conventional muscle (α1, β1, δ, ε/γ) AChRs and neuronal α7 AChRs in the brain. Choline, a precursor and metabolite of acetylcholine (and succinylcholine), is an extremely weak agonist of conventional muscle AChRs but is a full agonist of muscle α7 AChRs. Furthermore, no desensitization of the α7 AChR occurs even during the continued presence of choline, thus allowing a greater chance for potassium to efflux from within the cell. Muscle α7 AChRs are different from neuronal (autonomic ganglia and brain) α7 AChRs. Muscle α7AChRs, sensitize with choline, where as neuronal α7AChRs are desensitized with choline. The α7 AChR in muscle also has lower affinity for its antagonists, including pancuronium and

α-bungarotoxin. In the conventional AChRs, binding of even one of the α1-subunits by an antagonist results in inactivation of that receptor because acetylcholine needs both α1-subunits of the AChR for its activation. In the α7 AChR, however, even when three subunits are bound by an antagonist (e.g., muscle relaxant), two other subunits are still available for binding to agonist and causing depolarization. This feature may account for the resistance of α7 AChRs, as opposed to conventional AChRs, to the blocking effects of drugs such as pancuronium.

In clinical usage of NMBD in newborn and premature children the scenario is different. They require some time even triple the adult dose of Succinylcholine. For NDMB drugs new born are sensitive and less dose of drug is required since the motor end plate is ill developed with less number of receptor. This paradox of receptor sensitivity and clinical dose may be due to the volume of distribution and immature drug elimination system.

Re-expression of Immature AChRs in Adult Life

Denervation , burns, immobilization, chronic muscle relaxant therapy, stroke and sepsis lead to re-expression of the γ-subunit– and α7-containing acetylcholine receptors at the junctional and extrajunctional areas (The latter changes are potentially reversible if muscle immobilization/catabolism/inflammation is restored to normal) known as upregulation. The mature receptor have a life span of 2 weeks where as the newly developed immature extrajunctional receptor is only 24 hours.Sensitivity to relaxants can begin to change between 24 and 72 hours after an injury. Since the γ -subunit– and α7-containing acetylcholine receptors get distributed allover the muscle membrane and succinylcholine can keep the receptor channel open for longer time (increased sensitivity) large efflux of K+ ion take place leading to life threatening hyperkalemia. In this clinical state they are less sensitive to nondepolarizer and pretreatment with nondepolarizing relaxants in the usual doses will not prevent succinylcholine-induced hyperkalemia

Prejunctional Acetylcholine Receptors.

Nicotinic autoreceptors are localized at the presynaptic nerve terminal and are responsible for the increased release of ACh into the synaptic cleft during high frequency stimulation of the presynaptic nerve terminal.

They are nAChR a3b2. They are blocked by NDMR but not by Succinylcholine. The phenomenon like fade after NDMR , absents of fade after depolarizer and prevention of fasciculation induced by succinylcholin by NDMR are explained by this prejunctional block by NDMR. Muscrinic M1and M2 are involved in facilitation and inhibition of acetylcholine release respectively by modulating Ca+ influx.

Carotid body nAChR

The neuronal nAChR subunits a3–a5, a7 and b2, b4 have been found to be of importance in oxygen signaling from oxygen sensing chemoreceptor type-1 cells in carotid body against hypoxia and generate a hypoxic ventilatory response. NDMR block these recptors too and depress the hypoxic ventilatory response. For this reason that during recovery TOF response should be >0.9 for total recovery NDMRblock.

The understanding of Neuromuscular junction structure and physiology has come on long way. The lacunae in the current understanding in course of time will be fulfilled. At that time the present understanding may be modified or even changed! So constant up date is mandatory for better understanding and clinical practice.

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