Human Physiology
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Week - 06
Lecture - 04

Hello, welcome to another new class on human physiology. Hopefully, you are enjoying the Human Physiology classes. This week, we were discussing our skeletal system. After that, we remember that we discussed bones, such as different types of joints and muscles. So, in this class, we will see how neuromuscular transmission happens. So, basically, how the signals from the nerves are processed and transmitted to our skeletal muscles, followed by muscle contraction.

So, let us stay with it. So, what different concepts will be covered in this class? We will discuss the neuromuscular junction. We will discuss different types of neurotransmitters, followed by a detailed discussion of how neuromuscular transmission occurs. We will also discuss a few conditions, such as different types of drugs and a few diseases that can affect the neuromuscular transmission process.

So, let us see them one by one. So, what is a neuromuscular junction? It is basically a junction between the terminal branch of the nerve and the surrounding muscle fibers. So, basically, as you can see in this diagram, this is like a neuro junction, a classical example of a neuro junction, where there are neuron cells from the nerves. So, these are neuronal nerve cells, and these are like our skeletal muscle cells, okay. And the junction between these neuronal nerve cells and the skeletal muscle cells is called the neuromuscular junction.

So, here, as you can see, the skeletal muscle fibers are innervated by the motor nerve fiber, and each nerve fiber divides into many terminal branches. Here you can see that each kind of terminal nerve fiber is getting branched into several terminal branches. And then these branches are innervated within the skeletal muscle cells. At the neuromuscular junction, the end of the motor neuron is called the axon terminal. So, this end area, like this end area, is also called the axon terminal.

So, these end areas are called axon terminals. And as you can see, these axon terminals are kind of branched into clusters of synaptic bulbs. So, you can see here they are kind of branched in small clusters of synaptic bulbs, right? So, a cluster of synaptic end bulbs. And suspended in the cytosol within each synaptic end bulb, it has about 100 membrane-enclosed sacs. So, if we zoom in on one of these bulbs, for example, you will see that inside the synaptic bulb there are a lot of membrane-encapsulated vesicles, and these vesicles have molecules called neurotransmitters.

So, inside these vesicles, there are different neurotransmitters; mostly in cases of the neuromuscular junction, what we see is that we find a neurotransmitter called acetylcholine. So, we see a neurotransmitter called acetylcholine. This is one of the most important neurotransmitters in the neuromuscular junction. Now, let us take a little bit further to see the details of the structure of the neuromuscular junction; first, we should focus on the axon terminal. So, this is basically the axon terminal area.

So, this is basically the axonal terminal area, right? We should also discuss the motor end plate, okay? So, basically, the terminal branch of the nerve fiber is called the axonal terminal. So, as you can see, this is the terminal branch of the nerve fiber. And when the action comes closer to the muscle fiber, it basically loses the myelin sheath, and the portion of the axon is expanded like a bulb, which is also called the motor end plate. So, you can see that in this terminal area, there is no myelinated sheath, right? In the neuron class later, we will see exactly how the myelin sheath works and where the myelin sheath is placed. But here you can see that there is almost no myelin sheath.

So, there is no presence of any myelin sheath or any type of Schwann cells or oligodendrocyte cells; everything you can see here, nothing is there. And in the end area, see it kind of form like a bulb-like structure. So, this area can also be called the motor end plate. These axon terminals contain a lot of mitochondria. You can see there are a lot of mitochondria, and as you know, mitochondria supply ATP.

Apart from the mitochondria, you can also see a lot of synaptic vesicles. So, these are like small vesicles. And as we already said in the last slide, this vesicle has different neurotransmitters, mostly acetylcholine. Mostly you will find acetylcholine in cases of the neuromuscular junction. The ACH, or acetylcholine, is basically synthesized by the use of mitochondria that are present in the axon terminal.

And eventually, once it is synthesized, it can be stored inside the synaptic vesicles. So, we will see in the next few slides how acetylcholine is synthesized, but this neurotransmitter is basically synthesized with the help of mitochondria, and once this ACH is synthesized, it can eventually go inside these synaptic vesicles for storage purposes. Then the next structural pattern is similar to the synaptic cleft. So, basically, the synaptic cleft is nothing but a space or the junction between the axonal terminal and the neuronal nerve. This is like the neuronal nerve area and the muscle area, right? So, basically, the gap between the nerve and the muscle is called the synaptic cleft.

So, the membrane of the nerve ending can also be called the presynaptic membrane. So, this is basically what you can also call presynaptic. So, the presynaptic membrane, the membrane of the muscle fiber on this side, can be called the postsynaptic membrane. So, the membrane of the muscle on this side can be called the postsynaptic membrane. The synaptic cleft basically contains basal lamina.

It is like a thin layer of spongy reticular matrix through which the extracellular fluid diffuses. So, basically, in this synaptic cleft, you can see a thin kind of basal lamina structure, which is basically like an extracellular matrix, a thin extracellular spongy matrix through which the extracellular fluid and other molecules can diffuse. And also, here there is an enzyme that can be found, which is acetylcholinesterase or AchE. So, this is the enzyme acetylcholinesterase. This can be found in this subregion, such as the neuronal cleft or the synaptic cleft area.

So, basically, in this junction area, you can find this AchE enzyme, which is present in a very high number and has a very crucial role to play. In the next few slides, we will discuss the role of acetylcholine esterase, but just remember that it has a very important role to play. So, let us see what acetylcholine is. So, as you can see the structure of acetylcholine, this is a very important neurotransmitter that is present in the neuromuscular junction. And this is the

primary neurotransmitter that is responsible for the transmission of nerve impulses across the neuromuscular junction.

And most importantly, we have to now discuss how the synthesis of ACH, or acetylcholine, happens. So, the first thing you have to remember is that ACH, or acetylcholine, is synthesized in the presynaptic terminal or the axonal terminal area. So, basically, this area is the axon terminal area, and here mostly the synthesis of ACH, or acetylcholine, happens. Now, acetylcholine, like one of the precursor molecules for acetylcholine, is choline. And this choline is basically obtained from food.

So, basically through the diet, it is like after the food is digested, it absorbs choline. Now, there are some choline receptors on the membrane of the axonal terminal area, and because choline concentration is high outside and low inside, choline can basically diffuse into the axon terminal area. Now, let us see how this choline actually gets converted to acetylcholine, or ACH. Interestingly, from the mitochondria, this acetyl-like CoA can come out. So, from the mitochondria, acetyl CoA or acetyl CoA enzyme A can come out or be generated.

Now, once this acetyl CoA is generated from the mitochondria, what happens is very interesting. So, there is basically this enzyme, which is choline acetyltransferase. So, you can see this enzyme, which is the CHAT or choline acetyltransferase. What it does, basically, is catalyze the transfer of this acetyl group. So, basically, what this CHAT enzyme does is take this acetyl group from the CoA and convert it with the choline.

So, what we just said is that for the synthesis of acetylcholine, one of the most important precursor molecules is choline, which is basically absorbed from food or diet. The external terminal membrane area has choline receptors, and through diffusion, choline can enter the external terminal area. Now, acetyl CoA, or acetyl coenzyme A, can be generated from the mitochondria. There is one enzyme that is called choline acetyl transferase. What this choline acetyltransferase, or CHAT enzyme, does is basically take this acetyl group and combine it with the choline.

Then what will it eventually form? It will form acetylcholine, right? Because this acetyl group eventually, so what is basically happening is that this acetylcholine group is getting combined with the choline, forming acetylcholine or ACH. And once this acetylcholine is formed, it now needs to go into synaptic vesicles because acetylcholine generally does not stay freely floating inside the axonal terminal area; it is stored inside those terminal vesicles. So, how again is the next step, the first step we discussed, which is the acetylcholine synthesis? So, this is the first step which discusses the acetylcholine synthesis. Now, we have to discuss how the acetylcholine synthesized by this mechanism is getting transferred inside these vesicles. So, let us see here; let us see in this image.

So, basically, let us consider these as the synaptic vesicles. And we have a few ion transporters here. Now, what happened in the presynaptic-like area or the axonal terminal area is that it generated acetylcholine. So, that means the acetylcholine concentration is high here, right? So, eventually, acetylcholine will go inside, right? So, basically, eventually acetylcholine will come inside. So, actually, although acetylcholine is generated, the acetylcholine concentration is low outside, and because there are a lot of already stored acetylcholine inside the vesicles, the acetylcholine concentration is higher inside the vesicles.

And this is, as you can see, happening against its concentration gradient, right? So, you can see the acetylcholine is moving from a low concentration to a high concentration against its concentration gradient, and as we all know, if an ion moves or if a molecule moves against its concentration gradient, it needs participation in direct or indirect participation of energy. So, let us see how it actually moves against the concentration gradient. Generally, the H plus ion is more numerous here, while the H plus ion outside is lower in number and more abundant inside. So, what happens basically is that initially protons come inside these vesicles from the synaptic area. So, what we just said is that in the synaptic axonal terminal area, or the presynaptic area, in this region, a lot of proton ions are present.

So, a lot of proton molecules or proton ions are there, and this proton ion is there by the process of primary active transport. So, by the process of primary active transport, this proton ion comes inside, and here ATPase has an important role to play. Now, tell me, as the lot of H plus ions is coming in, the H plus concentration is building up inside, right? So, H plus concentration is too high inside these synaptic vesicles. Now, this H+ can go outside of the cells through its concentration gradient. So, H plus can move across these cells through their concentration gradient.

And while they are going, a secondary active transport process is happening. So, basically, secondary active transport is happening here, and acetylcholine is basically moving from a low concentration to a high concentration inside these vesicles. So, this is the way, like here the primary active transport is happening, and here secondary active transport is happening. In this process, secondary active transport of acetylcholine is basically moving from low concentration to high concentration and getting stored inside the synaptic vesicles. So, this is the most important component for this, like the whole chapter or the lecture.

So, we will quickly recapitulate once more. So, what we just said is about how acetylcholine synthesis initially occurs. So, from the food. It is kind of converted to choline, or choline is getting absorbed from the diet or food. Now, through this choline receptor, you can see here in the axonal membrane that it is getting diffused and has come to the axonal terminal area.

Now, from the mitochondria, acetyl coenzyme A is coming out. From this acetyl coenzyme A, the acetyl group is transferred to the choline by the activity of the choline acetyltransferase enzyme. So, once this acetyl group is transferred to the choline, it makes acetylcholine, or ACH. Now, this ACH is present in the synaptic vesicles; although some amount of ACH is being produced in this axonal terminal area, we have to consider that ACH is still low in concentration outside. Compared to the low concentration of ACH molecules outside, generally in these synaptic vesicle areas, the number of ACH molecules is much higher.

because it is continuously getting stored inside these synaptic vesicles. Now, how this low number of ACH will enter this high amount of ACH inside these vesicles will be against this concentration gradient, and as we all know, if the molecular movement or ionic movement goes against its concentration gradient, it occurs via the process of active transport. So, how does active transport happen? It involves both primary and secondary active transport. Generally, the H plus ion concentration is low in number inside this axonal terminal area and high in number inside the vesicle area. So, this H plus is first coming inside with the help of the ATPase, right? And once the H plus comes inside, it builds inside the synaptic vesicles, increasing their concentration.

Now, H plus from the high concentration inside the vesicle is going outside where the process is like diffusion, and when the H plus is going, it is helping the acetylcholine, which was low in number inside this axonal terminal area, to become high in number, which is present inside the vesicles. So, this is a process called secondary transport, like active transport, which does not directly need energy, but it also depends on something like primary active transport of the proton pump. So, hopefully it is clear to you how the ACH synthesis happens, followed by how the ACH molecule is basically transported and stored inside the synaptic vesicles. So now see the next steps in neuromuscular transmission. So once the ACH is synthesized and stored inside the acetylcholine, what will happen is that the acetylcholine needs to diffuse out from the neuron, right? And once we discuss during the neuron class, you will see that neuronal transmission happens.

So I'll just briefly tell you because we will discuss all of this in the neuron class. So, basically what happens is that it generates an action potential. So, it generates action potential where there are a lot of sodium pumps here. And these are voltage-gated sodium pumps. So, when this axonal area gets a voltage of about minus 55 millivolts, a lot of sodium ion channels, which are the voltage-gated sodium ion channels, get activated, right? A lot of sodium voltage-gated ion channels get activated, and this charge, the potential from minus 55 millivolts, gets converted to plus 30 millivolts.

So, basically, it is kind of a depolarization that happens. So, basically, a depolarization happens, and the potential from minus 55 millivolts gets converted to plus 30 millivolts. And this, plus a 30 millivolt charge, basically comes to the next transporter ion. Again, this transporter ion, the voltage-gated ion transporter, opens up, allowing a lot of sodium ions to come inside. Further, this cascade of events happens, and this way, the positive charge migration occurs.

And inside this terminal area, there are a lot of calcium ion channels. So, when this plus 30 millivolt charge, or the positive 30 millivolt charge, basically attenuates near this area, this calcium voltage-gated ion channel pumps open, causing calcium ions to come inside. And now you also have to see what happens once this calcium ion comes inside. So, let us see the next image. So, basically, let us consider that these are the synaptic vesicles and that we have inside them the neurotransmitter, which is acetylcholine.

Now, let us see if this is the synaptic bulb; this is the synaptic bulb from where the depolarization wave is coming, which is minus 55 millivolts to positive 30 millivolts, and due to this depolarization wave, what is happening is that a lot of voltage-gated sodium ion channels have opened. Right. So, sodium ions are basically coming inside, right? So, basically, sodium ions are coming inside, and as sodium ions are positively charged, further inside, the positive charge is building, which is about plus 30 millivolts, and this positive charge is affecting the next sodium channel pump to open, allowing more and more sodium to enter. So, it's like where the positive channel or the positive ions are migrating. So, once this positive ion migrates in this terminal area, there are also calcium ion channels.

So, whenever this positive 30 millivolt is coming, it also activates the calcium ion channels, and calcium basically floods inside. So, basically calcium, which is more outside of the cell, flooded inside of the cell. So, once calcium comes inside, you can see that inside, not outside, of these synaptic vesicles, there are a lot of SNARE proteins. So, we will discuss this again during our neuronal neurotransmission class, but you can see there are a lot of peptide or protein linkers in these synaptic vesicles, and there are also certain peptide or protein linkers in the

membrane in the axonal terminal membrane area. Now, initially these vesicles were floating at a far distance from the terminal membrane area.

What happens basically when calcium comes inside is that this calcium creates a cross-linking between these two proteins. So, what happens is that calcium creates a cross-linking between two proteins, and once the cross-linking happens, both of these basically get twisted. So, both the protein kinds get folded and twisted. And eventually, these vesicles are pulled close to the membrane. So, these vesicles are kind of pulled near the membrane, and once these vesicles are pulled near the membrane, they basically diffuse inside with this membrane, and the process that occurs is called exocytosis.

So, basically, exocytosis happens, and these membranes get ruptured and diffuse, allowing all these acetylcholine molecules to come inside the synaptic cleft or the synaptic junction area. So, this is the way in which there is importance of the calcium ion and how these calcium ions come in; we have already discussed that due to a depolarization wave, due to depolarization which is from minus 55 millivolts to plus 30 millivolts. So, due to this voltage transmission, eventually the calcium ions come in; calcium ions create a bridge between the proteins that are present outside of the synaptic vesicles and this axonal terminal membrane area. Eventually, it causes those pulling these vesicles toward the membrane area, eventually diffusing these vesicles. And through the exocytosis process, the ACH molecule comes outside.

So, let us now see what different steps we discussed. So, the first step we discussed was the synthesis of acetylcholine. So, we discussed acetylcholine synthesis, then we discussed acetylcholine transport and storage, right? So, we discussed acetylcholine transport and storage to the vesicles, to the synaptic vesicles. Next, we discussed the release of acetylcholine. Next, we discussed the release of acetylcholine. Now, we have to see how this release of the neurotransmitter, acetylcholine, basically creates neuromuscular transmission and eventually affects the surrounding muscle.

So, in the surrounding muscle, you can see there are a lot of nicotinic receptors. So, you can see that there are a lot of nicotinic receptors, and with these nicotinic receptors, acetylcholine basically initially creates a nicotinic acetylcholine receptor complex. So, basically what it does with this nicotinic receptor is that the acetylcholine binds and creates an acetylcholine receptor complex. And when it binds with the acetylcholine receptor complex, it basically activates a lot of ligand-gated sodium ion channels.

So, basically, these are like the ligand-gated. So, you can consider these as ligand-gated sodium ion channels, right? So, these are ligand-gated sodium channels. In the case of ligand-gated sodium ion channels, for example, it has a binding pocket. So, this ACH, once it comes and fits inside the initially closed gate or the membrane, will basically open, right? So, it basically will open up, and once this gate, or the like, the closed membrane opens up, what happens is that sodium ions flood inside. So, basically, there is a lot of sodium ion, and initially, the sodium concentration is higher outside and lower inside. So, basically, a ligand-gated diffusion happens and a lot of sodium ions come from the ECF into this neuromuscular cell or muscle cell.

So, after sodium ions are present, it can alter the resting membrane potential and create an electrical potential known as the end plate potential. So, let us see how the development of the end plate potential occurs. So, basically, initially the resting membrane potential of the neuromuscular junction was about minus 80 to minus 90 millivolts, and as a lot of sodium ions were coming inside. Because what we said is that these ligand-gated channels are nicotinic

receptors, and whenever this ACh binds to them, it opens up the gate, allowing a lot of sodium ions to come inside. So, initially the voltage at the resting potential was about minus 80 to minus 90 millivolts.

And now, when the sodium ions are coming inside, the charge is basically getting polarized to about minus 55 millivolts, and this is only called the end plate potential. So, basically this is called an end plate potential, which is around minus 55 millivolts. So, this end plate potential that is being generated is also called a graded potential. This is also called a graded potential. We will again see similar terms such as resting membrane potential, graded potential, and action potential in our neuronal class.

Again, we will discuss similar types of things, but in a different context. So, as we see, what basically happens is that first ACH is synthesized, then ACH is stored, and after that ACH is released in the synaptic junction. When ACH is released, there are nicotinic binding receptors with which it binds, and it opens up ligand-gated channels. ACH, like ligand-gated sodium ion channels, causes sodium ions to flood inside, and the resting membrane potential, which was about minus 90 millivolts, increases to about minus 55 millivolts.

Due to sodium ions, which are called the end plate potential. And this is also called threshold potential; this is also called threshold potential. And whenever there is this threshold potential generation, basically at this minus 55, it kind of opens up the voltage-gated ion channels near this muscle cell. So, basically near this sarcolemma, it contains a lot of voltage-gated sodium ion channel cells, and whenever the cells attain this minus 55 millivolts, which is also called the end plate potential or the threshold potential. All those voltage-gated sodium ion channels open.

So, basically, voltage-gated. So, these voltage-gated sodium ion channels open, and a large number of sodium ions come into the cell, causing the potential to increase from minus 55 millivolts to plus 30 millivolts. So, basically, it gets further depolarized. And in this way, the depolarization wave basically propagates, which is also called an action potential, and this propagation is essentially unidirectional in nature. So, in this way, the wave of depolarization generates this action potential in a unidirectional manner. And then this action potential basically propagates along the T-tubules, and simultaneously, what happens is that there are a lot of potassium ion channel pumps, and those potassium ion channel pumps get activated at around plus 30 millivolts.

So, what we said initially is that whenever the sodium ion channel pumps open, the voltage basically increases to plus 30 millivolts, and at this plus 30 millivolts, potassium ion channels open, and generally potassium ions are high inside the cell and low outside the cell. So, basically, potassium ions come outside. A lot of potassium ions come inside, causing the repolarization from plus 30 millivolts back to the resting membrane potential of minus 90 millivolts. At the same time, what happens is that this plus 30 millivolts activates the calcium ions and the voltage-gated calcium ion channels, right? So, basically, whenever there are a lot of calcium ion channels also inside and whenever this depolarization wave is going, which is plus 30 millivolts, it activates these voltage-gated calcium ion channels, causing a lot of calcium ions to come inside. causing a lot of calcium ions to come inside, and this calcium release is basically very important in terms of muscle contraction through the sliding filament mechanism.

So, basically, once these calcium ions come inside, what they do is bind with the troponin type of protein, and by the mechanism of the sliding filament mechanism, they contribute to muscle contraction. So this is the whole way: as you saw, initially, the neuromuscular and neuronal transmission happens there, the action potential gets generated, right? Then, acetylcholine gets synthesized and stored inside the vesicles. After that, due to the action potential, calcium ions come in, which basically helps in terms of bridging the SNARE proteins, assisting in the exocytosis and release of acetylcholine at the synaptic junction. Then acetylcholine binds to the nicotinic receptor, causing the ligand-gated sodium ion channels to open. The sodium ions basically come inside and increase the resting membrane potential from minus 90 millivolts to minus 55 millivolts, which is also called the end plate potential or threshold potential.

At this threshold, a potential action potential or depolarizing wave is created basically with the help of voltage-gated ion channels. So, a lot of sodium ions come in, the voltage increases from minus 55 to plus 30, and simultaneously, a repolarization wave is also created by the removal of potassium ions from the cell. At the same time, the positive 30 millivolt charge helps to open the voltage-gated calcium ion channels, and the calcium ions come inside the cell, which eventually bind with troponin and other proteins of the muscle, causing muscle contraction. So, this is the whole way in which muscle contraction occurs in neuromuscular transmission through this process. The last important thing is similar to the very important one, which is the destruction of acetylcholine.

So, whenever neurotransmission like this happens, there is a lot of extra acetylcholine that can be present in this synaptic cleft or the neuromuscular synaptic junctions. So, if these molecules of acetylcholine persist or stay here, they can cause further stimulation to this muscle cell, leading to continuous muscle contraction. And if it happens, the muscle can get like a muscle spasm and all other types of continuous excitatory movement. So, after the excitation step, muscle needs to be inhibited, right? So, basically, what happens is this additional ACH gets immediately degraded by this enzyme, which is called acetylcholinesterase. So, what this acetylcholinesterase does is basically degrade the ACH, right? So, it basically degrades the ACH back to inactive choline and acetate.

So, what it does is degrade the acetylcholine to choline and acetate, and further, this choline can again go inside the cells where the choline receptor is, and the process continues. So, in this way, it is very quick—within a millisecond—this kind of conversion of choline and acetate happens from acetylcholine, and the enzyme that is important for this is acetylcholinesterase. There are different factors on which neuromuscular junction and transmission kind of depend. We will quickly go through it; you can review this note to understand better. So, basically, this Curae, which is like a plant-derived poison, acts as a competitive antagonist at this nicotinic acetylcholine receptor.

And as you can understand, if it completely binds with this nicotinic acetylcholine receptor, what will happen is that ACH will not be able to bind, causing no muscle contraction. So, basically, muscle paralysis can happen. So, this type of plant-based poison can cause muscle-like paralysis. In the same way, this Botox toxin can also cause the presynaptic terminal cleaving of the SNARE protein, and that means there would not be any vesicle fusion, and if there is no vesicle fusion, the ACh will not come to this synaptic cleft. In the junction area, if there is no synaptic junction area in the presence of ACH, what will happen is that muscle paralysis can occur, and muscle spasms can happen because there would not be enough ACH present in the neuromuscular junction.

There are other drugs, such as ACH inhibitors and organophosphate poisons; you can read about these and see how they basically affect the neurotransmission and neuromuscular transmission processes. There are a lot of diseases, for example, myasthenia gravis, which is an autoimmune type of disorder where antibodies basically attack and destroy these nicotinic acetylcholine receptors at the motor plate, and what will happen in the same way is that if these receptors are damaged, ACH will not be able to bind. So, ACH binding will not happen, which will cause muscle weakness, muscle-like fatigue, and paralysis. So, temperature and pH are also highly responsible. So, basically, around 30 to 40 degrees of temperature are required for this efficient action, and a pH of about 7 to 8 is required for proper enzymatic and protein activity.

So, do you know that the neuromuscular junction is capable of significant plasticity and adaptation? And then finally, the activity question is if a person were exposed to a poison that completely blocked the acetylcholine receptor. So, the nicotinic acid is. So, this is the nicotine acetylcholine receptors at the neuromuscular junction; what will happen? So, can you tell me what will happen to the muscle if there is a poison that blocks the nicotine acetylcholine receptor in the neuromuscular junction? So, hopefully you like the human physiology class. So, this week we thoroughly discussed the skeletal muscles, the bone joints and muscles, and finally, how neuromuscular transmission occurs.

So, very soon we will meet with you again for another new class on human physiology. Thank you again.