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Physiology of the neuromuscular junction and related disorders

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ABSTRACT

The neuromuscular junction (NMJ) is a region of communication between the nerve and muscle cells. Non-anatomical functional contact occurs at this junction. The NMJ is divided into three regions: presynaptic, synaptic gap, and postsynaptic. When action potentials reach the terminal end of the nerve, acetylcholine (ACh) molecules in the presynaptic region are released into the synaptic gap. The released ACh stimulates nicotinic ACh receptors and depolarizes the motor endplate. This depolarization is transformed into actual action potential when a threshold value is surpassed. At this point, ACh in excess that required for neurotransmission is released. Subsequently, the ACh receptor is stimulated. The principal reason for excess production of ACh is to ensure a sufficient supply for neurotransmission. The NMJ is thought as a static structure. However, this structure has dynamic remodeling activity, which is significantly affected by drugs, toxins, aging, injury, and exercise. The net effect of pathologies involving the NMJ is to decrease the ability of neurotransmission. These pathologies can be congenital, acquired, or specific to presynaptic, synaptic, or postsynaptic regions. The etiological origins of NMJ pathologies include autoimmunity, congenital disease, pharmacological or toxic agents, and trauma. In this review, physiology and related disorders of the neuromuscular junction considered in the light of recent knowledge has been aimed.

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1. Introduction

Studies of the physiology of the neuromuscular junction (NMJ) commenced in 1853 when the French physiologist Claude Bernard first described neurotransmission; the French neurologist Alfred Vulpian further defined the NMJ, and the British physiologist John Newport Langley demonstrated the role of acetylcholine (ACh) in the NMJ (Glick, 2009; Cousin, 2013).

In the human body, there are over 400 striated muscles. The total mass of striated muscles is nearly equal to 40-50% of the total body weight (Widmaier et al., 2004; Martyn, 2009). Striated muscles contract and relax but tire more rapidly than smooth muscles. Unlike smooth muscle cells, striated muscle cells contain more than one nuclei. Structurally, striated muscles contain filaments of myofibrils called as myofilaments and myofibrils, which form muscle cells (muscle fibers). Muscle fibers form fascicles, and fascicles comprise anatomical muscle tissue (Widmaier et al., 2004; Martyn, 2009).

Each muscle fiber contains myofibrils, varying in number between a few hundred and a thousand. Each myofibril is composed of nearly 1500 myosin and 300 actin filaments. When observed under a microscope, myosin filaments are darker that is why called thick and actin filaments are lighter so they are called thin. Besides actin, myosin is responsible for the striated appearance of skeletal muscle (Widmaier et al., 2004; Martyn, 2009).

Actin fibrils are composed of actin, tropomyosin, and troponin molecules. Two actin fibrils join to form an actin filament. Myosin consists of tail, neck, and head regions, and hundreds of myosin come together to form a myosin filament. Actin and myosin filaments act in concordance to achieve contraction. The smallest component of an actin-myosin unit is called a sarcomere (Widmaier et al., 2004; Martyn, 2009).

Muscle fiber contains many mitochondria to provide energy. Ca^{++}, which has a significant role in contraction, is

stored in the sarcoplasmic reticulum (SR), which occupies a large space in the cell. The cell membrane that surrounds muscle fiber is called the sarcolemma. Invaginations of the sacrolemma, known as T-tubule, transfer action potential to myofibrils located deep within muscle fibers (Widmaier et al., 2004; Martyn, 2009).

Neural signals arising from the motor cortex are transmitted by alpha motor neurons originating from the anterior horn of the spinal cord. These are large neurons with axons, with fast signal transmission capacities. When a neuron approaches the muscle tissue, divides into 20-100 branches, and form an NMJ near the middle of the muscle fiber. Each one of branches innervates one muscle cell. The resulting functional unit is called a motor unit. The number of muscle fibers in the motor unit varies. For example, in sensitive muscle groups, such as the eye muscles, the motor neuron/muscle fiber ratio is 1:1, whereas the ratio is 1:100 in back muscles. The action potential generated in one motor neuron stimulates all muscle fibers contained in the motor unit (all-or-none principle) (Widmaier et al., 2004; Martyn, 2009).

2. Contraction of skeletal muscle

When action potential coursing through a motor neuron axon reaches the terminal end of the nerve, voltage-gated Ca^{++} channels in the presynaptic region open, and Ca^{++} rushes through the gates, triggering exocytosis of synaptic vesicles containing ACh. ACh binds to nicotinic acetylcholine receptors (AChRs). Ca^{++} and Na^{+} cations then enter the muscle fiber, and some K^{+} cations are expelled from the cell. This ion exchange causes regional depolarization, known as end-plate potential (EPP). When this local depolarization surpasses a certain threshold, it is amplified through the action of voltage-sensitive Na^{+} channels located in the perisynaptic region and postsynaptic membrane. The EPP is converted to an actual action potential through this process (Cross and Plunkett, 2008; Martyn, 2009).

Once an action potential forms, it is disseminated into the cell through T-tubules. This process stimulates voltage-dependent L-type Ca^{++} channel receptors located in the T-tubules (dihydropyridine receptors [DHPRs]). These receptors link to ryanodine receptors located in the SR. Stimulation of the DHPR results in physicochemical changes in the ryanodine receptor, leading to its activation. A small amount of Ca^{++} enters cells when the DHPRs are stimulated. This phenomenon activates the ryanodine receptor and triggers the entry of increased amounts of Ca^{++} into cells (excitation-contraction coupling) (Cross and Plunkett, 2008; Martyn, 2009).

The released Ca^{++} binds to troponin C, causing conformational rotation of troponin. Tropomyosin is normally found in the cleavage furrows of actin filaments and prevents binding of actin to myosin. The conformational rotation of troponin removes tropomyosin from these furrows and exposes active sites for myosin binding. The myosin head then binds to actin to achieve contraction. Via the action of adenosine triphosphatase (ATPase), adenosine triphosphate (ATP) provides the required energy for contraction. To be effective, ATPase requires the presence of Mg^{++}. When the muscle is relaxed, the angle between the myosin head and neck is 90°. With contraction, this angle narrows to 45-50°. Actin and myosin overlap and slide over each other, shortening the length of the sarcomere (sliding filaments mechanism) (Cross and Plunkett, 2008; Martyn, 2009).

3. Relaxation of skeletal muscle

To enable skeletal muscle relaxation, the following has to occur:

1-ATP binds to the myosin head after contraction (smoothing effect of ATP). In the absence of ATP, myosin cannot separate from actin, and muscle relaxation does not occur, as is the case in rigor mortis.

2-ACh released in the synaptic gap is degraded by the action of acetylchloinesterase.

3-Calcium ions are taken up into the SR by the activity of the calcium pump known as sarco/endoplasmic reticulum Ca^{++}-ATPase (SERCA).

SERCA plays a role in the above process. In this process, contraction and relaxation are ATP-dependent events (Cross and Plunkett, 2008; Martyn, 2009).

4. Neuromuscular junction

The NMJ is a region of communication between nerve and muscle cells. Non-anatomical functional contact occurs at the NMJ. The NMJ is a type of synaptic region, and transmission of signals is realized in the extracellular region (humoral-type transmission), mediated by the neurotransmitter ACh (Widmaier et al., 2004; Martyn, 2009; Bittner and Martyn, 2013).

From structural and functional perspectives, the NMJ can be divided into presynaptic, postsynaptic, and synaptic gap regions.

4.1. Presynaptic region

The presynaptic region is a terminal nerve ending where synthesis, storage, release, and retrieval of choline after hydrolysis are achieved. This region also contains synaptic vesicles, which are synthetized in the Golgi body located in the motor neuron cell and transported to the terminal end of the axon. There are nearly 300.000 vesicles in the nerve endings. Each contains 10.000 ACh molecules. Vesicles cluster in active zones, which are specialized membranous areas.

Voltage-gated-Ca^{++} channels located near these vesicles are responsible for rapid ACh release. Entry of Ca^{++} into the presynaptic membrane is prevented by Mg^{++}, cadmium (Cd^{++}), and manganese (Mn^{++}). (Widmaier et al., 2004; Martyn, 2009; Bittner and Martyn, 2013).

4.2. Synaptic gap region

The width of the synaptic gap is nearly 50 nm. In the synaptic gap, basal lamina, acetylcholinesterase, laminin, and collagen are found. Acetylcholinesterase is a carboxylase, which is synthetized by muscle cells. It is responsible for the degradation of ACh (Widmaier et al., 2004; Martyn, 2009; Bittner and Martyn, 2013).

4.3. Postsynaptic region

Muscle membrane specialization occurs in the postsynaptic region, with primary and secondary folds. AChRs are distributed unevenly in the membrane. Voltage-gated Na^{++} channels are found in the deeper parts of the folds and in the

perisynaptic domain (Widmaier et al., 2004; Martyn, 2009; Bittner and Martyn, 2013).

5. Neuromuscular transmission

When action potential approaches the terminal ending of the nerve, nearly one million ACh molecules are released into the synaptic gap, stimulating 250.000 AChRs. Following the stimulation of the receptors, Na^{++} and a small amount of Ca^{++} enter the cells. At the same time, K^{+} is expelled from the cell. The inner side of the muscle membrane is more negatively (-80 millivolts) charged than neurons (-70 millivolts), causing a higher amount of Na^{++} to enter the cells and a lower amount of K^{+} to exit the cells. Membrane depolarization then occurs, with ensuing formation of the EPP. At this time, higher amounts of ACh than required for neuromuscular transmission (NMT) are released, and AChRs are stimulated. Thus, the ensuing depolarization has a much higher amplitude than required. The fundamental justification of this process is to create sufficient ACh for NMT (Widmaier et al., 2004; Bittner and Martyn, 2013).

6. Nicotinic acetylcholine receptors

AChRs are ligand-gated ionic channels with a pentameric configuration. In receptors with a pentameric configuration, five protein subunits are aligned around a central ionic channel. They can be localized in postsynaptic, presynaptic, and perisynaptic regions. Cations, such as Na^{+}, K^{+}, and Ca^{++}, easily pass through these receptors. However, as anions are located at the entrance of the channel, negatively charged ions, such as Cl^{-}, cannot enter these channels (Martyn, 2009; Bittner and Martyn, 2013).

There are three clinically important AChRs subtypes:

1. $\alpha 3\beta 2$ is found in the presynaptic membrane. Although its functions are not fully understood, it is presumed to act as an autoreceptor.

2. $2\alpha 1\beta 1\delta\varepsilon$ is found in the postsynaptic membrane. It is the major mature nicotinic receptor involved in NMT.

3. $2\alpha 1\beta 1\delta\gamma$ and $\alpha 7$ are found in the postsynaptic membrane during fetal life.

These immature receptors are replaced by mature forms within a very short time after birth. However, in some pathological conditions (motor neuron injury, immobilization, burns, infection/inflammation, and life-threatening diseases), the receptors may be seen in postsynaptic and perisynaptic regions. $2\alpha 1\beta 1\delta\gamma$ and $\alpha 7$ AChRs are synthesized within hours of a pathological incident and can be found in all muscles within a few days (Zhou et al., 2009; Bittner and Martyn, 2013).

The channels of immature AChRs are smaller than those of mature forms, and their duration of patency is 2-10 times longer when compared with mature forms. Immature AChRs are also very responsive to ACh and succinylcholine (SCh), efficacious, even at doses of 1/10-1/100. Succinylmonocholine and choline, which are metabolites of SCh, can stimulate these receptors (Zhou et al., 2009; Bittner and Martyn, 2013).

7. Plasticity of the neuromuscular junction

Up to very recently, the NMJ was thought to be a static structure. However, recent studies have shown that it has dynamic and continual remodeling activity (Wilson and Deschenes, 2005; Deschenes et al., 2006) This activity can be affected by drugs (nondepolarizing neuromuscular blockers [NMBs]), toxins (botulinum, tetanus-clostridial, tetrodotoxin), aging, injuries, and exercise (Deschenes et al., 2006; Bittner and Martyn, 2013).

8. Factors affecting the functioning of the neuromuscular junction

8.1. Aging

Aging results in functional denervation, atrophy, and loss of muscular strength. The predisposition of elder people to contraction-induced injury and the age-related reduction in the regeneration of muscle contribute to atrophic changes. Despite morphological and physiological changes in the NMJ, elder people can sustain NMT (Bittner and Martyn, 2013).

8.2. Neuromuscular blockers

NMBs are used to induce muscular paralysis in operating rooms and intensive care units. These agents are divided into two groups of drugs: depolarizing, and nondepolarizing. Depolarizing agents mimic the effects of ACh. Only SCh is used in clinical practice. SCh first binds to AChRs and then induces depolarization and muscular fasciculation. As the separation of SCh from its receptor takes longer relative to that of ACh, it induces prolonged depolarization and flask paralysis. The synaptic gap does not contain cholinesterases. Thus, termination of the effect of SCh depends on its diffusion from the synaptic gap into the circulation. In the blood circulation, it is rapidly inactivated by plasma cholinesterases (Naguib and Lien, 2009; Bittner and Martyn, 2013). Nondepolarizing NMBs are competitive antagonists of AChRs. Reversal of their effects is based on increasing the amount of ACh in the synaptic region via inhibition of acetylcholinesterases. A novel agent, γ-cyclodextrin (sugammadex), can also reverse the effects of nondepolarizing NMBs, such as rocuronium and vecuronium, by directly binding them or encapsulating them (Naguib and Lien, 2009; Bittner and Martyn, 2013).

Apart from these agents, some drugs exert receptor desensitization effects on AChRs and/or Na^{+} channels and directly block the functions of these channels (Table 1; Bittner and Martyn, 2013). As a result, NMT slows down, and sensitivity to the effect of the NMBs develops. With receptor desensitization, the channel closes due to prolonged exposure of the receptor to an agonist agent. Although the exact mechanism underlying receptor desensitization is not known, phosphorylation of tyrosine amino acids in the receptor has been blamed. In direct channel blockade, NMBs occlude the pores of the channel. As the ACh binding sites are open, the increase in the amount of ACh also increases the number of channels with open pores and hence intensifies blockade. This mechanism explains why anticholinesterases are avoided in the presence of deep anesthetic blockade (Naguib and Lien, 2009; Bittner and Martyn, 2013).

9. Neuromuscular junction abnormalities

Pathologies affecting the NMJ decrease the ability of NMT. These pathologies can be inherited or acquired and can involve presynaptic, synaptic, or postsynaptic regions. Possible etiological factors include autoimmune, congenital disease, pharmacological or toxic agents, and trauma. Most of the pathologies are complex in nature, and more than one mechanism can be involved in its etiology (Bittner and Martyn, 2013; Butterworth et al., 2013).

Table 1. Agents demonstrating receptor desensitization and direct channel blockade effects on AChRs and/or Na^+ channels

Acetylcholinesterase inhibitors	**Calcium channel blockers**
Neostigmine	Verapamil
Edrophonium	Diltiazem
Pyridostigmine	Nicardipine
	Nifedipine
Agonists	**N-Methyl-D-aspartate receptor antagonist**
Acetylcholine	Ketamine
Decametonium	
Carbachol	
Alcohols	**Phenothiazines**
Ethanol	Chlorpromazine
Butanol	Trifluoperazine
Propanol	Prochlorperazine
Antiepileptics (acute use)	**Volatile anesthetics**
Carbamazepine	Sevoflurane
Phenytoin	Isoflurane
	Desflurane
Antibiotics	
Aminoglycosides	
Polymyxin	

9.1. Congenital myasthenic syndrome

Mutational changes in the NMJ can result in congenital myasthenic syndrome, which is inherited as an autosomal recessive trait. Mutations affecting the release and resynthesis of ACh, acetylcholinesterases, and AChRs have been reported. Clinically, patients present with symptoms of muscle weakness, hypotonia, respiratory distress, and ptosis. The diagnosis of congenital myasthenic syndrome is based on clinical and electrophysiological tests. Treatment includes anticholinesterases (pyridostigmine), ephedrine/salbutamol, and quinine (Amato and Russell, 2008; Ramani, 2012; Urban, 2012).

9.2. Myasthenia gravis

Myasthenia gravis is the most frequently seen postsynaptic NMT disorder. It is associated with the formation of autoantibodies against postsynaptic AChRs (20% of myasthenia gravis patients have no autoantibodies). As a result of complement-mediated degradation, the number of postsynaptic folds, Na^+ and AChRs channels, and amplitudes of EPP decrease. There is a concomitant compensatory increase in the release of ACh. Clinically, muscular fatigue and weakness are present. The ocular muscles are the first to be affected, followed by the extraocular, bulbar, and proximal muscles, sometimes resulting in the patient requiring mechanical ventilation. Anticholinesterase inhibitors (pyridostigmine and neostigmine) are used to treat the disease. Patients with myasthenia gravis are often sensitive to NMBs (Urban, 2012; Bittner and Martyn, 2013; Butterworth et al., 2013).

9.3. Lambert-Eaton myasthenic syndrome

In this acquired syndrome, patients produce autoantibodies against presynaptic Ca^{+2} channels. Subsequently, the amount of ACh released and the number of active zones decrease. Clinically, muscular weakness (proximal parts of the extremities), fatigue, and autonomic dysfunction are observed. Although oropharyngeal and ocular muscles are rarely affected, respiratory involvement can be seen, as in the case of myasthenia gravis. In some patients, small-cell lung cancer accompanies the clinical picture. Typically, increased muscle strength is noted after recurrent muscular activities. In common with myasthenia gravis, patients with Lambert-Eaton myasthenic syndrome often show sensitivity to NMBs (Urban, 2012; Bittner and Martyn, 2013; Butterworth et al., 2013).

9.4. Botulism

Botulism is a clinical syndrome caused by Clostridium botulinum, which is an anaerobic, gram (+) microorganism. The botulinum toxin released from this microorganism exerts potent, neurotoxic effects on the NMJ. Improper preparation of canned foods is the most frequent cause of exposure to this toxin, resulting in food poisoning. Blockade of the release of ACh leads to weakness of the neck, extremity, and torso muscles, eventually resulting in respiratory failure. The toxin is topically applied as a treatment for spastic strabismus, blepharospasms, and wrinkles. Systemic side effects have been reported with topical use (Glick, 2009; Bittner and Martyn, 2013).

9.5. Immobility

Prolonged immobility progresses with muscular atrophy and an increase in the number of $2\alpha1\beta1\gamma$ and $\alpha7$ AChRs. Resistance to nondepolarizing NMBs starts from day 4 of immobility. SCh-related hyperkalemic arrest has been reported after day 5 of immobilization at the earliest. As a general rule, SCh should not be used in patients immobilized for more than 48-72 h. The pathological changes resolve a few months after mobilization of the patient (Bittner and Martyn, 2013).

9.6. Malignant hyperthermia

Malignant hyperthermia is a rare, life-threatening musculoskeletal metabolism disease caused by calcium dysregulation in the skeletal muscle. Predisposed individuals generally have a mutation in the ryanodine receptor gene, with autosomal dominant inheritance. When these individuals are exposed to volatile anesthetics and/or SCh, severe and uncontrolled oxidative metabolism can be triggered in the skeletal muscle. Combined with the increase in body temperature that accompanies malignant hyperthermia, the condition can result in circulatory collapse if not treated. Dantrolen, a direct musculorelaxant agent, is used as therapy. It exerts its effect by preventing the release of Ca^{++} from the SR (Urban, 2012; Bittner and Martyn, 2013; Butterworth et al., 2013).

9.7. Organophosphate intoxication

Organophosphate-based insecticides and pesticides are used in agriculture and in chemical weapons (e.g., sarin nerve gas). They irreversibly inhibit the function of acetylchloinesterase, resulting in cholinergic neuroeffector effects/postganglionic parasympathetic effects (e.g., bradycardia, diarrhea, lacrimation, salivation), autonomic ganglionic effects (e.g., sympathetic ganglionic effects, hyperperspiration), NMJ effects (e.g., muscle weakness, paralysis, twitching, respiratory failure), and central effects (e.g., stupor, coma, seizures). In addition to supportive treatment, atropine and pralidoxime (cholinesterase reactivating agents) are used (Baker, 2009; Hines and Marschall, 2012).

10. Conclusion

In conclusion, it is important to be aware that the NMJ is an active rather than a static structure, which undergoes continuous remodeling and exhibits complex and dynamic NMT processes. The net impact of pathologies affecting its remodeling activity and NMT is a reduction in the ability of NMT.

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