Human Physiology
Prof. Nishikant Subedar
IISER-Pune

Lecture – 07
Molecular Mechanism of muscle contractility - Part 2

We will move on and try to understand how the calcium is made available. Okay, but for that we need to understand how the innervation of the skeletal muscle really takes place. So I am sure most of you can understand what this for those who cannot let me make it very simple. We are looking at the section of a spinal cord, spinal cord. Okay, your spinal cord at the level of say your neck and shoulder, this your spinal cord. Okay, and in the spinal cord you can see the, the grey, the H-shaped grey matter in which all the neuronal cell bodies are located.

And some of the neuronal cell bodies, in particular the ventral part of the grey matter, this is a neuronal cell body, this will give rise to an axon that will come out of the spinal cords and it will go and terminate on the skeletal muscle. So any fibre that you will find, any nerve fibre that you find ending on the skeletal muscle has its cell body located where, look at the image and tell me. It is in the grey matter of the spinal cord of the central nervous system, of the central nervous system. As I am talking, okay, I am controlling the muscles of my jaw which are the skeletal muscles which is voluntary and I am going to control it. That is why I am able to talk coherently.

That is happening because the cells, the motor cells are located in the brain. Okay, and there those cells keep on sending messages to the skeletal muscle of the jaw and then I am able to compose the sentence. Good. So where is the cell body located? In the central nervous system, it may be in the brain or in the spinal cord. Any problem there? Okay, so, so, it is a very interesting piece of information.

Don't lose it. For every skeletal muscle, it has to have the nerve supply, no nerve supply - the skeletal muscle is useless. The nerve supply has to be there, has to be there, okay. Nerve supply, muscle just doesn't do anything, okay. The muscle needs to be told and told by a nerve, told by an axon and that axon will have a cell body, a neuronal cell body and a nucleus and certain level that is located in the spinal cord or it may be located in the brain.

Okay so far. And it will give rise to an axon that will go, and I will call this as the innervation of the skeletal muscle and the neurotransmitter that it releases is called as?

Acetylcholine. Everybody should say that. Acetylcholine. Acetylcholine, what is it? Acetylcholine. Acetylcholine and since it is the neurotransmitter, there is acetylcholine, therefore, I can also say that this neuron which is sitting in the spinal cord is acetylcholinergic.

What did I say? Acetylcholinergic. Say that loudly. Acetylcholinergic. Acetylcholinergic. So it is acetylcholinergic neuron and the neurotransmitter that it will released on the muscle. Now here we have the entire scenario.

Okay, it is an amazing figure, but I will take you through. Actually we have seen all the steps, but I will take you, I will hold your hand and take you through. So here you have the same image, here you have a neuron and that neuron is innervating and the same neuron is innervating a skeletal muscle. Good. So if you stimulate this neuron, okay, now why should that neuron be stimulated? That neuron should be stimulated because my brain decides that I want to pick up the chalk and then my frontal cortex puts the information together and that instructs my hands, therefore my hands moves in the direction of the object, it picks up. Well, why it is happening? Because the information from the command that has been given by the frontal cortex has gone to the spinal cord, in the spinal cord the signals have finally focused on that neuron, that neuron which is here in my spinal cord at the level of my shoulder and that is a cholinergic neuron and then cholinergic neuron starts firing.

Okay and when that cholinergic neurons start firing, then the muscles in my hand - they respond. They respond by contracting, therefore I am able to lift an object. So who is commanding the muscles to contract? The nerve, the nerve, okay and how is the communication between the nerve and the muscle established? Neurotransmitter, what neurotransmitter? Acylcholine, good. So here you have the Neuro-muscular junction, or motor end plate and that neuron I can also call as a motor neuron.

What do I call it as? Motor neuron. Motor neuron, okay so it is a motor cholinergic neuron sitting in the spinal cord which sends a fiber that comes out of the spinal cord and innervates a skeletal muscle. And when this neuron fires or when this neuron brings action potential, this neuron at its terminal has synaptic vesicles which contain what neurotransmitter? Acylcholine. Acylcholine, okay and when the action potential arrives in this part of the, on the presynaptic side, hello, on the presynaptic side there are voltage-gated calcium ion channels. The calcium will enter and it will mobilize the trafficking of the synaptic vesicles. The synaptic vesicles will go and merge with the plasma membrane by the process of exocytosis. Exocytosis, say that again.

It is by the process of exocytosis that the synaptic vesicles will release their contents and

the acylcholine will flow, flow in the synaptic space. So in this space you will have the acylcholine molecule. That acylcholine molecule will just diffuse, okay diffuse and some will come in contact. So what do you have this - red line here indicates the plasma membrane of what? The skeletal muscle, okay and that plasma membrane has a system of proteins. Okay and that system of proteins I will call as acylcholine receptor. What will I call it as? Acylcholine receptor. That is relatively a big molecule. I will show you how big that molecule is, that receptor molecule has binding sites, okay and those binding sites will have affinity for acylcholine. So acylcholine will combine and once it combines this receptor here which is sitting on the plasma membrane will respond.

How will it respond? It will respond by opening, it is a channel protein, it is a channel protein, that channel protein will open and it is selective for sodium ions. What ions? Sodium ions. Now sodium ions will go from which direction to which direction? Say that loudly. Outside. Why from outside to inside? Inside.

Number two reasons, actually two reasons because outside is more, how many times more? 14.2 more. And number two, they will go in because it is a polarized membrane, outside is positive as compared to inside, inside is about minus 70 mV. So it is also the electrochemical gradient, got my point? Electrochemical gradient which will suddenly draw the sodium ions to inside and as a result, when the sodium ions go in, originally outside was plus, inside was minus but because of the sodium ions something interesting has happened. What is it? Reversal of polarity, depolarization or reversal of polarity. Then that actually is the current flows.

So current is flowing, can you see this depression here? Do you remember what that depression could be about? T-tubule, you remember? Hello? T-tubule. Okay, so it goes along with the T-tubule and as it goes, the action potential spreads along the plasma membrane and the voltage gets added up because of the opening of the sodium ion channels and finally, as you go inside you interact with a very interesting kind of protein. Very, very interesting kind of protein which the author is showing here. That protein is actually voltage gated calcium ion channel. What did I say? Voltage gated calcium ion channel. And that voltage gated calcium ion channel was in a particular conformation and that conformation was so because outside was plus and inside was minus. As the wave of depolarization arrives there is a change in the electric charge and as a result the voltage gated calcium ion channel will undergo a conformational change and it will open.

Voltage gated. Okay, okay. Now that protein which is sitting here, okay, inside is, inside very close to that is endoplasmic reticulum or sarcoplasmic reticulum. Are you with me? In that sarcoplasmic reticulum there is another receptor. Okay, another protein molecule. So what is actually happening is, the two membranes, one is the plasma membrane, T-

tube is lined by plasma membrane, that membrane and the endoplasmic reticulum come very close.

And there is a protein sitting here which is voltage gated calcium ion channel and on the endoplasmic reticulum there is another protein, I will give you the name for the first time. It is called as ryanodine receptor. Say that again - Ryanodine receptor.

Again. Ryanodine receptor. You did not say. Ryanodine receptor. What do you call it as? Ryanodine. Don't forget. Okay, so one protein is talking to another protein, one protein is sitting in the plasma membrane, the other is sitting in the sarcoplasmic reticulum and they are mechanically linked.

And when this guy, by this guy I mean that calcium. When this guy changes, this guy also changes because they are mechanically linked, they are mechanically linked. Not electrically, but mechanically linked. Actually, it is like something like one protein shaking hands with another protein molecule. And when the ryanodine receptor receives the information, it opens. And when ryanodine receptor opens, then the contents of the sarcoplasmic reticulum are now free to flow in the cytoplasm. The endoplasmic reticulum is a rich source for calcium ions.

The calcium ion concentration, the calcium ion concentration in the sarcoplasmic reticulum may be 1000 times more, 5000 times more, huge huge. So the moment it opens, the calcium ions, so here we have, okay, I have given you one name. Now the biologists have given different names for the same thing. Okay, but because textbook used it, therefore I have to tell you. I said calcium channel.

That particular calcium channel has affinity for an organic molecule called as dihydroxypyridine. Did you observe the statement? That protein molecule, that protein molecule which is sitting in the plasma membrane, which serves as what? Which serves as calcium channel, has affinity for what? Dihydroxypyridine. Therefore, this calcium channel is also called as DHPR, dihydroxypyridine receptor. What did I say? Dihydroxypyridine receptor, DHPR, very commonly used, DHPR.

Okay, it is calcium channel. So I will use my newly introduced language, DHPR talks to ryanodine receptor. And when it happens, the ryanodine receptor - and then suddenly if I draw your attention to the sarcoplasmic reticulum, every red dot represents a calcium ion. Now you can see here, now it has opened and as a result the calcium ions have started diffusing outside. Now the calcium ions are suddenly available and as a result of that you can imagine the interaction between the cross bridges. Cross bridges will be excited and then you will get the what? What cross-bridge cycling, what will you get? Cross-bridge

cycling. But at the same time, you do not want the interaction to go on forever.

So you want to stop it. So how do you stop it? You stop it because in the endoplasmic reticulum, in the wall of the endoplasmic reticulum, there is yet another protein system which we call as calcium pump. What do I call it as? Calcium pump. Pump. And can you, can somebody please guess what would be the function of calcium pump? Pumping back. Pumping back calcium from lower concentration to higher concentration and for that which work needs to be done - you will need energy or ATP.

So this is an ATP driven system in which the calcium ions will be picked up from the cytoplasm and it will be taken within the reticulum and as the calcium ion concentration drops then there is no more calcium available. So there is complete cessation of the interaction between the actin-myosin filaments and the cell or the muscle cell will relax. Have you gone through the entire cycle of events? How the availability of calcium will ensure that the muscle contracts and just read there, the skeletal muscle, DHPR also known as L-type calcium channel is a voltage gated calcium etc.

Okay, I will move on now. Yeah. So what regulates this calcium pump? The answer to that, you do not really need any regulation, it keeps on working 24 by 7. As long as there is calcium and as long as the difference between the two is critical, okay. I mean not that every calcium ion will be picked up, that is not possible But as long as it is within the threshold, it will keep on picking up the calcium ions from the cytosol and putting it in the lumen of the sarcoplasmic reticulum, okay. If it finds a calcium ion floating around, it will pump it in, right.

Yeah, please. Okay, okay, good you asked. L stands for long lasting. Okay, now you can ask why long lasting. The answer to that question is, you see, as you go deeper into the calcium channels, for that matter, as you go anywhere in biology, you find that there is not one standard protein, there are half a dozen of them. Okay, so you have actually, if you remember, I mentioned that in the, on the presynaptic side, there are calcium channels, they are slightly different type and we call them as P and Q type of calcium channels. Okay, these ones are, DHPR ones, okay, they are, they open, they remain open for a little longer. Therefore, the phrase has come long lasting. And therefore, we have taken that L and we start calling them as L type, which happens to be same as DHPR. Have I sorted out this thing? So, if you go into the literature, you will get even 3, 4, 5 more types of calcium channels and they have been named accordingly. These ones are called as L type. I have also given the reason why they are called as L type, okay.

Make sense? Yeah, go ahead. The DHPR, it is a voltagegated calcium channel, is it also

ligand gated by dihydropyridine? No, no, no, no, no, no, no, no, it just binds. It cannot, it is good you asked. The dihydrozepyridine cannot open the channel, it binds. And binding improves its efficiency of… No, it does not, okay. Dihydrozepyridine is not a molecule that will operate the calcium channel, no, it just binds, that's all.

It binds there, okay. And this binding is, you know, why this binding is very important? I will tell you why. Very interesting. I will ask you a biochemical problem.

I will give you 10 grams of muscle, okay. And I will ask you to, as a biochemistry student, please separate for me this DHPR receptor. You understood the problem? Okay, I am giving a problem, okay, okay, I will give you 10 grams.

Now I will give you a clue. DHPR. Now, use your intelligence and tell me how will you use it. It will bind it to some agarose beads and do the column chromatography. Something like that or I can use labeled dihydropyridine, okay, and then so it will have affinity, so it will separate. Then I will separate the rest of the things and then maybe I will change the pH so that the dihydro pyridine separates from the molecule. Are you getting the argument now? So it binds but that does not mean that it operates, no, it does not change. I mean, it is not a replacement for the molecule will respond only to the voltage changes. Got the answer? Sir, there is no biological function of dihydropyridine itself.

There is no biological function, there is no, okay, okay. She is asking a very interesting question, is there a biological function for DHPR? The answer to that question, there is no biological function, but I will give you a clue, I will give you a clue. Can you use it as a drug? Hey, think about it. It has an affinity to modulate a channel. Are you getting argument? So such things give us the clue to finding out if DHPR antagonists have certain medicinal value.

We will talk about it later. You know there is a, there is a plant. That plant is called as, can you read for me? Ryanodine is a poisonous alkaloid found in South American plant Ryania speciosa. Got it? And people without knowing anything about its pharmacology have been using the extract of that plant as what? Read there? As an insecticide. Now, can you, with your common sense, can you put two and two together and figure out why this substance was being used as an insecticide? They did not know how it works, okay. But now we know that, that when you use that, it enters into the body and then binds to ryanodine receptors in insects. So, this also proves that insects have ryanodine receptors.

Hello? Are you getting the argument? Insects also have what? Ryanodine receptors. Okay. So, so this, this is a plant, from this plant you get an extract, from that you can

isolate this alkaloid and this, you can label that alkaloid and then you can use that alkaloid to separate ryanodine receptors from skeletal muscle. Are you with me? And therefore, now you also know why ryanodine receptor is called as a ryanodine receptor. Okay, it is named after the molecule which has affinity for the protein.

What is the protein - ryanodine receptor. It has affinity for what? An alkaloid. Where do we get it from? Please read. Will you read for me the name of the plant? Ryania speciose what is it? So, so this is the ryanodine molecule that you get from here which has affinity for that and this is the dihydroxypyridine which has affinity for the DHP receptor.

This is interesting. Well, a disclaimer, I do not understand much of that figure. Okay. Anybody of you can, can you throw light on this? This is a calcium ATPase of muscle sarcoplasm. You remember that pump we talked about? Pump, where was it located? On the membrane of the sarcoplasm, we had a protein system.

Okay, now this is that protein system that really looks like. This is the crystal structure of the calcium pump, calcium ATPase and can you see the author has written here something. What does it mean? On that molecule, that is where the ATP can combine and then it can change the molecule. This is the lumen of the endoplasmic reticulum or sarcoplasmic reticulum and your aim, whenever you want to relax the muscle, your aim is to draw all the calcium and put it back here as much as possible and this is possible because of this huge protein molecule which we call as simple language as calcium pump. But when you say calcium pump, we generally have a very simplistic idea as I say so. But how complicated is the biology? To just to give you an overview of that, I just put this image to show how this pump is and its function is to remove the calcium ions.

This is the sequence of events you can read. This is from here the action potential - depolarization of T tubules and finally you have the calcium being pumped back into the endoplasmic reticulum. Going back. So this is the skeletal muscle, this is the skeletal muscle plasma membrane and from here somewhere is the neuron and the neuron has a terminal, presynaptic terminal, it releases acetylcholine and these are the acetylcholine molecule, it is okay. And in the plasma membrane you have this, what is it? Acetylcholine receptor.

Please say that once. Acetylcholine receptors. Acetylcholine receptors. Are you with me? Now the acetylcholine receptor comes into many forms. The one that you get in the plasma membrane of a skeletal tissue, that receptor also has affinity for nicotine, nicotine, you get it? Nicotine, okay. Nicotine has affinity for this molecule, okay.

Therefore, I can also call it as nicotinic acetylcholine receptor. Get my language? Don't forget, don't forget. Nicotinic, say that again. Nicotinic acetylcholine receptor. So located on every skeletal muscle, at the site of neuromuscular junction on the plasma membrane, you are going to get a protein system which will combine with acetylcholine. But acetylcholine receptors are of different types.

I am not going to tell you about all that right now. The one that is present on the skeletal muscle is called as what? Is called as, is called as, please read for me here. Nicotinic acetylcholine receptor. Now please nicotine will not function like acetylcholine. Are you getting it? No, do not think that I can replace acetylcholine with nicotine - no that does not happen. But actually nicotine, when it binds, it remains bound for very long time.

So when the acetylcholine or acetylcholine like compound binds with it, this is the closed channel and when acetylcholine comes, the channel opens and the sodium ions will go through it and then when the sodium ions goes through it, then the membrane will be depolarized. I just put there for your information. So okay, okay, okay. Just one more additional point.

Nicotinic acetylcholine receptor, say that once. Nicotinic acetylcholine receptor. It is made up of protein system made up of 5 subunits, 1, 2, 3, 4, 5. How many subunits? 5 subunits. Out of them, alpha, beta, gamma or some of those names. Two of them have sites where acetylcholine can combine.

And once acetylcholine combines, the channel opens and it will allow the ions to flow. You can see those 5 subunits there, hello. Can you see that cartoon there? Okay, and can you see those 2 tiny circles where acetylcholine can combine? Once acetylcholine combines, then that protein will undergo conformational change and the channel will open and the sodium ions will go in. Now this system is so highly conserved. What system? This system, acetylcholine. So highly conserved that whether you are a fish, whether you are a fish or a frog or a bird or a reptile or a mammal or a primate or whatever, it is always a neuron in the spinal cord or in the brain that will give rise to a fiber, a cholinergic fiber, it will come out of the spinal cord or the central nervous system, it will innervate this skeletal muscle and at that motor end plate it is always acetylcholine, it will always combine with the receptor and the receptor is always nicotinic type.

A system that is very well conserved across millions of years in the vertebrates. The neuromuscular system for the innervation of the skeletal muscle is what? Highly conserved, it is acetylcholine and what kind of acetylcholine receptor would that be?

Nicotinic. Nicotinic, I think I will stop now. Yes, please. Is there any channel to remove the sodium from inside to outside? Very good question, very good question. His question is by this method you will have, you will soon have the cell flooded with sodium ions. How do you deal with it? The answer to that question is sitting here somewhere is the sodium potassium pump, which will keep on pumping the sodium ions to the outside potassium to the outside in the ratio of 3 sodium to 2 potassium.

Please ask your question again. Why does it do? I will ask you counter question, I will get the answer from you. Where do you get the nicotine from? Drug? No, wait, where did the drug get it from? Plant, it is a plant, it is a tobacco plant.

So the plant is making nicotine. Follow through andI will get the answer from you. The tobacco plant is synthesizing the nicotine molecule which is there plenty. Which is abundantly present in its leaf. Why is the plant making the nicotine molecule? So I will remind you about that ryanodine. So why is the tobacco plant making nicotine? So if an insect eats it, the insect will die. So it is a protective mechanism. So we have that mechanism which the plant has evolved to protect itself, we suddenly find that I can, it can also stimulate the reward system in my brain and therefore I get addicted to it.

I have answered all your questions. Yeah, but what does that do? I did not know it. So does it inhibits the action. Oh no, it modulates, nicotine modulates the action of the receptor. It partly opens the channel, okay.

And after some time it desensitizes the channel. It does not do what Acetylcholine does. It is completely different.