University of Jordan
Faculty of Medicine
Department of Physiology & Biochemistry
Medical students, 2018/2019,
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Muscle physiology and the basis of contraction

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Introduction:

Three types of muscle are found in our body. Skeletal, cardiac, and smooth muscle cells. These cells are found where mechanical activity is needed. Movements of the whole body or parts of it need contraction of skeletal muscles. Pumping of blood in vessels need contraction of cardiac muscle. Emptying the content of hollow organs requires contraction of smooth muscle in that particular organ.

Muscle cells have been classified according to their characteristics, first (according to their appearance under the microscope) in **striated** (cardiac and skeletal muscle) and **unstriated** (smooth muscle) fibers. Second, (according to their innervation): **voluntary** (have somatic innervation), an example: skeletal muscle, and **involuntary** (have autonomic innervation), example: cardiac and smooth muscle.

Structure of skeletal muscle:

One muscle is composed of many **muscle fibers** that are lying parallel to each other and bundled together by a connective tissue. The most dominant structure in muscle fibers is the presence of **myofibrils**. Each myofibril consists of regular arrangement of cytoskeletal elements known as **thick and thin filaments.** Which give the striated appearance in skeletal muscle.

The special arrangement of thick and thin filaments in lighter and darker (I and A) bands, gives the striated appearance in skeletal muscle. The **I** band is formed only from thin filaments. While the **A** band is formed from thick filaments with the portion of thin filaments that overlap on both ends on thick filaments. The area of thick filaments that is not overlapped by thin filaments is known as **H zone**.

In the middle of I band, there is a dense vertical structure (flattened disc-like structure that hold thin filaments) known as **Z disc**. The area between 2 Z discs is known as **sarcomere**, which represents the functional unit in skeletal muscle contraction. In the A band a similar system hold thick filaments known as **M line**.

The cross sectional arrangement in the area where is an overlap between thin and thick filaments shows 6 thin filaments around one thick filament and 3 thick filaments around one thin filament.

Thick filament (1.6 μm length) is composed of several hundreds of **myosin** molecules that are held together in a specific arrangement. A myosin molecule is composed of 2 identical subunits. Each has a globular head that projects out to one end and a tail that is intertwined with the tail of the other molecule. Each myosin head has 2 binding sites. One can interact with thin filaments and the other is myosin ATP-ase site. The heads and the portions of tail that are protruding from thick filaments are known as **cross bridges**.

Thin filament (1.0 μm length) is composed of three proteins, actin, tropomyosin and troponin. **F-Actin** helix forms the backbone of a double stranded structure of the thin filaments. Each strand is formed of polymerized G-actin. On actin molecules there is a site that can interact with myosin head (**myosin binding site**). It is believed that this site is an ADP molecule bound to G-actin. The bases are inserted to Z disc. The ends lie in the space between thick filaments.

Tropomyosin is protein molecules that wrap around the F-actin helix. In resting state this protein covers the active site (myosin binding site) on actin and prevents interaction of actin with myosin head.

Troponin is a complex structure of 3 subunits, which plays a role in controlling muscle contraction. One subunit has affinity for actin (troponin I), the other has affinity for tropomyosin (troponin T), and the third has affinity for Ca++ (troponin C).

Interaction of thick and thin filaments to induce contraction, and the role of Ca++:

The myosin binding sites on actin is the place where myosin heads bind to actin. In the absence of troponin –tropomyosin complex, myosin can bind strongly to actin in the presence of ATP and Mg++. When troponin-tropomyosin complex is added the binding is inhibited. From these it was suggested that in relaxed muscle, troponin-tropomyosin

complex inhibits or physically covers the binding site on actin and prevents the interaction between myosin heads and actin.

In the presence of high Ca++ concentration, the inhibitory effect of tropomyosin-troponin complex on myosin and actin binding was inhibited (so, binding was induced). From this it was suggested that during muscle contraction Ca++ binds to troponin C (up to 4 Ca++ bind to one molecule of troponin C), this produce conformational changes that results in the displacement of tropomyosin away from the active sites on thin filaments. The uncovered active sites can interact with myosin and induce contraction in the muscle. This theory shows the relation between contractile and regulatory proteins (troponin and tropomyosin), and explains the role of Ca++ on muscle contraction.

During contraction, the two Z lines become closer. This results by pulling thin filaments inward toward the center of sarcomere. This will result in a decrease in the H zone, I band and the whole sarcomere length. This happens after binding of myosin heads to the active site on actin. After this binding, myosin bends between the head and the arm of cross bridges, which pulling the thin filament toward the center of the sarcomere. Bending (tilting) of myosin head is known as *power stroke*. Then the head detach from the actin and bind to another active site on actin, which located closer to the base of thin filament and the cycle is repeated many times. The result of this mechanism is more overlap will be obtained between thick and thin filaments by pulling thin filaments inside. This theory is known as “sliding theory” or “walk-along” theory.

According to this theory, after many cycles of (binding, power stroke, detachment, then binding again) that are taking place between cross bridges and actin, a shortening of the sarcomere will be induced in the muscle by sliding thin filaments toward the sarcomere center.

https://www.youtube.com/watch?v=GneonFlcZG8

Requirement of energy for contraction:

We have mentioned that myosin head has an ATP-ase site. At this site ATP binds, where it splits into ADP and Pi. This needs Mg++ to attach the ATP before ATP-ase can split ATP molecule. This breakdown of ATP occurs before the head links to actin. The resulted ADP and Pi remain bound to myosin and the generated energy from splitting is stored within the cross bridge. During relaxation of the muscle, the head is energized. When the muscle fiber is excited, the increase in Ca++

concentration in the sarcoplasm, pulls tropmyosin-troponin complex out of their blocking position. This will enable myosin head to attach to actin. When attached, myosin head can use stored energy to bend. After this power stroke, the head releases ADP and Pi from their site. At this point, the detachment of myosin head will take place ONLY when another ATP molecule binds to myosin head. After detachment the new molecule is cleaved, the head returns to its position and energized by splitting ATP. The cycle continues as long as we have high Ca++ concentration inside the sarcoplasm (cytosol) to keep active sites on actin ready for interaction with myosin.

ATP is necessary for the detachment of cross bridges from actin. Not enough ATP will cause muscle to stiff because of the inability cross bridges to detach from actin. This phenomenon is called **rigor mortis** (a stiffness of skeletal muscle after 3-4 hours of death).

Source of energy for muscle contraction:

During muscle activity ATP is needed to provide energy for the power stroke. In addition to that, Ca++ is pumped into the sarcoplasmic reticulum by the activity of Ca++ pump. This pump needs ATP for its operation. Pumping of Na+ and K+ through sarcolema maintains the ionic composition of cytosol and permits optimal activity of muscle cells. All these activities need a direct use of ATP. In muscle the amount of ATP is sufficient for only few seconds.

3 ways by which muscle cells supply additional ATP as needed:

1. Transfer of high energy phosphate from **creatine phosphate** to ADP:
   Creatine phosphate contains a high-energy phosphate bond. This bond can be transferred to an ADP molecule to form an ATP by the activity of an enzyme known as creatine kinase. The amount of creatine phosphate in muscle is 5 times that of ATP. For that the muscle needs more efficient supply for longer activities of muscle.

2. Oxidative phosphorylation: This takes place in the muscle when a sufficient supply for O2 is present. This pathway provides rich supply of ATP (from one glucose molecule processed by oxidative phosphorylation, 36 ATP molecules are yielded). This source is slow and needs constant supply of O2. This way can be sufficient for ATP supply when there is a

moderate demands for ATP, such as during light and moderate exercise (walking, jogging, or swimming).

3. Glycolysis: high amount of glycogen are stored in muscle cells. The breakdown of glycogen to glucose which can be broken down by glycolysis into two pyruvic acid molecules to yield 2 ATP molecules. Pyruvic acid can undergo further degradation by oxidative phosphorylation. Glycolytic pathway is much faster than oxidative phosphorylation in generating ATP molecules. And it is operating anaerobically (there is no need for O2). On this process, fast muscles are depending on for energy supply.

Although it is very useful during intense exercise when the O2 supply is reduced, but it can lead to a muscle **fatigue** because of accumulation of lactic acid in muscle which results in inhibition of enzymes (involved in energy-producing pathways or excitation-contraction coupling) and depletion of energy reserves.

Muscle mechanics:

We have seen that muscle contraction induces shortening in the sarcomere, which results by pulling thin filaments toward the center of the sarcomere. This contraction is seen in the whole muscle as a change in length. When a muscle contracts by changing its length without changing its tension, the contraction is said to be *isotonic*. If a muscle develops tension without changing its length, the contraction is said to be *isometric* (which can be recorded by using electronic force transducer to measure tension).

Tension and sarcomere length relation:

The tension that can develop on muscle depends on the length of sarcomere and the length of the muscle. When sarcomere length is more than 3.6 μm (length of one thick filaments and 2 thin filaments), the tension that can develop is almost zero. When the sarcomere length decreases, the tension increases as the overlap increases and cross bridges that can be recruited for muscle contraction increases. This increase reaches a maximum after which more overlap will reduce developed tension. The *maximum tension* that can develop is at the sarcomere length of 2.0-2.2 μm (this known as optimal length). Below this length (from

2.0-1.6 μm) an interaction between thin filaments and cross bridges from the other half of sarcomere may result in a decrease in tension.

From this we can conclude that more overlap between thin and thick filaments located in the same half of sarcomere will induce more tension. This tension is reduced by decreasing the overlap in the same side, or increase in the interaction of thin filaments with cross bridges from the other side of thick filaments (increasing overlap with the other side).

Tension and whole muscle length relation:

We have seen that maximum tension develops at a sarcomere length of 2.0 –2.2 μm. This corresponds with the resting length of the muscle. At its normal length, the muscle also responded with the maximum *active tension* (tension induced by stimulation). By stretching muscle (increasing its length), before stimulation we increase the inactive (passive) tension (due to elastic property) in the muscle. When the muscle stimulated at this new length will develop less active tension. That corresponds to the increase in sarcomere length beyond 2.2μm.

Velocity of contraction and load:

Skeletal muscle contracts with maximum velocity when it is not loaded. By loading the muscle, the velocity of contraction decreases as the load increases.

Muscle twitches and characteristics:

One a nerve of nerve-muscle preparation is electrically stimulated, the muscle will respond by a contraction then followed by relaxation. The whole recordings from the beginning of stimulation until the end of muscle relaxation is known as *simple muscle twitch*. The simple muscle twitch can take less time in muscles composed of fast fibers such as ocular muscle, or longer time in muscles composed of slow fibers such a soleus muscle. These muscles not only differ in their speed of contraction but also in their color and composition. Fast fibers are large fibers, have extensive sarcoplasmic reticulum, contain large amount of glycolytic enzymes, and fewer mitochondria. These fibers also have less extensive blood supply. Slow fibers are smaller, have more extensive blood supply, and contain more mitochondria. These fibers also contain larger amount of myoglobin (iron containing molecule similar to hemoglobin that can combine with O2), which stores O2 until needed by fibers for oxidative

phosphorylation. The presence of large amounts of myoglobin gives the slow fibers a reddish appearance. For this reason, slow muscles are known as red muscles while the muscles containing fast fibers are white muscle.

Skeletal muscles are innervated by motor neurons that originate from the central nervous system (CNS). Each neuron innervates a certain number of muscle fibers. Muscle fibers that are innervated by single nerve fiber are called **motor unit**. The number of muscle fibers in motor unit depends on the function of the muscle. Some muscle that controls fine movements such as laryngeal muscles have only two or three muscle fibers in motor unit. Movements that do not need fine control of muscle contraction may contain up to 100 muscle fibers in one unit.

Summation of simple muscle twitches:

Two types of summation are known in the muscle:

1. Frequency summation (wave summation) and tetanization:
   When muscle stimulated by more than one stimulus, this will result in successive and complete simple twitches if the time between 2 successive stimuli is more than the duration of simple muscle twitch. Increasing the frequency of stimulation (shorten the time between stimuli) permits excitation by another stimulus while the muscle is in simple muscle twitch. This may result in summing of the successive contractions. When frequency of stimulation is more increased and the muscle responds by contraction without any relaxation, we can say that the muscle is in **tetanization**.

2. Motor unit summation (multiple fibers summation): If only few nerve fibers in a nerve that innervates a muscle are stimulated, this will induce shortening in the muscle that corresponds to contraction of motor units that are innervated by stimulated nerve fibers. When the number of nerve fibers stimulated increases, this will recruit more motor units in contraction. The increase in contraction will result in an increase in the amplitude of simple muscle twitch. In human body this

summation is important for gradation of forces during contraction.

Staircase effect (Treppe):

When a muscle contracts after a period of rest, the simple muscle twitch has certain amplitude. After several contractions, the amplitude of simple muscle twitches increases. This is known as *Treppe* or *staircase effect*. This effect is probably due to an increase in Ca++ concentration inside the cytosol with each muscle stimulation and inability of sarcoplasmic reticulum to recapture Ca++ immediately.

EXCITATION-CONTRACTION COUPLING:

Skeletal muscle is voluntary. It contracts upon stimulation by motor neurons. Large and myelinated nerve fiber that originates in the anterior horn of spinal cord after entering the muscle branches in axonal terminals that end about near the midpoint of muscle by forming a **neuromuscular junction** (motor end plate). At that point, nerve terminal ends into a small invaginated part of the muscle membrane called *synaptic gutter* (*synaptic trough*). At the bottom of synaptic gutter, muscle membrane has small folds called *subneural clefts*, which increase the surface area of synaptic gutter. The small space (20-30nm) between the terminal and muscle membrane, where the neurotransmitter is released to stimulate muscle, is called *synaptic cleft* (*synaptic space*). In this space large quantity of *acetylcholinesterase* (an enzyme that destroys Ach after its release into synaptic cleft) is found. The enzyme can be inactivated by drugs, such as neostigmine, physostigmine, and diisopropyl fluorophosphates, which result in increased Ach concentration in synaptic cleft, and prolonged action of this transmitter.

Secretion of Ach from the terminal:

When the impulse reaches the terminal, this will cause Ach to be released into the synaptic cleft. The mechanism of release includes activation of voltage gated Ca++ channels, which results in Ca++ influx into the axon terminal. The increased Ca++ concentration in the terminal will cause the vesicles containing Ach to dock and fuse with the terminal membrane, and release their content into synaptic cleft by a process called **exocytosis**. About 125 vesicles release their content after one stimulus. Stimulation the release of neurotransmitter for long time may result in depletion of vesicles containing neurotransmitter. This will induce fatigue of neuromuscular junction.

Once released into synaptic cleft, Ach binds to its receptor (a complex protein of 5 subunits, 2α, β, δ, γ subunits). Two molecules of Ach bind to the α subunits and cause activation of chemical gated ion channel, which induces Na+ influx (diffusion) into the muscle fiber and causes local change in the membrane potential at the end plate known as *end plate potential*. The receptor is a subject of inhibition by curariform drugs such as D-tubocurarine, which can affect transmission of impulse from the nerve terminal to the muscle membrane by blocking the action of Ach on its receptor.

Transmission can also fail by destruction of chemical gated ion channels. This appears in myasthenia gravis (an autoimmune disease that generate antibodies against acetylcholine gated ion channels on the muscle) which results in muscle paralysis. The paralysis can be partially ameliorated by anticholinesterase drugs (neostigmine or physostigmine), which increase Ach in synaptic cleft.

In addition to its stimulation by Ach, the receptor can also be stimulated by many compounds, including methacholine, nicotine, and carbachol. These compounds produce prolonged activation of receptor (due to the absence of destroying enzymes that can destroy these compounds), which results in muscle spasm.

Generation and spreading of action potential to the interior of the muscle:

The end plate potentials generated by activation of chemical gated channels will induce activation of voltage gated Na+ channels. The activation of these channels will induce an action potential, which spreads over the sarcolema. At the surface of muscle membrane, they are small openings for tubules that run deeply (in transverse direction) in the muscle cell known as **transverse tubules** (T-tubules). These tubules contain extracellular fluid. They transmit action potential to the interior of the cell closely to myofibrils, where it stimulates release of Ca++ into the cytosol (sarcoplasm). The whole process by which membrane generate an action potential that causes **release of Ca++** which results in muscle contraction is known as **excitation-contraction coupling**.

The arrangement of T tubules and sarcoplasmic reticulum at the Z lines of the sarcomere permits release of Ca++ in close vicinity to contractile proteins of the myofibrils. These structures form a triad (2 sacs (terminal cisternae) of sarcoplasmic reticulum and one T tubule).

The gap between sarcoplasmic membrane and T tubule is spanned by a protein structure called **foot protein**. The part of foot protein in sarcoplasmic reticulum serves also as Ca++ channel and known as **ryanodine receptor**. The part of foot protein on T-tubules is known as **dihydropyridine receptor**. Dihydropyridine receptors are voltage sensors. The change in voltage of T-tubules will induce conformational changes in the whole foot protein, which results in activation of ryanodine receptors and rapid release of Ca++ from the sarcoplasmic reticulum into the sarcoplasm, which binds to troponin C and causes muscle to contract.

At the membrane of sarcoplasmic reticulum, there are also highly active Ca++ pumps. These pumps concentrate Ca++ inside the sarcoplasmic reticulum by 10.000 folds (Ca++ concentration in sarcoplasmic reticulum = 10^{-3}molar, in the sarcoplasm during rest = 10^{-7} molar, and during excitation of muscle = $2X10^{-4}$molar). The rapid uptake of Ca++ by these active pumps results in muscle relaxation.

SUMMARY:

https://www.youtube.com/watch?v=C3cZN9nV9KY

SMOOTH MUSCLE CELLS:

These muscles have their characteristics, which may differ from those of skeletal muscles. In addition to that, smooth muscle cells may also differ from organ to organ in their organization, physical dimension responses to stimuli, and innervation. Generally they are divided into multi-unit smooth muscle and single unit smooth muscle.

In multi-unit: each muscle fiber operates independently of all other fibers. In single unit muscles, their function needs cooperation of many muscle fibers to perform a function. Muscle fibers, in this type, are connected to each other by gap junctions to synchronize their contraction (functional syncytium).

Organization of contractile proteins in smooth muscle and mechanism of contraction:

The organization of contractile proteins is different from that in skeletal muscle. The actin filaments are attached to a dense structure inside the muscle known as **dense bodies**. These actin filaments radiate between dense bodies. In the midway between dense bodies, few myosin

filaments are found where they overlap with actin filaments. The mechanism of contraction in smooth muscle cells also involves actin myosin interaction but with different mechanism than that found in skeletal muscle. When smooth muscle is stimulated, it takes longer time than striated muscle to induce contraction (long latent period), the total contraction time is about 30 times more than that in skeletal muscle. These appear because of the slow attachment and detachment of contractile proteins, which results in slow cycling of cross bridges.

The mechanism of contraction in smooth muscle also involves an increase in Ca++ concentration but the source could be different than in skeletal muscle. The source in skeletal muscle is only from the endoplasmic reticulum, which has high representation in skeletal muscle, while in smooth muscle the main source is extracellular and some contraction can be induced also by the release of Ca++ from intracellular stores (sarcoplasmic reticulum), which is moderately developed in smooth muscle (not well as in skeletal muscle).

The release of Ca++ into the cytosol induces activation of a protein known as **calmodulin** by forming calmodulin-Ca++ complex (4 Ca++ bind to one calmodulin). The activated calmodulin-Ca++ complex will induce activation of an enzyme called **myosin kinase**. This enzyme will **phosphorylate** regulatory chain on **myosin head**. The phosphrylated myosin can interact with actin to induce contraction.

The relaxation in smooth muscle cells also involves a decrease in Ca++ concentration by increased activity of Ca++ pumps located at the plasma membrane and sarcoplasmic reticulum. In addition to that, the mechanism of relaxation also involves dephosphorylation of myosin heads by an enzyme called **myosin phosphatase**.

In some instances, the smooth muscle contracts and their contraction is sustained. This is known as **latch phenomenon**. This is due to a much decrease in cycling frequency of cross bridges. Which is probably due to a decrease in myosin phosphatase activity, that results in a decreased dephosphorylation of myosin head (remain longer time activated). Little ATP molecules are consumed during this phenomenon.

Membrane potential and action potential in smooth muscle cells:

The resting membrane potential in smooth muscle is less negative than in skeletal muscle. It is about -60 to –50mV in smooth muscle. The

characteristics of action potentials are also different in smooth muscle. Many types of action potential are found on smooth muscle fiber:

1. Spike potentials (have short duration): these can be elicited by external stimulus.

2. Action potential in with plateau: similar to the action potential that found in cardiac muscle. The onset is rapid as in spike potential, but repolarization takes longer time. This type of action potential has importance in organs where longer contraction period is needed such as in uterus. The longer action potential and the plateau in this type are due to activation of Ca++ channels. These channels are activated slowly and their opening is maintained for longer time than Na+ channels.

3. Slow wave potentials: some smooth muscle cells are self-excitatory. This property is due to rhythmic variations in membrane potential that appear at muscle membrane. These rhythmic variations are known as **slow waves**. These waves are probably caused by changes in Na+ pump activity, or changes in conductance of ion channels. Slow wave are not action potentials and they cannot induce contraction in smooth muscle. When the peak of these slow waves rises above threshold, they can generate spike potentials, which result in contraction of smooth muscle.

Neural and hormonal control of smooth muscle contraction:

Muscle cells are innervated by autonomic fibers. The terminals of these fibers are ending diffusely between cells and not forming organized synapses such as the motor end plate in skeletal muscle. The transmitter in autonomic fibers is found in varicosities of the fine terminals of nerve fibers. The released transmitters from these varicosities act on their receptors to induce activation or inhibition of the contraction in smooth muscle.

In addition to neural control, some smooth muscle membrane has receptors for hormones, neuropeptides, or other factors. These also control the activity of smooth muscle cells when their receptors on smooth muscle are activated.

The mechanism by which smooth muscle cells are activated may include activation of Ca++ channels or activation of phospholipase C. The later results in the formation of IP3 (inositol trisphosphate) and release of Ca++ from the sarcoplasmic reticulum. The mechanism of inhibition may include formation of cAMP or cGMP, which induces phosphorylation of some proteins that activate K+ channels or proteins involved in relaxation, or inhibition of proteins involved in contraction.