**Course: Electrophysiology of Heart**

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**Lecture2:Physiology of voltage gated channels**

Hello everyone. So, today's lecture is about physiology of voltage gain. So, today's lecture is about physiology of voltage gated channels. So, last lecture we discussed about the ion channels, ion membrane potentials. So, today we will discuss about the physiology of voltage gated channels, where we will discuss about the ion channels, the structure of the voltage gated channels, and today specifically we will deal with the sodium channels. Now, before moving on to the structure of the voltage gated channels, a little bit about the history.

Now, in 1937 John Z. Young was the person who discovered ion currents and the membrane potentials, specifically the nerve action potential in the squid, that is giant axon of squid. Then following this in 1940s Kenneth Collet, he discovered voltage clamp mechanism to study the interior of the cell, like the interior of the cell is electro negative and the exterior is electro positive. In 1970s with the invention of patch clamp mechanism by Erwin Neher and Bert Sackmann, these two were the Nobel prize winners, we got to know about the various potentials of the ions.

And 2003 there was discovery of the x ray crystallography to study the detailed structure of the various ion channels, sodium channels, potassium channels, chloride channels, calcium channels. So, with this brief history we will move on to the types of ion channels. Now, ion channels are mainly two types, we have voltage gated channels and ligand gated channels. Now, ligand gated channels they specifically respond to a ligand, that is a chemical binding substance and voltage gated channels they respond to the change in the membrane potential or the voltage. So, thus voltage gated channels they respond to the voltage, that they are the transmembrane proteins that forms the ion channels, they are activated by the changes in the membrane potential, which bring about the conformational changes.

And there is gating mechanism or opening or closing of the gates seen in the voltage gated channels. So, ligand gated channels the most common example we see with the

acetylcholine nicotinic receptor mechanism and voltage gated channels, we have various examples of voltage gated channels like sodium, we have potassium, chloride, calcium, most studied these are the voltage gated channels and today we will study about the sodium ion channel. So, the functions of voltage gated channels in general the voltage gated channels these is mainly meant for the conduction of the electrical signals. Now, why this electrical signals conduction is required, it is mainly required for the contraction of muscle, signal transmission, gene expression, protein degradation, information processing. So, these are the main few important functions which are done by the voltage gated channels apart from there are few others other functions also like maintaining homeostasis.

Then we have secretion, then we have ciliary motility ciliary functions mainly. So, these are the functions also which are done by the electrical signals transduction which are done by the voltage gated channels. So, by definition voltage gated channel is a channel that opens and close in response to the changes in the electric membrane potential across the cell membrane in which it is situated since it is a transmembrane protein. Now, there will be changes in the membrane potential that cause the conformational change and there will be opening and closing of the gates and there will be propagation of the action potential. Now, these voltage gated channel dynamics these are usually studied with molecular dynamics simulation tools.

Now, if we see the structure of a voltage gated channel they mainly we have three parts, if we go from downwards that there is a voltage sensitive gate or voltage sensor voltage sensitive gate or voltage sensor it contains lots of positive charges. And this voltage sensor is mainly responsible for the conformational change for bringing the about the conformational change in the channel protein. Then we have a selectivity filter which selectively filters the ions for example, in case of sodium ion channel it will filter the sodium ions. Then we have a transmembrane pore or conducting pathway we have a conducting pathway. So, transmembrane pore selectivity filter then voltage sensitive gate or voltage sensor.

So, these are the three main paths of a voltage gated channel. Now, specifically if we talk about sodium channels. So, sodium channel voltage gated channel first described by William Agnew this was first described in the organism EEL that is electroplax organ of electrophores electricus where it was successfully first successfully purified the sodium channel protein it was done by William Agnew. So, this we have to remember now generally a sodium channel if we are talking about a sodium ion channel. So, it has got principle alpha subunit and also it has got beta subunits mainly the beta 1 and beta 2.

Now, alpha subunit is very much responsible for the function that is the pore is present

in the alpha subunit. So, the functional component is basically alpha, but the beta subunits it is not like that they are of no use the kinetics and the voltage. The kinetics and the voltage which are required for the gating mechanism that is mainly done by the beta subunits, but we will most we will put more focus on the alpha subunit. So, alpha subunit as we can see if we just see this diagram this is the extracellular space and this is the ICF. So, this is the your alpha subunit and this is the beta subunits.

So, alpha subunit is bearing a p side this is the phosphorylation side. So, here also we have this p side now this phosphorylation side is mainly because it modifies the activity of the channel protein. Rest we have four domains we have domain 1, domain 2, domain 3 and domain 4. Now, these are the trans membrane domains which are present in the alpha subunit. So, four domains are there.

So, we have four domain now each domain consist of six segments S 1, S 2, S 3, S 4, S 5, S 6. Now, whenever I am talking about segment I will write it as S the symbol will be given as S, S 1 for segment 1 and D for domain. So, segment 1 domain 1, S 1, D 1. So, each domain is having six segments. So, totally we have therefore, 24 trans membrane segments.

This is more or less common in all the ion channels. So, most specifically or most abundantly studied ion channel is sodium. So, that is why we are talking we have taken sodium channel as the first subject. So, 24 segments are present in the alpha subunit. Now, the second important thing is between the S 5 and S 6 you can see this.

This is known as the P loop or the selectivity filter or we can tell also as the pore. So, S 5 and between S 5 and S 6 we have the selectivity filter between S 5 and S 6 we have the pore that is the trans membrane pore situated. And in all the domains domain 1, domain 2, domain 3, domain 4 we can see the S 4 segment is yellow in color which is bearing a positive charge. This is nothing but the voltage sensor. So, with this topic four domains are present each domain consisting of six segments.

So, 24 segments trans membrane segments are present in the alpha subunit which is mainly the functional component of the channel protein with two auxiliary beta 1 and beta 2 subunits which are mainly maintaining the kinetics and voltage for the gating mechanism. So, next we move on to the pore forming segments as I already been told you that is between S 5 and S 6 also between S 5 and S 6 we have the selectivity filter. Voltage sensor is present in the S 4 which contains lots of positive charges and this sensor has got intrinsic ability to sense the change in the membrane potential. So, whenever it will sense the changes in the membrane potential it will bring about the conformational change in the channel protein and thus the charged residues in the sensor

trigger the conformational change. Now one thing we have to remember that at resting membrane potential which we had already discussed that whenever the membrane is at rest the membrane potential which is generated or which is measured that is across the cell membrane that is resting membrane potential.

So, for example, minus 70 milli volt is the resting membrane potential minus 90 milli volt is the resting membrane potential for cardiac cell. So, at resting membrane potential this ion channels are closed they only open whenever there is a voltage change that means whenever there is a depolarization. So, this we have to remember now if we specifically talk about the voltage sensor this is the voltage sensor that means it is the S 4 segment. So, we can see this S 4 segment this is the inactivation gate and this is bearing the positive charges. So, this is intracellularly we have the negative electronegativity and outside we have the positive charges.

Now the voltage gated channels how they act or how they work now first they act in three modes that is closed open and inactivated state they have three states for each channel proteins. Now whenever there is a closed state the voltage gated channels from closed state it will move on to the open state that means the channels will open. Now closed state means this is at resting membrane potential the membrane potential is at resting stage. So, at closed state there is no depolarization whenever there will be depolarization the channels will open. So, this is voltage dependent.

So, when the channel gets open for here it is obviously the sodium channels then it will move on to the inactivated state. So, this inactivated state is time dependent, time dependent means it will like open it will remain open for hardly 10 to 50 milliseconds. So, this ion channels will remain open for 10 to 50 milliseconds and then it will become inactivated automatically, why it will get inactivated automatically I will cover it in the further slides. So, this inactivated state from this inactivated state you cannot open the channels again until it reaches the closed state again which means from inactivated state again the membrane should respond to the voltage changes. So, because this is voltage dependent.

So, the cell has to repolarize again. So, from positive to negative it should become and it should come closer to the resting membrane potential it should come to the resting membrane potential again further to open the channels. So, the channels usually work in 3 states that is closed state, open state and inactivated state. So, for the close from closed state to open state there has to be a depolarization since it is voltage dependent that is the membrane potential will rise from negative towards positive from open state to inactivated state it is time dependent that is it will open it will remain open for 10 to 50 milliseconds. And again from inactivated state the ion channels usually should repolarize

and because that this phase is also voltage dependent.

So, that again the ion channels could open. So, I have to move the inactivated state from inactivated state to the closed state again to open the ion channels. With this now we will see the molecular mechanism of this gates ion channels. Now, with this diagram this is the these are the phospho by lipid layer phospho lipid bilayer of the cell membrane. So, we are talking about only say suppose one domain of alpha subunit we are not talking about the beta subunit.

So, we have 6 segments in the alpha subunit of one single domain. So, S 1, S 2, S 3, S 4, S 5 and S 6. So, what we can see this S 4 is consisting of the number of positive charges. So, this is S 4 as and I told you that S 4 is nothing but the voltage sensor in the ion channel specifically sodium ion channel. Now, between S 5 and S 6 this is the P loop or the selectivity filter and from here as you can see this is the pore the sodium ions will pass through.

Now, here we have few things this is the hydrophobic amino acids which is usually termed as IFMT moiety these are the hydrophobic amino acids which is present in the S 6. And we have few amino acids also named here you can see alanine and asparagine and this is the amine. So, these are also amino acids present in the other segments of the domain. So, we have two hydrophobic amino acids that is one FM 1FMT moiety and other we have alanine and asparagine. Now, these amino acids have a very peculiar characteristics they try to attract each other and form a bond.

So, initially there would not be any bond because this green color is the activation gate this green color is the activation gate. Now, this green color activation gate is lying in between this two amino acids moiety. So, that is why this two amino acids moiety cannot form a bond and actually this two amino acids moiety form the inactivation gate or closing gate. So, this is the inactivation gate and the closing of the gate. So, this activation gate is between the two moieties that is IFMMT and the alanine asparagine moiety.

So, that is why the bonding does not occur, but whenever there will be change in the conformational change in the channel protein the linking of this two proteins will occur and there will be closing of this gate. So, as we can see now this is the condition at rest I told you at resting membrane potential usually there the ion channels they are closed. Now, whenever there is change in the membrane potential that means whenever the interior become more positive, if the interior is becoming more positive what will happen this S 4 segment will move upward. Now, when this S 4 segment will move upward this activation gate which is green in color it will come down and down thus the

gate will open and sodium will enter. So, this is what happens when due to the depolarization the S 4 segment has moved outwards towards the extracellular side and it has pulled the activation gate downwards and so the activation gate opens and hence sodium will enter.

Now, as soon as the sodium enters as I told you now this activation gate is not coming in between this hydrophobic amino acids moiety. So, this hydrophobic amino acids moiety that is IFMT and alanine and asparagine this will try to get link or come closer to each other and form a bond. So, whenever this will form a bond this gate will get closed and there will be no further entry of sodium ions. So, when this will again reverse this will again reverse whenever there will be change in the membrane potential that means whenever there will be repolarization that means when the electro positivity of the inside membrane will turn into electro negativity. Whenever there will be further electro negativity the S 4 segment will come downwards from the extracellular portion towards inside this green color gate will again come in between the two hydrophobic amino acid moiety.

And thus the gate the closed gate I mean the gate which is inactivated because of this bonding will come to the resting membrane potential state that is closed. Now, whenever I am talking about coming together of this two moieties this is actually causing the inactivation. So, this you should not confuse closed state means this is happening as resting membrane potential. Open means this is happening at depolarization and inactivation is mainly because of the pulling together of IFMT and alanine or asparagine moiety. So, from inactivation gate to become closed state again there has to be repolarization which need to occur.

So, this is the molecular mechanism of voltage catered sodium channels how it opens or the gating mechanism. Now, this is the simplified further diagram that is at the closed state the resting membrane potential channel is closed this is the RMP. At the open state the channel is open because of the depolarization. So, this is the molecular mechanism of voltage catered sodium channels how it opens or the gating mechanism. Now, this is the simplified further diagram that is at the closed state the resting membrane is open because of the depolarization.

There is influx of sodium ions and generation of the nerve impulse and inactivated state for a brief period following the activation the channel does not open in response to a new signal. So, that is the purpose of the inactivation that when a even if you give any stimulus there would not be any further action potential generation, because we reach the inactivated state. And this within few milliseconds this activation occurs because the sodium channels are very fast as it has been described in the ion membrane potential

previous lecture the sodium channels are very fast voltage gated channels. So, that is why it is very the inactivation occurs also very rapidly the rapidly it opens the rapidly it gets closed. So, Hodgkin and Huxley these are the two scientists who have worked on the gating mechanism of the sodium channels.

The sodium channels are the first voltage gated channels which are to be cloned. So, this channels are very important. So, these are the first voltage gated channels which are to be cloned these channels are very abundantly studied. And they mediate as I had already told the fast depolarization not only through heart, but also muscle as well as nerve. And these channels the sodium channels are blocked by the toxins like tetrodotoxin, then you have conotoxins, oxytocin.

So, these are acting on the extracellular side of the membrane the blocker of the sodium channels. Also we have a very clinical role of the sodium channels the sodium channels are usually blocked by the local anesthetics. So, local anesthetics we use like procaine, then lidocaine these are the local anesthetics which usually block the sodium channels. And because of this blocking there will be no further impulse transmission. So, the impulse transmission is hindered because of blocking of the sodium channels.

So, these are the voltage gated sodium channels. And the inhibition of this channels is usually dependent on the frequency of the stimulation how it is stimulated. So, that is why these is the actions of this anesthetics is user dependent. So, what are the applied aspects of this sodium channels? Now, the dysfunctional sodium channels results in various diseases in relation to the electrophysiology of heart we need to remember two important conditions that is Brugada syndrome and long cutis syndrome. So, Brugada syndrome is a disease with an autosomal dominant pattern of transmission. In 1998 the first pathogenic mutation was seen with this SCN 5 8 gene.

And this gene mainly encodes for the alpha subunit of the cardiac sodium channels. So, this cardiac channels mainly to be specific N a V 1.5. So, this we have to remember. So, the Brugada syndrome they mainly affect the alpha subunit of the sodium channels cardiac sodium channels specifically N a V 1.

5. And it presents with a typical ECG pattern with a right bundle branch block and then persistent elevated ST segment at the right precordial leads. So, we have the right bundle branch block and the persistent ST segment elevation in the right precordial leads which usually leads to sudden deaths. Now, these terms we will further describe in the upcoming lectures or when we will study the electrocardiography in details. So, some of these arrhythmias usually they occur they might occur in response to exercise or stress or even they might occur at rest also or even after high meals due to the high vagal tone.

So, if you ask about what is the treatment there is no specific treatment for the right Brugada syndrome.

Right now what is the treatment of choice for Brugada syndrome is ICD that is implantable cardio cardio water defibrillator. So, they are mainly used whenever there is ventricular fibrillation noted. Then we next have long QT syndrome. So, the congenital long QT syndrome is a life threatening cardiac arrhythmia syndrome. So, as the name been suggest long QT means the QT interval of the ECG gets prolonged that is very much prolonged.

So, it is typically characterized by the prolongation of the QT interval. Now, this can happen because of the delayed repolarization due to the potassium outward channels or because of the increased depolarization of the sodium channels, inward sodium channels or even the calcium currents. All the major three channels are usually involved in this long QT syndrome whether it is potassium channels, sodium channels or calcium channels. Specifically if we talk about sodium channels it is SCN 5A specifically seen in the heart these are most commonly involved besides we have KCN Q 1 and KCN H 2 these are the potassium channels which are involved, but we will study that later. So, sodium channels SCN 5A is mainly involved for the long QT syndrome.

So, the long QT syndrome the alpha sub unit is mainly affected that is the inward sodium channels which conducts the depolarizing sodium inward current. And they mainly present with the ventricular tachycardia, polymorphic ventricular tachycardia which further move on to the ventricular fibrillation and finally, sudden cardiac death. So, in this way we have various sexiness syndrome, atrial fibrillation which are also related to the various sodium channels. So, these two Brugada syndrome and long QT syndrome you have to remember. Now, there are two other conditions also that is paramyotonia congenita and hypercalamic periodic paralysis.

So, these are not related to the cardiac muscle these are mainly related to the skeletal muscle. So, when we talk about the sodium channels of skeletal muscles the gene which encodes for them is SCN 4A. And when we talk about the cardiac muscle, so the gene which encodes for them is SCN 5A. So, this we have to remember. So, what happens this also in case of hypercalamic periodic paralysis and paramyotonia congenita in both the cases the alpha subunit of sodium channel gets disrupted.

The domains are different in case of hypercalamic periodic paralysis and the paramyotonia congenita. But in both the cases the alpha subunits you have to remember that alpha subunits of the sodium channel voltage gated sodium channel gets disrupted. So, they mainly presents with the muscle weakness and the electromyographic study

EMG studies usually could detect them. So, with this we conclude today's lecture. In this today's lecture we studied how the voltage gated ion channels operate, what are the molecular mechanisms of the opening and the closing of the voltage gated channels specifically sodium.

The other channels potassium and calcium this we will deal in the later lectures. And we also studied the various sodium channel dysfunctions in which the Brugada syndrome and Long QT syndromes are important. So, with this I would like to conclude today's lecture. Thank you.