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NEUROMUSCULAR JUNCTION IN CELL NECROSIS AND APOPTOSIS

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Abstract

The neuromuscular junction (NMJ) is a unique, specialized chemical synapse that plays a crucial role in transmitting and amplifying information from spinal motor neurons to skeletal muscles. NMJ complexity ensures closely intertwined interactions between numerous synaptic vesicles, signaling molecules, ion channels, motor neurons, glia, and muscle fibers, making it difficult to dissect the underlying mechanisms and factors affecting neuro-degeneration and muscle loss. Muscle fiber or motor neuron cell death followed by rapid axonal degeneration due to injury or disease has a debilitating effect on movement and behavior, which adversely affects the quality of life. It thus becomes imperative to study the synapse and intercellular signaling processes that regulate plasticity at the NMJ and elucidate mechanisms and pathways at the cellular level. The neuromuscular junction (NMJ) is the site of communication between motor nerve axons and skeletal muscle fibres, it is composed of four specialized cell types; Motor neurons, schwann cells, muscle fibre and the recently discovered kranocyte, the functions of the NMJ is to transmit signals from the motor neuron to the skeletal muscle fibre quickly and readily to ensure precise control of skeletal muscle contraction and therefore voluntary movement. The ability of the transmission is aided by specialized architecture (multiple active zone junctional folds) that promotes high level of transmitter released, large and reliable postsynaptic responses to transmitter binding and rapid termination of signaling event. The site where the terminus of a motor neuron (axon) meets with skeletal muscle firbre is quite complex. The motor neuron is a myelinated one, each of the branch produced by the nerve fibre is in close proximity with the muscle fibre membrane and each of the branches of the motor neuron invaginated in the muscle fibre but however, lie totally outside the skeletal muscle fibre membrane (sarcolemma).The motor nerve (axon) contain many sac-like structure known as mitochondria, suspended also in

the intracellular fluid of the axon terminus are many tiny dots/sacs referred to as vesicles in which are stored the synthesized acetylcholine (ACh) molecules. These dots are better known as synaptic vesicles several hundred are usually within each branch of the nerve terminus. The vesicles contains membrane protein called synapto-brevin which when fuses with the axon membrane protein synthaxin brings about exocytotic release of its (ACh) contents into the synaptic cleft and the binding of the released neurotransmitter to its receptors brings about muscle contraction.

Keywords: neuromuscular junction, apoptosis, end-plate potential, acetylcholine, neurotransmitters.

GENERAL OVERVIEW ON NEUROMUSCULAR JUNCTION IN CELL NECROSIS AND APOPTOSIS.

The neuromuscular junction (NMJ) is a large cholinergic synapse responsible for muscle functioning. Any structural changes or degeneration of this vital junction could result in motor neuron cell death and muscle atrophy. The NMJ comprises three integral components working in tandem a presynaptic motor neuron terminal, the intrasynaptic basal lamina1 in the synaptic cleft, and the postsynaptic muscle membrane (Punga and Ruegg, 2012). In response to stimuli, activated multimeric neuronal voltage-gated channels open, leading to a calcium influx into the cell. The calcium induces the acetylcholine (ACh)-filled synaptic vesicles to fuse with the neuronal cell membrane, releasing the neurotransmitter into the synaptic cleft. ACh binds to the highly dense nicotine acetylcholine receptors (nAChR) on the post synaptic muscle leading to the depolarization of the membrane through activation of the sodium channels. This depolarization further activates voltage- gated L-type calcium channels and ryanodine receptors in the muscle, triggering calcium release from the sarcolemma. The released calcium binds to troponin C, inducing the motion of the motor protein elements. The figure below shows a schematic of synaptic transmission at the NMJ. Even though the preformed AChR clusters on the post synaptic membrane may have a role in determining where innervations of muscle fibers occur, (Ziskind-Conhaim and Bennett, 1982) studies have shown that the extending axons of motor neurons form synapses at a different location. (Anderson and Cohen, 1977) There is species-dependent pre- and post- synaptic differentiation with differences observed in NMJ.

The NMJ was initially explored as a synapse to understand synaptic plasticity, development, and function due to its size and easy access. However, the rising prevalence of neuro- degenerative and muscular disorders also necessitated a model to study the pathophysiology underlying diseases, such as amyotrophic lateral sclerosis (ALS) and myasthenia gravis, where the denervation at the NMJ results in the weakening of limbs and increasing unsteadiness in gait (Gonzalez-Freire, *et al*., 2014).Understanding the pathogenesis of these disorders at the NMJ is

hence critical for early diagnosis and effective treatment. This pressing imperative to investigate new treatment strategies underlines the need for better models that can provide some much needed insights into the mechanisms involved in NMJ disruption. Advances in healthcare have led to shifts in age demographics with the percentage of the older population increasing each year. Age-associated degeneration of the NMJ has thus increased the urgency for the development of robust NMJ models to understand the mechanism involved in motor neuron death and muscle loss apart from evaluating novel therapeutic interventions for the same. Fabrication of NMJ has also been aimed toward tissue engineering the stretch reflex arc and may have applications in the field of robotics and prosthetics (Guo, *et al*., 2012). Muscle and nerve communicate at the level of a specialized region, namely the neuromuscular junction (NMJ), a synaptic connection where the peripheral nervous system contacts skeletal muscle fibers, governing crucial vital processes, such as body voluntary movements and breathing (Rudolf, et al., 2019). Nerve activity guarantees not only muscle contraction but can induce myoblast orientation (Schiaffino and Reggiani, 1994) and strictly influences fiber type specification and myosin isoforms expression. Skeletal muscle fibers can be generally classified as fast or slow twitch, based on their contractile and metabolic properties (Olson, and Williams, 2000). These properties are dependent on the pattern of motor nerve stimulation. The skeletal muscular cell contraction is in response to the stimulation from a nerve firbre which supplies the muscle usually half way along its length.

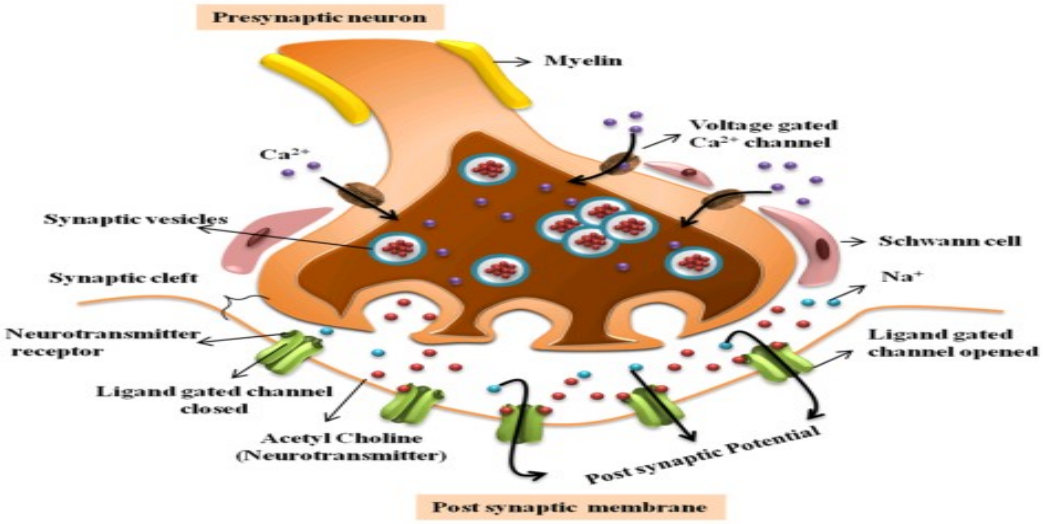

Figure 1. The NMJ: influx of calcium (Ca^{2+}) into the motor neuron leads to release of the neurotransmitter, ACh into the synaptic cleft. ACh binds to the receptors on skeletal muscle membranes opening up ion channels, leading to muscle depolarization and contraction.

When the action potential spread from the nerve along the sarcolemma it is conducted deep into the muscular cell through a special network of channels that run through the sarcoplasm and release calcium from the extracellular store. The calcium triggers the binding of the myosin to the actin filament next to it form the so called so-called cross bridges. ATP provides the energy for the two filament to slide over each other pulling the z -line at each end of the sarcolemma closer to one another shortening the sarcomere. If enough fibres are stimulated to do this at the same time the whole muscle will shorten (contract) this is what called the sliding theory.

The muscle relaxes when nerve stimulation stops, calcium is pumped back in the intracellular storage area which break the cross-bridge between the actin and myosin filament. They then slide back in the starting position lengthening the sarcomeres and retaining the muscle to its original length. The axons of the motor neurons carrying impulses to the skeletal muscle to produce contraction is divided in number of fine filament terminating in minute pads called synaptic knobs. The space between the synaptic knobs and the muscular cell is called the synaptic cleft or space. (Witzemann, 2006).

However, NMJ, allows efferent signals from the nervous system to contact muscle fibres causing them to contract .In vertebrate neuromuscular junction is always excitatory, therefore, to stop contraction of muscle, inhibition must occur at the level of efferent motor neuron. In other words, the inhibition must occur at the level of spinal cord. Release of Acetylcholine (ACh) vesicles from the pre-synaptic terminal occur only after adequate depolarization of the efferent nerve. Once a motor nerve action potential reaches the pre-synaptic nerve terminal it causes an increase in the intracellular calcium concentration by causing an increase in ion conductance through voltage gated calcium channels. This increase in calcium ion concentration allows the acetylcholine vesicle to fuse the plasma membrane at the pre-synaptic membrane in the process called exocytosis thus releasing Acetylcholine into the synapse. ACh is present in the synapse it is able to bind to nicotinic Acetylcholine receptors (AChRs) increasing conductance of certain cations; sodium (Na^+) and potassium (K^+) into the post-synaptic membrane and producing excitatory end plate-current. As cation flows into the post-synaptic cell, this causes depolarization, as the membrane voltage increases above normal resting potential -10mv for intracellular of fluid nerve ending and -80mv of the motor end plate of the post-synaptic membrane. If the signal is of sufficient magnitude, then an action potential will be generated post-synaptically. The action potential will propagate through the sarcolemma to the interior of the muscle fibres eventually leading to an increase in intracellular calcium levels and

subsequently initiating the process of Excitation-contraction coupling. Once the coupling begins it allows the sarcomeres of the muscle to shorten, thus leading to the contraction of the muscle. Any malfunction in any of the myoneuronal transmission results to any of the common neuromuscular junction diseases or disorders.

NEUROTRANSMITTERS, SYNAPSES, AND IMPULSE TRANSMISSION

As noted earlier, synapses are the junctions where neurons pass signals to other neurons, muscle cells, or gland cells. Most nerve-to-nerve signaling and all known nerve-to-muscle and nerve-to-gland signaling rely on chemical synapses at which the presynaptic neuron releases a chemical neurotransmitter that acts on the postsynaptic target cell. In this section we discuss the types of molecules that function as transmitters at chemical synapses, their origin and fate, and their effects on postsynaptic cells. Because the ability of neurotransmitters to induce a response depends on their binding to specific receptors in the postsynaptic membrane, we introduce the major classes of receptors in this section; individual receptors are examined in more detail in the next section. We also briefly discuss electric synapses, which are much rarer, but simpler in function, than chemical synapses. Many Small Molecules Transmit Impulses at Chemical Synapses Numerous small molecules synthesized in the cytosol of axon terminals function as neurotransmitters at various chemical synapses. The “classic” neurotransmitters are stored in synaptic vesicles, uniformly sized organelles, 40 – 50 nm in diameter. With the exception of acetylcholine, the classic neurotransmitters are amino acids or derivatives of amino acids. Nucleotides such as ATP and the corresponding nucleosides, which lack phosphate groups, also function as neurotransmitters. Each neuron generally produces just one type of classic neurotransmitter. Following their exocytosis from synaptic vesicles into the synaptic cleft, neurotransmitters bind to specific receptors on the plasma membrane of a postsynaptic cell, causing a change in its permeability to ions. Structures of several small molecules that function as neurotransmitters. Except for acetylcholine, all of these are amino acids (glycine and glutamate) or derived from the indicated amino acids. The three transmitters synthesized from tyrosine, which many neurons secrete neuropeptides, a varied group of signaling molecules that includes endorphins, vasopressin, oxytocin, and gastrin. Neuropeptides are stored in a different type of vesicle than classic neurotransmitters. Exocytosis of both types of transmitter is triggered by a localized rise in cytosolic Ca^{2+}, but neuropeptides are released outside the synaptic zone. The effects of neuropeptide transmitters are very diverse and often long-lived (hours to days).

The following discussion deals mainly with the release and actions of the classic neurotransmitters such as will be seen later in this work.

ACETYLCHOLINE BIOSYNTHESIS

Acetylcholine is synthesized from acetyl coenzyme A (CoA) and choline in a reaction catalyzed by choline acetyltransferase: Synaptic vesicles take up and concentrate acetylcholine from the cytosol against a steep concentration gradient, using a H^+/acetylcholine antiporter in the vesicle membrane. Curiously, the gene encoding this antiporter is contained entirely within the first intron of the gene encoding choline acetyltransferase, a mechanism conserved throughout evolution for ensuring coordinate expression of these two proteins. Stimulation of the motor neuron releases the acetylcholine(ACh) a neurotransmitter which diffuses across the synaptic cleft and binds to the acetylcholine receptor on the post-synaptic membrane on the motor end plate (The area of the muscle membrane directly across the synaptic cleft) Acetylcholine (ACh) causes contraction of muscle cell, the acetylcholine is produce in the nerve ending by the enzyme synthesized from the cell body, the acetyl CoA comes from the mitochondria in the nerve ending while the choline is transported by choline transporter protein on the nerve ending membrane from the extracellular fluid of the GIT into the nerve ending where it react with the acetyl CoA to form acetylcholine in the nerve ending ,the vesicle in the nerve ending has a carrier protein sensitive to acetylcholine which then bind with synthesizes Ach and package them in the vesicle. The synthesis of this special neurotransmitter starts with the enzyme acetyl choline transferases.

The Biochemical illustration of the reaction or for the synthesis will be seen bellow. This starts also with reaction between the choline and acetyl CoA catalyzed by the enzyme cholineacetyl transferase.

BIOSYNTHESIS OF ACETYLCHOLINE.

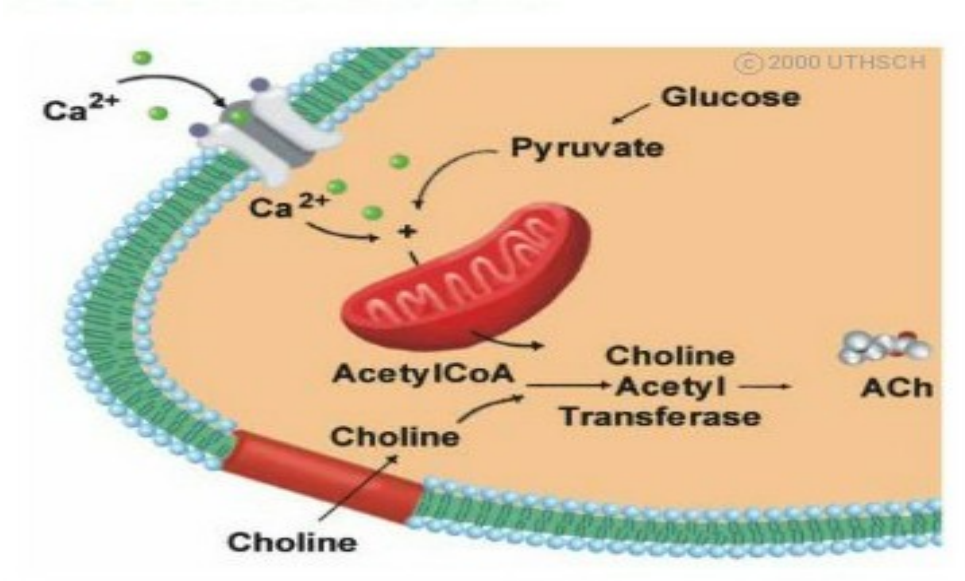

Figure 2: biochemical process of acetylcholine biosynthesis (Lin S, *et al.*, 2008)

However, acetylcholine is a neurotransmitter produced by neurons referred to as cholinergic neurons. In the peripheral nervous system acetylcholine plays a role in muscular movement as well as regulation of smooth muscle and cardiac muscle. In the central nervous system (CNS) Acetylcholine (ACh) is believed to be involved in the following; learning, memory and mood. Acetylcholine is synthesized from Choline and Acetyl CoA through the enzyme choline transferases and becomes packaged into a membrane bound vesicles in the nerve ending, after the arrival of a nerve signal at the termination of an axon the vesicle fused with the cell membrane causing the release of acetylcholine into the synaptic cleft. For the nerve signal to continue, Acetylcholine must diffuse to another nearby neurons or muscle cell where it will bind and activate a receptor protein. Moreover, Acetylcholine (ACh) having been produced in the neurons which reaches the pre-synaptic region of the synaptosome where the acetylcholine is stored in packets, when a nerve impulse reaches there, post-synaptic region Acetyl choline is picked up by the specific receptor which produce a nerve impulse. Thus, the nerve impulse is transmitted through the Synapses by the chemical messenger, Acetylcholine. As soon as the nerve impulse is generate in the post-synaptic fibre the Acetylcholine (ACh) is hydrolyzed by acetyl cholinesterase into choline and acetate.

SOURCE AND STRUCTURE OF CHOLINE.

The choline is the precursor for the biosynthesis of acetylcholine, it comes from phospholipid, called phosphotidylcholine or lecithin and will react with coenzyme A (Acetyl CoA)to form acetylcholine and CoA, and the acetylcholine is broken down in acetic acid and choline molecules, the two; product; acid and choline molecules now moves into the axon terminus where the duo are used to synthesized more Acetylcholine molecule which after synthesis are packaged in vesicles and are released as the case may be on the arrival of another action potential.

RECEPTOR OF ACETYLCHOLINE (ACh).

There two main types of cholinergic receptors namely; the nicotinic receptor and the muscarinic receptors. The nicotinic receptors are located at the synapses between two neurons two neurons and the synapses between the neuron and the skeletal muscle cell. Upon activation, a nicotinic receptor act as channel for the movement of ions in and out of the neurons, directly resulting in depolarization of the neuron. The muscarinic receptors in the other hand are located at the synapse of nerve with smooth or cardiac muscle, which trigger a chain of chemical reaction of event referred to as signal transduction. For cholinergic neuron to receive another impulse, Acetylcholine must be released from the receptor to which it is bound. This will only happen if the concentration of acetylcholine in the synaptic cleft is very low, low synaptic concentration of acetylcholine (ACh) can be maintained through hydrolysis reaction catalyzed the enzyme acetyl cholinesterase.

CLINICAL APPLICATION.

The post-synaptic receptors for acetylcholine (ACh) may be competitively blocked by succinylcholine, a structural analogue of acetylcholine, then, acetylcholine cannot act, producing muscle relaxation during surgery Also cholinesterase inhibitors, e.g. neostigmine will allow prolong action acetylcholine(ACh) and therefore, nerve impulse is sustained ,these are drugs used in myasthenia gravis.

NEURONS, NERVE TERMINALS AND MOTOR UNITS.

Neurons

Like all organ systems, the nervous system can do its specialized functions because the cells that make up the nervous system are specialized. The cells in the nervous system are specialized both in how they work individually and how they are connected to each other. The nervous system

contains two kinds of cells: neurons are the cell type (primarily) responsible for communication and integration in the nervous system.

Ganglia, which protect the neurons, but also modify their action. Neurons (nerve cells) have three parts that carry out the functions of communication and integration: dendrites, axons, and axon terminals. They have a fourth part the cell body or soma, which carries out the basic life processes of neurons. Neurons have a single axon is the output of the neuron. Axons are long (up to several feet long), but thin -sort of like a wire. They are designed both in shape and function to carry information reliably and quickly over long distances (communication). Axons usually branch to connect to go to different neurons. Axon terminals at the end of axons make the actual connection to other neurons. Axons carry information from the senses to the CNS (Central Nervous System, brain and spinal cord), from one part of the CNS to another, or from the CNS to muscles and glands, which generate the behaviors you do. Neurons usually have several dendrites (from the Greek dendron, for tree branches) are the input to a neuron. Dendrites are designed both in shape and function to combine information the information they get (integration). Most neurons have several dendrites, each of which may branch up to six times to collect signals from the axon terminals from other neurons that cover it. They are covered with synapses (connections) from many other neurons and combine the signals they get from these synapses.

NERVE TERMINALS

Acetylcholine is stored in vesicle in the presynaptic membrane, they are usually aligned at higher concentration snear release site called active zone ,this is the which with fusion of the vesicles and plasma membrane will allow the released of neurotransmitter in synapse. The postsynaptic membrane has many folds in which acetylcholine (ACh) receptors are located in high concentration. The distance across the synapse is between 20nm to 60nm citation. The release of the neurotransmitter require calcium influx through channels that present at higher densities at the active zone. The vesicle undergo a process usually described as docting, following by priming and then finally fusion and release contents into the synapse Axon terminals also called synaptic bonton are distal termination of the branches of an axon, an axon itself is a slender projection of nerve or neuron that conducts electrical impulses called action potential away from

the neuron body's cell or soma in order to transmit those impulses to other neurons. However, Neurons are interconnected in complex arrangements and uses electrochemical to transmit impulses one neuron to the other next to it. Axon terminals are separated from neighboring neuron by small gap called a synapse across which impulses are sent. The axon terminal and the neuron to which it is attached sometimes referred to as the pre-synaptic neurons.

MOTOR UNITS

Each muscle fibre is stimulated by only one synaptic knob but each motor nerve has many synaptic knobs, it stimulates a number of muscle fibre and the muscle fibre which is supplied constitute a motor units. Nerve impulses causes a serial contraction of motor units in a muscle and each unit contract to its full capacity. The strength of the contraction depend on the number of motor units in action at particular time some motor units contain large number of muscle fibre (one nerve serves many muscle cells). Lin S, et al., (2008). This arrangement is associated with large-scale, powerful movement such as in the legs or upper arms. The time delicate control of muscle movement is achieved when one motor unit contains very few fibre as in the muscle controlling eye movement. This has been demonstrated in neurons treated with drugs that block Na^+ channels and thus prevent conduction of action potentials. When the membrane of axon terminals in such treated cells is artificially depolarized, an influx of Ca^{2+} ions into the neurons occurs and exocytosis is triggered. Patch-clamping experiments show that voltage-gated Ca^{2+} channels, like voltage-gated Na^+ channels, open transiently upon depolarization of the membrane. Synaptic Vesicles Can Be Filled, Exocytosed, and Recycled within a Minute. Two pools of neurotransmitter-filled synaptic vesicles are present in axon terminals: those docked at the plasma membrane, which can be readily exocytosed, and those in reserve in the active zone near the plasma membrane. Each rise in Ca^{2+} triggers exocytosis of about 10 percent of the docked vesicles. However, this is but one of the series of steps involved in forming synaptic vesicles, filling them with neurotransmitter, moving them to the active zone near the plasma membrane, docking them at the plasma membrane, and then, after vesicle fusion with the plasma membrane, recycling their membrane components by endocytosis.

Recycling of synaptic-vesicle membrane proteins is rapid, as indicated by the ability of many neurons to fire fifty times a second, and quite specific, in that several membrane proteins unique to the synaptic vesicles are specifically internalized by endocytosis. Endocytosis usually involves clathrin-coated vesicles, though non-clathrin-coated vesicles may also be used. After the endocytic vesicles lose their clathrin coat, however, they usually do not fuse with larger, low pH

endosomes, as they do during endocytosis of plasma-membrane proteins in other cells. Rather, the recycled vesicles are immediately refilled with neurotransmitter.

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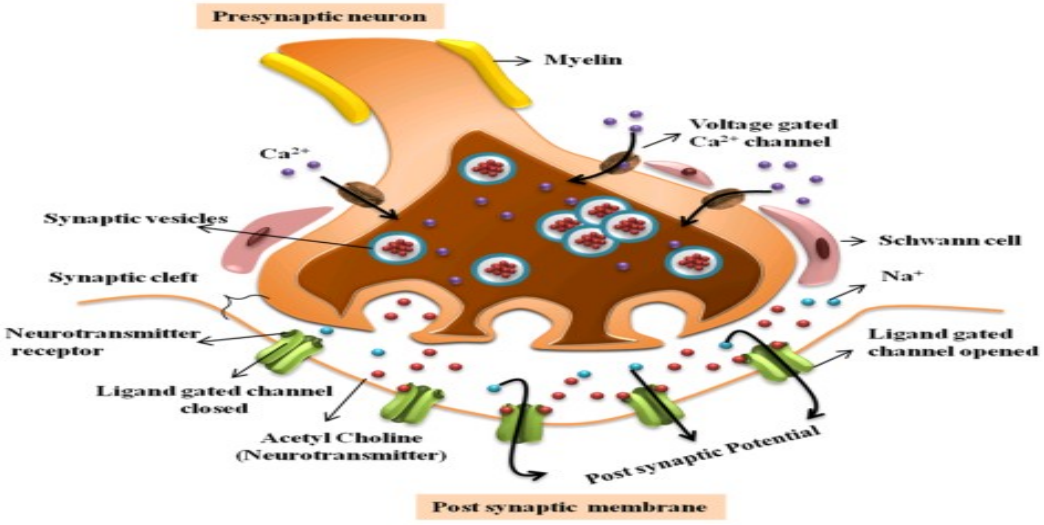

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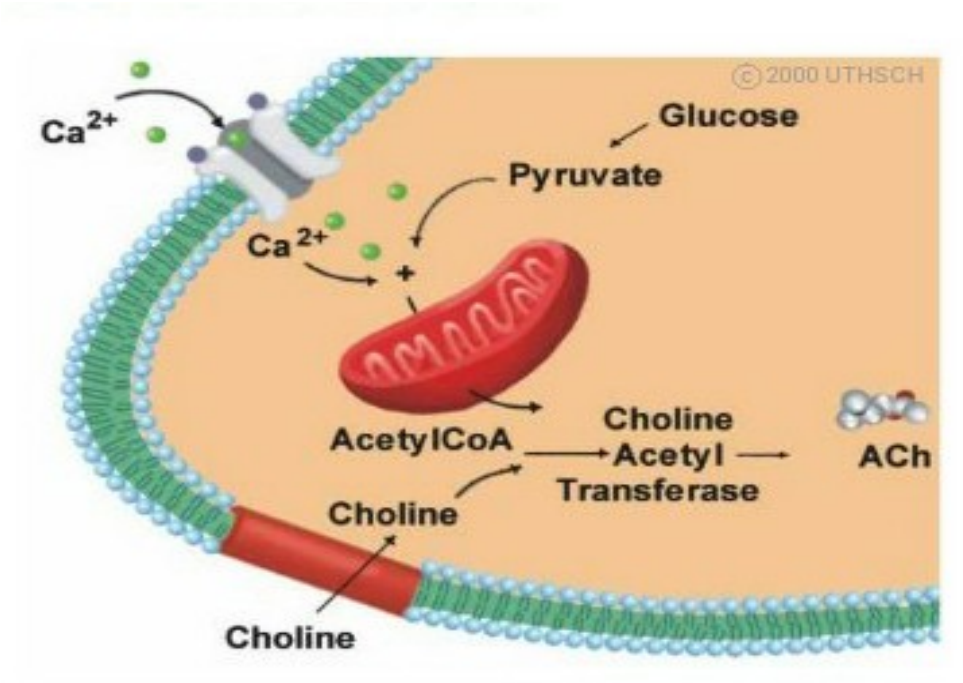
BIOSYNTHESIS OF ACETYLCHOLINE.



However, acetylcholine is a neurotransmitter produced by neurons referred to as cholinergic neurons. In the peripheral nervous system acetylcholine plays a role in muscular movement as well as regulation of smooth muscle and cardiac muscle. In the central nervous system (CNS) Acetylcholine (ACh) is believed to be involved in the following; learning, memory and mood. Acetylcholine is synthesized from Choline and Acetyl CoA through the enzyme choline transferases and becomes packaged into a membrane bound vesicles in the nerve ending, after the arrival of a nerve signal at the termination of an axon the vesicle fused with the cell membrane causing the release of acetylcholine into the synaptic cleft. For the nerve signal to continue, Acetylcholine must diffuse to another nearby neurons or muscle cell where it will bind and activate a receptor protein. Moreover, Acetylcholine (ACh) having been produced in the neurons which reaches the pre-synaptic region of the synaptosome where the acetylcholine is stored in packets, when a nerve impulse reaches there, post-synaptic region Acetyl choline is picked up by the specific receptor which produce a nerve impulse. Thus, the nerve impulse is transmitted through the Synapses by the chemical messenger, Acetylcholine. As soon as the nerve impulse is generate in the post-synaptic fibre the Acetylcholine (ACh) is hydrolyzed by acetyl cholinesterase into choline and acetate.

SOURCE AND STRUCTURE OF CHOLINE.

The choline is the precursor for the biosynthesis of acetylcholine, it comes from phospholipid, called phosphotidylcholine or lecithin and will react with coenzyme A (Acetyl CoA)to form acetylcholine and CoA, and the acetylcholine is broken down in acetic acid and choline molecules, the two; product; acid and choline molecules now moves into the axon terminus where the duo are used to synthesized more Acetylcholine molecule which after synthesis are packaged in vesicles and are released as the case may be on the arrival of another action potential.

RECEPTOR OF ACETYLCHOLINE (ACh).

There two main types of cholinergic receptors namely; the nicotinic receptor and the muscarinic receptors. The nicotinic receptors are located at the synapses between two neurons two neurons and the synapses between the neuron and the skeletal muscle cell. Upon activation, a nicotinic receptor act as channel for the movement of ions in and out of the neurons, directly resulting in depolarization of the neuron. The muscarinic receptors in the other hand are located at the synapse of nerve with smooth or cardiac muscle, which trigger a chain of chemical reaction of event referred to as signal transduction. For cholinergic neuron to receive another impulse, Acetylcholine must be released from the receptor to which it is bound. This will only happen if the concentration of acetylcholine in the synaptic cleft is very low, low synaptic concentration of acetylcholine (ACh) can be maintained through hydrolysis reaction catalyzed the enzyme acetyl cholinesterase.

CLINICAL APPLICATION.

The post-synaptic receptors for acetylcholine (ACh) may be competitively blocked by succinylcholine, a structural analogue of acetylcholine, then, acetylcholine cannot act, producing muscle relaxation during surgery Also cholinesterase inhibitors, e.g. neostigmine will allow prolong action acetylcholine(ACh) and therefore, nerve impulse is sustained ,these are drugs used in myasthenia gravis.

NEURONS, NERVE TERMINALS AND MOTOR UNITS.

Neurons

Like all organ systems, the nervous system can do its specialized functions because the cells that make up the nervous system are specialized. The cells in the nervous system are specialized both in how they work individually and how they are connected to each other. The nervous system contains two kinds of cells: neurons are the cell type (primarily) responsible for communication and integration in the nervous system.

Ganglia, which protect the neurons, but also modify their action. Neurons (nerve cells) have three parts that carry out the functions of communication and integration: dendrites, axons, and axon terminals. They have a fourth part the cell body or soma, which carries out the basic life processes of neurons. Neurons have a single axon is the output of the neuron. Axons are long (up to several feet long), but thin -sort of like a wire. They are designed both in shape and function to carry information reliably and quickly over long distances (communication). Axons usually branch to connect to go to different neurons. Axon terminals at the end of axons make the actual connection to other neurons. Axons carry information from the senses to the CNS (Central Nervous System, brain and spinal cord), from one part of the CNS to another, or from the CNS to muscles and glands, which generate the behaviors you do. Neurons usually have several dendrites (from the Greek dendron, for tree branches) are the input to a neuron. Dendrites are designed both in shape and function to combine information the information they get (integration). Most neurons have several dendrites, each of which may branch up to six times to collect signals from the axon terminals from other neurons that cover it. They are covered with synapses (connections) from many other neurons and combine the signals they get from these synapses.

NERVE TERMINALS

Acetylcholine is stored in vesicle in the presynaptic membrane, they are usually aligned at higher concentration snear release site called active zone ,this is the which with fusion of the vesicles and plasma membrane will allow the released of neurotransmitter in synapse. The postsynaptic membrane has many folds in which acetylcholine (ACh) receptors are located in high concentration. The distance across the synapse is between 20nm to 60nm citation. The release of the neurotransmitter require calcium influx through channels that present at higher densities at the active zone. The vesicle undergo a process usually described as docting, following by

priming and then finally fusion and release contents into the synapse Axon terminals also called synaptic bonton are distal termination of the branches of an axon, an axon itself is a slender projection of nerve or neuron that conducts electrical impulses called action potential away from the neuron body's cell or soma in order to transmit those impulses to other neurons. However, Neurons are interconnected in complex arrangements and uses electrochemical to transmit impulses one neuron to the other next to it. Axon terminals are separated from neighboring neuron by small gap called a synapse across which impulses are sent. The axon terminal and the neuron to which it is attached sometimes referred to as the pre-synaptic neurons.

MOTOR UNITS

Each muscle fibre is stimulated by only one synaptic knob but each motor nerve has many synaptic knobs, it stimulates a number of muscle fibre and the muscle fibre which is supplied constitute a motor units. Nerve impulses causes a serial contraction of motor units in a muscle and each unit contract to its full capacity. The strength of the contraction depend on the number of motor units in action at particular time some motor units contain large number of muscle fibre (one nerve serves many muscle cells). Lin S, Landmann L, Ruegg M. A, Brenner H. R (2008). This arrangement is associated with large-scale, powerful movement such as in the legs or upper arms. The time delicate control of muscle movement is achieved when one motor unit contains very few fibre as in the muscle controlling eye movement. This has been demonstrated in neurons treated with drugs that block Na^+ channels and thus prevent conduction of action potentials. When the membrane of axon terminals in such treated cells is artificially depolarized, an influx of Ca^{2+} ions into the neurons occurs and exocytosis is triggered. Patch-clamping experiments show that voltage-gated Ca^{2+} channels, like voltage-gated Na^+ channels, open transiently upon depolarization of the membrane. Synaptic Vesicles Can Be Filled, Exocytosed, and Recycled within a Minute. Two pools of neurotransmitter-filled synaptic vesicles are present in axon terminals: those docked at the plasma membrane, which can be readily exocytosed, and those in reserve in the active zone near the plasma membrane. Each rise in Ca^{2+} triggers exocytosis of about 10 percent of the docked vesicles. However, this is but one of the series of steps involved in forming synaptic vesicles, filling them with neurotransmitter, moving them to the active zone near the plasma membrane, docking them at the plasma membrane, and then, after vesicle fusion with the plasma membrane, recycling their membrane components by endocytosis.

Recycling of synaptic-vesicle membrane proteins is rapid, as indicated by the ability of many neurons to fire fifty times a second, and quite specific, in that several membrane proteins unique to the synaptic vesicles are specifically internalized by endocytosis. Endocytosis usually involves clathrin-coated vesicles, though non-clathrin-coated vesicles may also be used. After the endocytic vesicles lose their clathrin coat, however, they usually do not fuse with larger, low pH endosomes, as they do during endocytosis of plasma-membrane proteins in other cells. Rather, the recycled vesicles are immediately refilled with neurotransmitter.

GENERATING THE END PLATE PONTENTIAL AND EXCITATION CONTRACTION COUPLING.

End plate potentials (EPPs) are the voltages which cause depolarization of skeletal muscle fibers caused by neurotransmitters binding to the postsynaptic membrane in the neuromuscular junction. They are called "end plates" because the postsynaptic terminals of muscle fibers have a large, saucer-like appearance. When an action potential reaches the axon terminal of a motor neuron, vesicles carrying neurotransmitters (mostly acetylcholine) are exocytosed and the contents are released into the neuromuscular junction. These neurotransmitters bind to receptors on the postsynaptic membrane and lead to its depolarization. In the absence of an action potential, acetylcholine vesicles spontaneously leak into the neuromuscular junction and cause very small depolarizations in the postsynaptic membrane. This small response (~0.4mV) is called a miniature end plate potential (MEPP) and is generated by one acetylcholine-containing vesicle. [Boron, and Boulpaep, 2012). It represents the smallest possible depolarization which can be induced in a muscle. To some extent, this has been narrated before but for the sake of record, let me still repeat it. travelling along the a motor nerve ,an action potential arrives at the axon terminus, the arrival of this impulses changes the chemical nature of both extracellular fluid in which axon is embedded and the intracellular arena of the axon terminus. The both chemical changes in the gated calcium ion channel in the cell membrane of the axon terminus. Now more than usual, there is massive movement of calcium ion from the extracellular fluids into the axon terminus. For reasons very poorly understood at the moment energy is imparted into the synaptic vesicle containing Acetylcholine (ACh) molecules and an average of 60 of the vesicles, moves and briefly attach to the membrane of the axon terminus and by exocytosis empty their content (Acetylcholine molecules) into the synaptic cleft or space, The Ach molecules quickly attach to their nicotinic receptor in the muscle plasma membrane. At this juncture, chemical changes within the vicinity causes the opening of the sodium gated ion channel in the muscle plasma

membrane and the influx of massive quantity of the sodium ion into the post-synaptic (muscle fibre) membrane. This rapidly depolarizes the post-synaptic membrane and therefore, the action potential from the motor neuron the muscle fibre membrane. This action potential having cross to the skeletal muscle fibre membrane, what happens? The next event in the transmission process is the conversion of the action potential from its electrical nature into mechanical event which result in the contraction of the muscle fibers, the mechanism of this process is best explained using excitation-contraction coupling.

1.3.1. Miniature end plate potentials (MEPPs)

Miniature end plate potentials are the small (~0.4mV) depolarizations of the postsynaptic terminal caused by the release of a single vesicle into the synaptic cleft. Neurotransmitter vesicles containing acetylcholine collide spontaneously with the nerve terminal and release acetylcholine into the neuromuscular junction even without a signal from the axon. These small depolarizations are not enough to reach threshold and so an action potential in the postsynaptic membrane does not occur, [Purves, *et al.,* 2008]. During experimentation with MEPPs, it was noticed that often spontaneous action potentials would occur, called end plate spikes in normal striated muscle without any stimulus. It was believed that these end plate spikes occurred as a result of injury or irritation of the muscles fibers due to the electrodes. Recent experiments have shown that these end plate spikes are actually caused by muscle spindles and have two distinct patterns: small and large. Small end plate spikes have a negative onset without signal propagation and large end plate spikes resemble motor unit potentials (MUPs). Muscle spindles are sensory receptors that measure muscle elongation or stretch and relay the information to the spinal cord or brain for the appropriate response. Partanen J (1999).

Threshold potential ("All or None")

When an action potential causes the release of many acetylcholine vesicles, acetylcholine diffuses across the neuromuscular junction and binds to ligand-gated nicotinic receptors (non-selective cation channels) on the muscle fiber. This allows for increased flow of sodium and potassium ions, causing depolarization of the sarcolemma (muscle cell membrane). The small depolarization associated with the release of acetylcholine from an individual synaptic vesicle is called a miniature end-plate potential (MEPP), and has a magnitude of about +0.4mV. MEPPs are additive, eventually increasing the end-plate potential (EPPs) from about -100mV up to the threshold potential of -60mV, at which level the voltage-gated ion channels in the postsynaptic membrane open, allowing a sudden flow of sodium ions from the synapse and a sharp spike in

depolarization. This depolarization voltage spike triggers an action potential which propagates down the postsynaptic membrane leading to muscle contraction. It is important to note that EPPs are not action potentials, but that they trigger action potentials. In a normal muscular contraction, approximately 100-200 acetylcholine vesicles are released causing a depolarization that is 100 times greater in magnitude than a MEPP. This causes the membrane potential to depolarize +40mV (100 x 0.4mV = 40mV) from -100mV to -60mV where it reaches threshold. (Kohara, *et al*., 2002). Once the membrane potential reaches threshold, an action potential occurs and causes a sharp spike in membrane Action potential phase polarity. There are five phases of an action potential: threshold, depolarization, peak, repolarization, and hyperpolarization. Threshold is when the summation of MEPPs reaches a certain potential and induces the opening of the voltage-gated ion channels. The rapid influx of sodium ions causes the membrane potential to reach a positive charge. The potassium ion channels are slower-acting than the sodium ion channels and so as the membrane potential starts to peak, the potassium ion channels open and causes an outflux of potassium to counteract the influx of sodium. At the peak, the outflux of potassium equals the influx of sodium, and the membrane does not change polarity. During repolarization, the sodium channels begin to become inactivated, causing a net efflux of potassium ions. This causes the membrane potential to drop down to its resting membrane potential of -100mV. Hyperpolarization occurs because the slow-acting potassium channels take longer to deactivate, so the membrane overshoots the resting potential. It gradually returns to resting potential and is ready for another action potential to occur.

During the action potential before the hyperpolarization phase, the membrane is unresponsive to any stimulation. This inability to induce another action potential is known as the absolute refractory period. During the hyperpolarization period, the membrane is again responsive to stimulations but it requires a much higher input to induce an action potential. This phase is known as the relative refractory period. Once the action potential has finished in the neuromuscular junction, the used acetylcholine is cleared out of the synaptic cleft by the enzyme acetylcholinesterase. Several diseases and problems can be caused by the inability of enzymes to clear away the neurotransmitters from the synaptic cleft leading to continued action potential propagation. The calcium ions has entered the sarcoplasm through the pores in the cisternae of the transverse tubules and more calcium ions that have been pumped out of the sarcoplasmic reticulum in the sarcoplasm, enter into the myofibrils and there initiate skeletal muscle fibre and contraction of the muscle. Calcium ion are not attracted to troponin subunits of the Actin filament and tucks out the troponic C component of the troponin tropomyosin complex which

covered the active sites on the G actin molecules .the active site now uncovered is attracted to the cross bridge head of the myosin filament in the famous power stroke mechanism.as soon as the contractile process is over, the remaining calcium ions in the sarcoplasm are pumped into the sarcoplasmic reticulum which the Acetyl Cholinesterase in the synaptic cleft(space) destroys the remnant of the Acetylcholine the end of the contractile membrane.

ENERGY SOURCES FOR MUSCLE CONTRACTION.

Initial energy for muscle contraction come from ATP which is readily available within the environment. The ATP provide for the attachment and detachment of the cross bridge heads and the active site on the actin filament. The ATP already there is just enough for process to go for a short while .As the event is going on more ATP is being generated. Creatinine phosphate provides the platform for generating ATP from ADP Creatinine phosphate contain enough phosphate bonds which on hydrolysis will yield enough energy necessary to drive any biological processes. The creatinine phosphate high energy content is due to the fact that it store excess energy released from the mitochondria and in the way supply energy for contraction when the source is depleted. Another source of energy comes from the continuous conversion of glycogen stored there in the muscle tissues. This may be the ultimate source or supplier of energy for skeletal muscle contraction.

NEUROMUSCULAR JUNCTION (NMJ) IN NECROSIS AND APTOSIS

AGING AND NMJ DEFECT

Another physio-pathologic condition where the functional communication between muscle and nerve is compromised is aging. As mammals grow older, many functional and structural changes occur in skeletal muscle and NMJ, leading to gradual loss of mobility (Sengupta-Ghosh, et al., 2019). It is widely accepted that not all muscles share the same susceptibility to age-related alterations. Several studies indicate diversity in muscle fibers susceptibility to the denervation process, based on motor neuron type. The NMJ associated with faster-contracting motor neurons are more susceptible to age-related structural changes than those associated with slower-contracting motor neurons (Da Cruz *et al*., 2012). One of the first evidences of the aging process occurring in NMJ is the formation of new branches of terminal axons that form new synaptic sites on muscle fibers (Sengupta-Ghosh *et al*., 2019). NMJ undergo remodeling processes with cyclical extension and retraction of motor nerve terminals, leading to an increase in the complexity of the nerve terminal arborization (Sengupta-Ghosh, et al., 2019). In particular, this

remodeling process is characterized by the increase in size and complexity of the branches of terminal axons, associated with fragmentation (Suzuki, et al., 2009) and folds reduction of postsynaptic sites (Rudolf, et al 2014). It has been demonstrated that oxidative stress, along with compromised mitochondria and increased intracellular calcium, amplifies the presynaptic decline in NMJ, accompanied with a decreased number of synaptic vesicles, similarly to what observed in ALS mouse models (Dobrowonlny, et al., 2018). This initial NMJ dysfunction is followed by a neuro-degeneration promoted by increased production of inflammatory cytokines and loss of trophic support. Moreover, the evidence that some denervated fibers are not successfully re-innervated during aging raise the prospect that this alteration is at the base of the progressive decline in muscle mass and strength with aging, a condition known as sarcopenia. The triggers of the NMJ alterations during aging are still debated. It is still unclear whether NMJ changes in aged muscles are caused by alterations in motor neurons or rather in skeletal muscle fiber (Wokke, et al., 1990). One of the possible causes is the death of a fraction of motor neurons, occurring in humans between the age of 60 and 90 (Bolliger, et al., 2010). It has been suggested that motor neuron death provokes a temporary denervation of downstream muscles and induces a collateral reinnervation process. This process is driven by Perisynaptic Schwann cells that grow from the axon stump to guide surviving motor neurons that sprout their axons to create new synaptic terminals on muscle fibers (Lozano, et al., 2016). Nevertheless, in advanced age, motor neurons show impaired capacity to re-innervated denervated fibers, suggesting that alterations in different components of nerve-muscle circuit impinge the potential compensatory mechanisms. Other studies suggest that changes in the end-plate morphology and NMJ remodeling that occur with aging precede the loss of fast motor units and suggest the involvement of retrograde signaling. It has been reported that proteolytic cleavage of agrin, a proteoglycan involved in NMJ development, maturation, and AChRs clustering (Courtney and Steinbach, 1981), induces early onset sarcopenia in young adult mice (Andonian and Fahim, 1989) whereas the injection of a neurotrypsin-resistant agrin fragment stabilized NMJ and improved the phenotype of neurotrypsin-overexpressing mice. Other studies employed the effect of Muscle Electrical Stimulation (ES) to improve muscle functionality and to counteract NMJ decline during aging. ES treatment in old sedentary people improved muscle performance, increasing fiber size, stimulating satellite cells and modulating the degeneration of the mitochondrial apparatus. Moreover, it has been reported that ES treatment increases the number of fast twitch fibers and counteracts neuromuscular disabilities from age related NMJ degeneration in paraplegic patients. At molecular level ES treatment activates signaling pathways that decode for a specific calcium

signaling involved in metabolic and structural adaptation of muscle fibers, such as the Calcineurin-NFAT and CamKII pathways that control the maintenance or switching of muscle fiber type [108]. Moreover, it has been described that biphasic electrical stimulation significantly increases the number and size of AChRs clusters available for NMJ formation during innervation and that electrical stimulation promotes axonal growth and sensorimotor functional recovery after injury (Son, et al., 1996).

FACTORS THAT INFLUENCE NMJ STABILITY DURING AGING

Among the factors that can affect the stability of the NMJ there are metabolic changes associated to mitochondrial dysfunction and lifestyle. Several recent evidence demonstrated that mitochondrial energy metabolism is altered during muscle aging, which is also accompanied by a reduction in the rate of oxygen consumption (Gonzalez-Freire, et al., 2018). In human skeletal muscle, as well as in animal models, the mitochondrial capability to produce ATP decreases with age. It is widely recognized that mitochondrial dysfunctions have a causal link with cellular ROS accumulation and therefore, as organisms grow old, there is an increase of intracellular ROS and consequently an accumulation of damaged cell components in all the tissues. Since muscle and nerve are highly metabolic active tissues, their mutual communication is affected by mitochondrial dysfunction. Another factor playing a crucial role in sarcopenia progression is lifestyle. Physical inactivity and impaired nutrition stimulate loss of muscle mass and NMJ defect, exacerbating sarcopenia (Jackson and McArdle 2011). It has been demonstrated that exercise induces muscle hypertrophy and NMJ remodeling, and improves recovery of peripheral nervous terminal after nerve injury. In old mice exercise training is able to minimize NMJ expansion that turn back to levels comparable to young mice.

How skeletal muscle and NMJ send retrograde signals to motor neurons represents an intriguing field of research. Skeletal muscle has been identified as an endocrine organ that produce and secrete growth factors, cytokines, and peptides, collectively indicated as myokines, which make muscle capable to communicate with other tissues and organs, including bone, intestine, adipose tissue, liver, pancreas, and brain. One of the critical players that integrates muscle fiber function with motor neuron signaling following exercise is Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha. PGC-1α expression activates a broad NMJ gene program and improves postsynaptic NMJ architecture (Tintignac et al., 2015). It has been demonstrated that loss of skeletal muscle PGC-1α hampers acetylcholine receptor (AChR) clustering and the

transcription of NMJ genes including AChRs, muscle-specific kinase, and utrophin. Among other factors, Insulin-like Growth Factor-1 (IGF-1) plays a pivotal role in muscle growth, differentiation, and regeneration. In mammalian organisms the majority of IGF-1 is produced and released by the liver as systemic growth factor, while a minor fraction is produced by other tissues and participates to autocrine and paracrine signals. In skeletal muscle, the binding of IGF1 with its receptor activates anabolic, anticatabolic, and antiapoptotic signaling pathways that preserves muscle mass and strength. It has been demonstrated that the overexpression of IGF-1 isoforms selectively in skeletal muscle maintains muscle mass and activates several pathways that promote the clearance of dysfunctional mitochondria and the maintenance of NMJ stability in senescence mice. Moreover, it has been demonstrated that IGF-1 overexpression is sufficient to down regulate the levels of two cytokines, namely IL-1β and IL-6, in aged mice, counteracting the chronic inflammation process typical of aging (inflammaging). In this context, a crucial role is played by senescent Schwann cells that overexpress IL-6 cytokine and negatively affect the nerve microenvironment during muscle innervation.

MOTOR NEURON DISEASES: AMYO LETERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig□s disease) is a motor neuron disease that causes a disabling condition whereby the degeneration of motor neurons causes progressive muscle weakness and leads to death usually within five years of disease onset (Billlee, et al., 2008). Most cases of ALS occur sporadically, however a small percentage of cases are inherited. Several ALS genes have been identified, and mutations within them can lead to familial ALS, including Cu/Zn superoxide dismutase (SOD1), TDP-43 and Fused in sarcoma (Fus). In addition to familial ALS, Fus mutations are also implicated in sporadic ALS cases (Belzil and De Jesus-Hernandez et al., 2010). Both Fus and TDP-43 are DNA- and RNA-binding proteins. While TDP-43 has been intensively studied, the role of Fus in the ALS etiology is largely unknown. Fus is a ubiquitous multi-domain RNA-binding protein involved in many processes of RNA metabolism including transcriptional regulation, mRNA splicing and mRNA shuttling between the nucleus and the cytoplasm (Zinszner, et al., 1997). In neurons, Fus is also implicated in the transportation of mRNA for local translation in dendrites (Fuji et al., 2005). Fus mainly localizes in the nucleus, but has also been shown to present at lower levels in the cytoplasm of most cell types, including neurons and. glial cells (Andersson et al., 2008). The ALS-related mutations are clustered in the carboxyl-terminus of Fus and exhibit an abnormal nucleo-cytoplasmic redistribution and cytosolic inclusions in the motor neurons in familial ALS

patients (Kwiatkowski, et al., 2009). Studies at the cellular level have shown that the nuclear localization sequence (NLS) located in the C-terminus of Fus is necessary and sufficient for nuclear targeting of Fus. ALS mutations within the NLS significantly impair the nuclear targeting function of the sequence, leading to the cytoplasmic accumulation of mutant Fus. Mutations in the NLS have also been shown to promote Fus co-localization with stress granules in the cytosol (Bertolotti, et al 1996). However, it is still unclear how the mutations cause the toxicity. Moreover, wild-type Fus aggregation has also been reported in several ALS cases. Thus, it is important to determine whether the cytoplasmic or nuclear fraction of Fus is critical to cause the toxicity in ALS. The tentative hypothesis in the field is that cytoplasmic mislocalization and inclusions are important during the disease process. However, this remains to be thoroughly tested. Amyotrophic lateral sclerosis (ALS) is progressive neurodegenerative disease characterized by the loss of motor function. Several ALS genes have been identified as their mutations can lead to familial ALS, including the recently reported RNA-binding protein fused in sarcoma (Fus). However, it is not clear how mutations of Fus lead to motor neuron degeneration in ALS. One of the best examples of impaired interplay between nerve and muscle is ALS, a fatal disease characterized by motor neurons degeneration, muscle atrophy, weakness and, ultimately, muscle paralysis with respiratory failure. During ALS progression muscle denervation is accompanied by changes in muscle fiber profile with a preference loss of fast-twitch fiber, due to higher vulnerability of fast-fatigable innervating motor neurons and to specific signals released by PSCs (De Winter, et al., 2006). ALS is epidemiologically classified into two forms: sporadic (9095%) and familial (5 10%) form. Among the familial cases, approximately 20% correlate with mutations in the sequence of the Superoxide Dismutase 1 gene (SOD1), which encodes for an important antioxidant enzyme. In addition to SOD1 mutations, other gene defects have been reported to cause ALS, including senataxin (SETX), alsin, dynactin, synaptobrevin/VAMP (vesicle-associated membrane protein)-associated protein B (VAPB), TDP-43, FUS/TLS, profilin (PFN1), MATR3, CHCHD10, TBK1, TUBA4A, NEK1, C21orf2, and CCNF (Yang, et al., 2001). However, even in these cases, where a well-defined mutation has been linked to the disease, a clear correlation between the genetic defect and the pathophysiology of the disease has not yet been disclosed. Much of what we presently know about the role of mutant genes in ALS is based on studies of transgenic animals in which the potential genes involved in ALS are overexpressed under the control of specific promoters. Nevertheless, the failure to translate the positive results obtained in animal models into successful trials in human has cooled the enthusiasms and raised important questions on the validity of either animal

models or methodological approaches. Thus, robust criteria and guidelines for preclinical animal research in ALS are necessary (De Giorgio et al., 2019). One of the experimental models that has been widely used in ALS-related studies is the transgenic mutant SOD1 mouse. Although the SOD1 mutant mice present some limitations compared to ALS patients it remains, along with other ALS-related mice, an ideal model for preclinical tests and proof-of-concept studies (Clement, et al., 2003). The obvious loss of motor neurons in the spinal cord initially focused attention on how mutant SOD1 may act within motor neurons to provoke neuronal degeneration and death. Among pathogenic events, glutamate-induced excitotoxicity, oxidative stress, protein aggregation, and mitochondrial dysfunction within motor neurons have been proposed. However, the mutant gene products are widely expressed, raising the possibility that the toxic cascade may be achieved wholly or in part by mutant SOD1 action in non-neuronal cells. Notably, restriction of SOD1 mutant expression selectively to post-natal motor neurons failed to produce detectable sign of pathology or motor-neuron disease (Clement, *et al.,* 2003), suggesting that other cell types may be involved in ALS-associated neuro-degeneration. Indeed, analysis of chimeras generated between wild type and SOD1 mutant mouse embryonic cells revealed that wild type non neuronal cells in adult chimeric animals extended the survival of SOD1 mutant motor neurons (Wong and Martin, 2010). The generation of mouse models expressing human mutated SOD1 gene (SOD1G93A), exclusively in skeletal muscle, added new insights into the potential primary targets of the mutant SOD1 toxic protein. Muscle-specific expression of mutant SOD1G93A caused accumulation of reactive oxygen species (ROS), mitochondria dysfunction, muscle atrophy, NMJ dismantlement, microgliosis, and neuron degeneration (Rudolf, et al., 2016) . All together this evidence suggests that local toxic effect of SOD1G93A is a primary determinant of ALS-associated muscle pathology and that retrograde signals from muscle to nerve may contribute, in a sort of dying back phenomenon, to synapse and axon damage. In fact, several other recent evidences confirmed the hypothesis that motor neurons are not the only primary targets of SOD1G93A-mediated toxicity and revealed that early changes of neuromuscular transmission start long before motor symptoms onset. Fischer and colleagues demonstrated that pathological changes of motor neuron disease begin at the distal axon and they observed denervation and reinnervation changes in muscle tissue without any pathological signs in neurons cells. Furthermore, Schafers group characterized two different types of motor neurons, the losers or denervated branches, and the compensators, or reinnervating branches, which display different susceptibility to the toxic properties of SOD1G93A mutant gene product. Although a conclusive link is still missing, it is intriguing to speculate that loser and compensator

neurons are subject to different influences from neighboring interneurons, astrocytes, or microglia and from the vasculature and muscle fibers they innervate, all of which might provide either toxic or protective factors.

This evidence supports the notion that NMJ dismantlement can occur independently from motor neuron degeneration and may represent an early pathogenic signature of muscle-nerve communication defects.

DISORDERS ASSOCIATED WITH NEUROMUSCULAR JUNCTION AND TREATMENT.

Nerves connect with muscle at the neuromuscular junction ,there the ends of nerve fibre connect to special sites on the muscle membrane called motor end plates, these plates contains receptors that enable the muscle to respond to acetylcholine, a chemical messenger (neurotransmitter) released by the nerve to transmit a nerve impulse across the neuromuscular junction. After a nerve stimulates a muscle at this junction, an electric impulse flows through the muscle causing to contract after transmitting the impulse, Acetylcholine is broken down so that it does not continue to stimulate the muscle any malfunction in any of this process or step result to diseases or malfunctioning of the Neuromuscular Junction, They are two way in which these diseases are classified based on its mechanism of action or how the action of the disease affects normal functioning of the Neuromuscular Junction and they are thus; Immune-mediate diseases toxic/metabolic and congenital syndrome. Another classification divides the disease based on location of disruption. They are various disorders in the neuromuscular junction and they are thus; Myasthenia Gravis and Eaton-Lambert syndrome. All these disorders associate with malfunctioning of the neuromuscular junction (NMJ). Some other disorders of the neuromuscular junction results in over activity of the muscle as in the following; stiff-person syndrome and Isaacs syndrome.

Toxic/metabolic

Metabolic diseases are usually a result of abnormal functioning of one of the metabolic processes required for regular production and utilization of energy in a cell. This can occur by damaging or disabling an important enzyme, or when a feedback system is abnormally functioning. Toxic diseases are a result of a form of poison that effects neuromuscular junction functioning. Most commonly animal venom or poison, or other toxic substances are the origin of the problem.

Neuromuscular junction diseases in this category include snake venom poisoning, botulism, arthropod poisoning, organophosphates and hypermagnesemia. (Zalewska *et al.*, 2004). Organophosphates are present in many insecticides and herbicides. They are also the basis of many nerve gases (Sha and Layzer 2007). Hypermagnesmia is a condition where the balance of magnesium in the body is unstable and concentrations are higher than normal baseline values.

Congenital

Congenital syndromes affecting the neuromuscular junction are considered a very rare form of disease, occurring in 1 out of 200,000 in the United Kingdom. Engel *et al* (2012). These are genetically inherited disorders. Symptoms are seen early since the affected individuals carry the mutation from birth. Congenital syndromes are usually classified by the location of the affected gene products. Congenital syndromes can have multiple targets affecting either the presynaptic, synaptic or postsynaptic parts of the neuromuscular junction. Harper C. M (March 2004). For example, if the malfunctioning or inactive protein is acetylcholinesterase, this would be classified as a synapse congenital syndrome. (Engel, *et al* (2012))

Presynaptic

The diseases that act on the presynaptic membrane are autoimmune neuromyotonia, Lambert-Eaton syndrome, congenital myasthenia gravis and botulism. All of these disorders negatively affect the presynaptic membrane in some way. Neuromyotonia causes antibodies to damage the normal function of potassium rectifier channels, while Lambert-Eaton syndrome causes antibodies to attack presynaptic calcium channels. Congenital myasthenia gravis is a large group of diseases, since the genetic defects can affect any point in the chain of events leading to successful transmission across the junction. One discovered type of congenital myasthenia gravis can affect the junction presynaptically by a mutation in the gene encoding choline acetyl transferase. (Beytía, *et al*., 2012). This protein is an enzyme that is responsible for catalyzing the reaction that combines acetyl-coenzyme A with choline, yielding acetylcholine. There are many mechanisms through which presynaptic function can be impaired. Most often this causes a decrease in the release of acetylcholine. It can also impair vesicle exocytosis by interfering with the complex guiding vesicle fusion and release of contents. Mechanism of action can also impair the calcium channels that induce exocytosis of the vesicles. Other ion channels can also be

disrupted, such as the potassium channels causing inefficient repolarization at the presynaptic membrane as in neuromyotonia. (Van Lunteren and Moyer, 2005).

At the synaptic cleft, the neurotransmitter normally diffuses across the synapse to eventually contact postsynaptic receptors. However, after exiting the presynaptic membrane, the neurotransmitters can be hindered by a subset of diseases that interfere with the transmission of the neurotransmitter across the synapse. The mechanism currently known that operates via the synaptic cleft causing impairment of normal functioning is another congenital myasthenia gravis. (Levitan, et al., 2015). This mechanism is the only currently known disease that acts on the synapse. (Glass and Bowen, *et al*, (1996). It acts by impairing the function of the enzyme (acetylcholinesterase) that breaks down acetylcholine causing it to become very hypertonic at the synapse. (Glass and Bowen, *et al*, 1996) This increase in acetylcholine in the synapse disrupts normal functioning of the junction.

1.4.1. Postsynaptic

The highest number of diseases affect the neuromuscular junction postsynaptically. In other words, it is the most susceptible to negative intervention. (Van Lunteren E, Moyer M (2005). The targets of these postsynaptic diseases can be multiple different proteins. Immune mediated Myasthenia Gravis being the most common, effecting the acetylcholine receptors at the post synaptic membrane. All the diseases that affect the postsynaptic membrane are forms of myasthenia gravis. Here is a list of the diseases: Myasthenia Gravis, Neonatal Myasthenia Gravis, Drug Induced Myasthenia Gravis and several types of congenital myasthenia where the product of the mutated gene is a postsynaptic protein (Van Lunteren and Moyer 2005).

MYASTHENIA GRAVIS.

Myasthenia gravis is associated with severe muscular weakness because of a decrease in the number of acetylcholine receptors in the muscle cell. In myasthenia gravis, the end plate potential (EPP) fails to effectively activate the muscle fiber due to an autoimmune reaction against acetylcholine receptors, resulting in muscle weakness and fatigue. (Hoch, *et al,* 2001)

Myasthenia gravis is caused most commonly by auto-antibodies against the acetylcholine receptor. It has recently been realized that a second category of gravis is due to auto-antibodies against MuSK.

If the endplate potential is smaller, the endplate potential will fail to reach threshold. If it fails to reach threshold, there will be no action potential in the muscle cell and no contraction of the muscle, which causes muscular weakness. Neostigmine and other inhibitors of AChE are used to treat patients with myasthenia gravis. (Kohara et al. 2002). These agents make the amount of acetylcholine that is released to effectively reach the remaining acetylcholine receptors. This is one of the common disease state of the skeletal muscle system especially among the causation population. May be it is just as common in the black population but diagnosis being a problem in Africa. The incident rate is unknown, skeletal muscle so affected are weak and tired and therefore, do not have effective contraction. Hence, there is a practical paralysis of the neuromuscular junction and therefore, impulses are not effectively transmitted. It is theorized that myasthenia gravis is an autoimmune disorder in which a person so affected has developed auto antibodies against his or her own nicotinic Acetylcholine receptors like other autoimmune disorders the incident of this immune problem may be more common in females. The autoantibodies so produced destroys the majority of the individual nicotinic acetylcholine receptors so that even if the person produces enough acetylcholine (ACh) at the pre-synaptic terminus, there is still deficiency of the functions of the acetylcholine (ACh) at the post-synaptic (muscle fibre membrane, Therefore, are not really totally transmitted from the axon terminal to the muscle fibre plasma membrane, action membrane therefore, are not transmitted and as such end plate potential are not really properly generated.

1.4.3. TREATMENT

Drugs that antagonize Acetyl cholinesterase, e.g. Neostigmine and Physiostgmine can be used to treat this, these drugs inactivate acetylcholinesterase so that there may be summation of the little quantity of acetylcholine (ACh) released from the pre-synaptic membrane. The little quantity collected are able to stimulate the few available nicotinic receptors. Even though the activity of such person's skeletal muscle are not optimum, the person can manage to live with the problem just as a person are now seen to be living with HIV/AIDS.

1.4 4. LAMBERT -EATON SYNDROME

This is an autoimmune disorder that impairs communication between the nerve and muscle, causing weak nerve to communicate with muscle by releasing a chemical messenger (neurotransmitter) which interact with receptor on muscle (at the neuromuscular junction) and stimulates muscle to contract. Lambert -Eaton syndrome is caused by antibodies that interfere with the release of the neurotransmitter, Acetylcholine rather than attacking the Acetylcholine

receptor (AChR) as it occur in the Myasthenia Gravis. A different condition, Lambert-Eaton myasthenic syndrome, is usually associated with presynaptic antibodies to the voltage-dependent calcium channel. It is possible for these conditions to coexist. (Sha and Layzer, 2007).Lambert - Eaton syndrome usually precedes, occurs with or develops after contain cancers. (Titulaer, *et al*., 2011). It most commonly occurs in men with tumor in their chest, especially lung cancer.

TREATMENT

Guanidine, a Drug that increases the release of acetylcholine often lesson symptoms but may inhibits the bone marrow's production of blood cells and impair liver function. Corticosteroids and plasma exchange (filtering of toxic substances, including abnormal antibodies from the blood) help some people. (Titulaer, *et al*., 2011).

BOTULISM

Neurotoxin may act on the neuromuscular junction either post-synaptically or pre-synaptically as there are different forms of toxins that the neuromuscular junction (NMJ) is sensitive to (reference 14) Common mechanisms of action include blockage of acetylcholine released at the synapse thus, causing the NMJ to become abnormal in function. (Kohara, et al. (2002).

TREATMENT

Symptomatic treatment involves use cholinesterase inhibitor at the Acetylcholine receptor (AChR).Immunosuppressive treatment of botulism include the following; Thymectomy

Medical therapy: corticosteroid, non-steroidal immunosuppression. Short- term treatment: plasmapherisis, IVIG.

SUMMARY.

Neuromuscular Junction is the entire space between the nerve ending and the skeletal muscle fibre, it is simply the arena where the nerve ending meets with the skeletal muscle cell fibre which facilitate muscle contraction. In the nerve ending are vesicle which contain Acetylcholine (neurotransmitter) and proteins, the neurotransmitter is not synthesize right there in the nerve ending rather it is transmitted from the cell body, also the vesicle in which it is been stored is form from the death cell body, the protein in the nerve ending helps in signal transport, enzyme synthesis and also serve as choline carrier from the GIT. However, this movement of chemical

signal or electrochemical message from the nerve ending (presynaptic) to the post-synaptic (muscle fibre) is simply termed neuromuscular transmission. This movement of electrochemical signals starts pre-synaptically and been transferred down to the post-synaptic membrane(the motor end plate) as depolarization is set on the motor neuron of the nerve end plate potassium ion channel influx is generated on the pre-synaptic membrane leading to generation of another ion channel of calcium to create resting potential -10mv in the nerve ending intracellular fluid, as both are positively charged they repel each other allowing the protein on the vesicle synapto-brevin to fuse with transporter protein on the nerve ending membrane(the syntaxin) creating pocket like protraction along the nerve ending membrane through which acetylcholine diffuses in the post-synaptic membrane. At chemical synapses, impulses are transmitted by the release of neurotransmitters from the axon terminal of the presynaptic cell into the synaptic cleft. Their subsequent binding to specific receptors on the postsynaptic cell causes a change in the ion permeability and thus the potential of the postsynaptic plasma membrane Classic low-molecular-weight neurotransmitters are imported from the cytosol into synaptic vesicles by a proton-coupled antiporter. V-type ATPases maintain the low intravesicular pH that powers neurotransmitter import. Arrival of an action potential at a presynaptic axon terminal opens voltage-gated Ca^{2+} channels, inducing a localized rise in the cytosolic Ca^{2+} level that triggers exocytosis of synaptic vesicles. Following neurotransmitter release, vesicles are endocytosed and recycled. Multiple cytosolic proteins including synapsin recruit synaptic vesicles to the active zone of the plasma membrane adjacent to the synaptic cleft. The principal V-SNARE in synaptic vesicles is VAMP (synaptobrevin), which tightly binds the principal plasma-membrane T-SNAREs-syntaxin and SNAP25 with the assistance of Rab3A and other docking and fusion proteins. Synaptotagmin in the synaptic-vesicle membrane is thought to be the key Ca^{2+} sensing protein that triggers exocytosis. Stimulation of excitatory receptors by neurotransmitter binding causes depolarization of the postsynaptic plasma membrane, promoting generation of an action potential. Conversely, stimulation of inhibitory receptors causes hyperpolarization of the postsynaptic membrane, repressing generation of an action potential. Neurotransmitter receptors that are ligand-gated channels induce rapid (millisecond) responses, whereas those that are coupled to G proteins induce responses that last seconds or more. Depending on the specific receptor, the same neurotransmitter can induce either an excitatory or inhibitory response. Removal of neurotransmitters from the synaptic cleft occurs by enzymatic degradation, re-uptake into the presynaptic cell, or diffusion. Chemical synapses allow a single postsynaptic cell to amplify, modify, and compute excitatory and inhibitory signals received from multiple

presynaptic neurons. Such integration is common in the central nervous system. Postsynaptic cells generate action potentials in an all-or-nothing fashion when the plasma membrane at the axon hillock is depolarized to the threshold potential by the summation of small depolarizations and hyperpolarizations caused by activation of multiple neuronal receptors. At electric synapses, ions pass directly from the presynaptic cell to the postsynaptic cell through gap junctions. These synapses are much less common than chemical synapses.

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