Organic Chemistry In Biology And Drug Development
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Lecture - 44
Neurotransmitters

Welcome back, in this session we will be discussing the chemistry and medicinal aspects of neurotransmitters.

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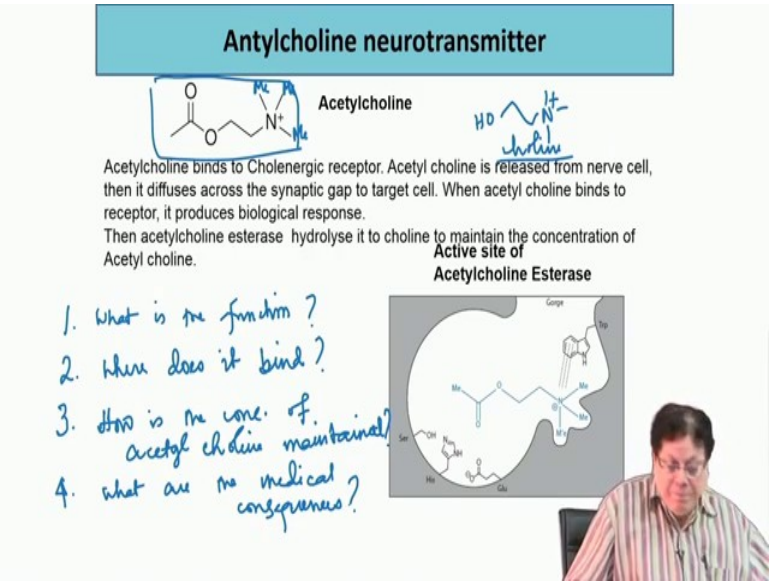

Neurotransmitters are small chemicals that are generated from the neurons and they have a receptor site very close by, in the next adjacent neuron or muscle cell, and then they bind there and send a signal. That creates a cascade of reactions. Thus neurotransmitters are small molecules belonging to the class of chemical messengers.

Neurotransmitters are chemical messengers which are secreted by neuronal cells and they have a nearby binding pocket in the adjacent neuronal cells or muscles muscular cells, after binding, they produce some signals which then carries out a cascade of reaction and exerts the desired effect.

Now chemical messengers are basically of two types, one is neurotransmitter another is hormone. Hormones are usually secreted from the glands like pituitary gland or adrenal glands and they actually traverse through the bloodstream to different sites and then exert

the effect by binding to their receptors. Primarily the difference between chemical messengers and hormones is based on the site where they are produced. Secondly, neurotransmitters basically do not traverse long distance, they bind to nearby sites; whereas, hormones are distributed to different parts of the body.

So, today we are going to talk about a neurotransmitter which is called acetylcholine. Now the structure of acetylcholine is given here; very simple; it is an ester and then it is basically an ethanol amine, but the amine is present as a quaternary salt, there are three methyl groups here.

So, it is an ethanol amine derivative and the alcohol is esterified by the acetyl group, hence the name acetylcholine. What is choline? Choline will be OH and then N, these 3 methyls and plus. Where does it bind? It has got a receptor and a binding pocket, but exactly what are the characteristics of that binding pocket. Next question is that how is the concentration of acetylcholine maintained? And then what are the medical consequences? Because now we are going to the topic where we will pick out different molecules and then their chemistry or biochemistry will be discussed. Then accordingly we will try to design drugs. The molecules that we will be talking about, either they are short fall or their excessive secretion will cause some harmful effects so you need medicine to treat that. So, first of all we have to see the chemistry of this acetylcholine and accordingly we will then decide, what are the molecules that can modulate the function of this neurotransmitter. So, what are the medical consequences? That is the next question.

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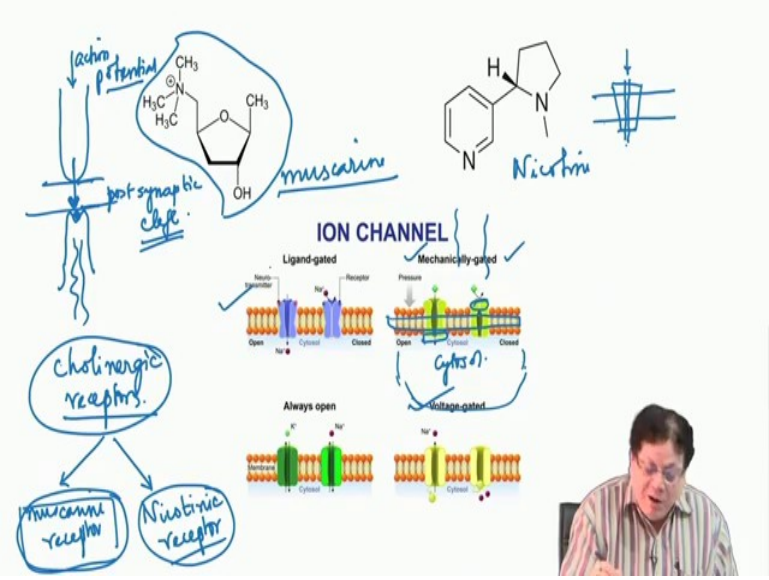

As I said, all neurotransmitters are secreted by the neurons; now acetylcholine has receptors which should be nearby; that means, a nearby neuron or it could be a muscular cell. Now suppose this is your neuron. So, acetylcholine is secreted from here and that secretion is dictated by what is called an action potential; when to fire or when to generate acetylcholine that depends on this action potential and then you have adjacent neuron; suppose and it has got a binding pocket here. This is called the post synaptic cleft.

So, acetylcholine drops into this area and then it goes and binds to this active site pocket and that creates a signal. It has been found that acetylcholine has two types of receptor sites. Now these receptor sites are named according to the neurotransmitter or according to the hormone like estrogen is a hormone. So, where it binds it will be called an estrogen receptor, similarly that the receptors where acetylcholine binds is called cholinergic receptors.

Now these cholinergic receptors again are of two types. There are some natural compounds like this called muscarine, if this natural product muscarine is taken, then this muscarine goes and binds to a particular type of receptor which are meant for acetylcholine. So, they are called muscarine receptors; but they actually belong to the class of cholinergic receptors. So, cholinergic receptors are those were acetylcholine binds, but they are subdivided into two, one is called muscarine receptor; that means, one

is distinguished by the fact that only those receptor sites can be occupied by muscarine. And the other is the site which is called nicotinic receptors.

So, nicotine will not bind to the muscarine receptors or muscarine will not bind to the nicotine receptors, but both belong to the cholinergic receptor systems. Now what is the signal that we are talking about? What it does actually when acetylcholine binds? The cell membrane is basically a lipid bilayer where the hydrophobic parts are pointing towards each other and that actually constitutes the membrane. That means, the inner part of the membrane is very hydrophobic and then these are the head groups. So, one is pointing towards the outside and another is pointing towards the cytosol. Suppose this is the cell so, these will be pointed towards cytosol and these head groups are pointed outwards.

Now, in these membranes, there are many proteins which are embedded in the membrane and these proteins are called membrane bound proteins. And these membrane bound proteins have an exterior surface, which is interacting with external molecules and it has also some interior part which is pointing towards the cytosol.

So, now, the question is what is the purpose of these different membrane proteins? They have different functions, but one type of function of the membrane proteins is that they allow foreign molecules to enter through them, by forming channels

It can provide a channel or a pore through which foreign molecules or ions can enter into the cell or can go out of the cell. These pores are sometimes closed, sometimes they are open, when it is required that some ions need to enter or get out of the cell then it opens up. So, basically this is acting as a log gate and the opening and the closing can be dictated by several ways. One is called mechanically gated, mechanical means you apply some pressure from outside and that will open the gate. So, the channel is open when we apply pressure; if you release the pressure, then the top portion join with each other and close that pore.

So, this is called mechanically gated; that means, the gate is opened only when there is a mechanical pressure from outside. Then there are different types of voltage gates; that means, the electrical potential controls the opening and the closing process, but the one that we are interested in here is what is called ligand gated ion channel. These nicotinic receptors are basically ligand; they activate the ligand gated ion channel opening.

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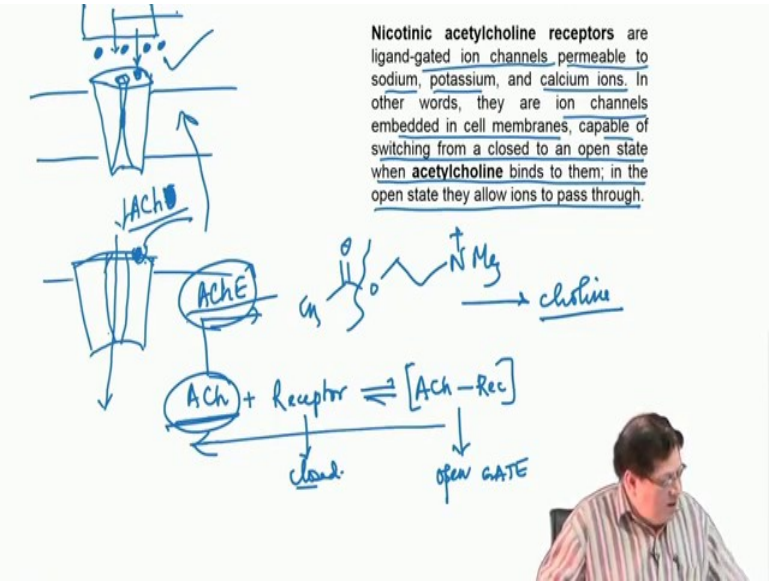

Here it is written, nicotinic acetylcholine receptors are ligand gated ion channels permeable to sodium, potassium and calcium ions so; that means, they allow the inflow or outflow of these ions. In other words they are ion channels embedded in cell membranes capable of switching from a closed to an open state when acetylcholine binds to them, in the open state, they allow ions to pass through. So, suppose this that membrane protein; it is a multimeric protein. So, inside there is a channel.

This channel is basically very narrow, this is close suppose, now this membrane protein has a binding site where acetylcholine binds. So, when acetylcholine binds, the membrane protein will be like this. So, earlier it was going inwards and locking the passage blocking the gate now it becomes open and now the ions can freely move into the cell or outside the cell, but this will happen as long as acetylcholine is bound to the receptor side.

So, as soon as the acetylcholine is detached from here, this goes into the resting state; that means the closed state. So, this opening is by ACh (acetylcholine). The nicotinic receptors are ligand gated ion channels; ligand means here ligand is acetylcholine. So, it sits on the membrane protein, which are ion channel proteins and then it opens up the channel and the ions pass through it, sodium, potassium or calcium. And then when it is detached, then it goes to again the state where the gate is locked. So, basically it controls the passage of sodium, potassium or calcium.

Now the question is what really controls this? There must be a neuron here which drops in the acetylcholine into the synaptic cleft and then that goes here. So, there is an equilibrium between ACh plus this receptor, that will have an equilibrium process; ACh receptor that is the bound state. So, in the bound state, you have the open gate and here you have the receptor closed here; so, there is this equilibrium going on.

Now if you do not want the signal to be to be produced for a long time because that is also not good, it has to be maintained properly that when it is required acetylcholine goes drops from the neuronal cell in the synaptic cleft and then binds to its receptor and opening the gate. But you want this signal to be produced for a certain length of time and then it should be stopped; you do not want all the time.

How to stop that? You have an enzyme called acetylcholine esterase that is what is AChE (acetylcholine esterase). Remember acetylcholine has a structure $OCOCH_3$ and then NMe_3 plus. So, acetylcholine brings it back to the choline Remember that acetylcholine sits on to the nicotinic, if it is nicotinic receptor and then receives on to the nicotine receptor and then opens up the gate this is called ligand gated ion channel and then the ions move in or out.

And then how to stop this signal, because if then you have to either hydrolyse, if you hydrolyse this acetylcholine by this enzyme acetylcholine esterase then the equilibrium will be shifted on this side. So, you will have the resting state of the receptor; that means, the gate is closed; that means, the flow of ions will also stop. So, this is for the nicotine receptor, we will come back to this acetylcholine esterase once we are through with the muscarinic receptor.

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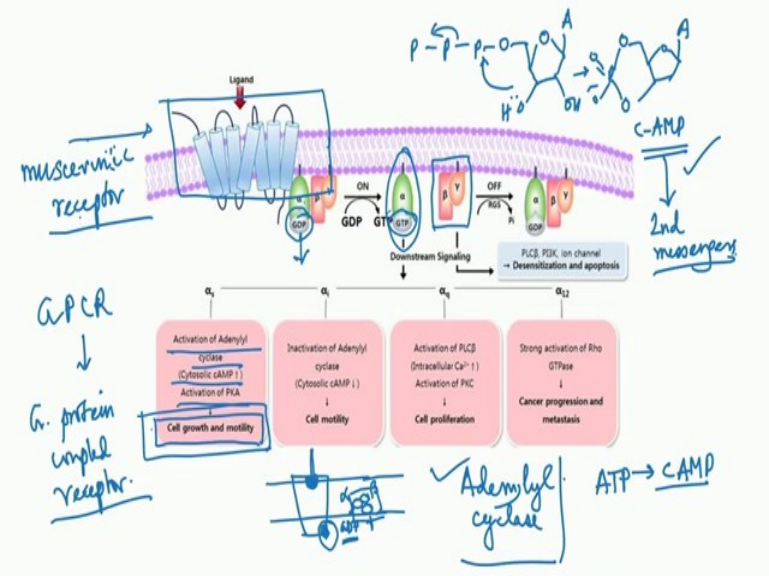

The muscarinic receptor is little bit different here; this is the membrane protein and in the membrane protein, remember the receptor site is in the membrane protein. The ion channel is also maintained by the membrane protein. There is another type of receptor which is the muscarinic receptor. It has got these different monomers of the protein. So, it is a complex, it is a multimeric system. Muscarinic receptor is a GPCR, G protein coupled receptor; this is a different kind of receptor, it activates the G proteins. Earlier when the ligand acetylcholine sits on to the nicotinic receptor site then it is a ligand gated ion channel. In the muscarinic site, it sits on to the membrane protein which is a G protein coupled receptor; that means, when it sits on to the receptor there is a system called G protein which will be activated.

What is this G protein? If this is your membrane, you have this G protein coupled receptor. So, there is a receptor site, your acetylcholine sits here. What is G protein? G protein is a trimer of alpha, beta and gamma, these three subunits. So, it is a hetero trimeric protein which is also moving into the membrane going from here to there.

One part is called alpha unit, another is called beta unit, another is called gamma unit. And in the alpha unit something is attached which is called GDP; that means, guanosine diphosphate. So, when this acetylcholine binds to the muscarinic receptor, there is a binding pocket here in this G protein; why this is called G protein because the alpha subunit is attached to GDP.

As soon as this binds acetylcholine, these goes and alpha binds to the other active site pocket which is intracellular. So, the ligand is bound to the receptor, the alpha subunit is bound to the inner side of this membrane and this is the G protein and now what happens as soon as it binds there is a reaction where GDP is now replaced by GTP.

So, earlier it was GDP; now GDP comes off as it binds and then GTP goes and binds to the alpha sub-unit, but that actually dissociates the whole thing from the this membrane, the alpha sub-unit falls off along with GTP and the protein. Now this protein which was earlier consisting of alpha beta gamma has the beta gamma unit intact, but the alpha is now attached to GTP. And then there is a reaction when that alpha GTP then activates another protein which is called adenylyl cyclase. Adenylyl cyclase coverts ATP into cAMP.

So, first you have this alpha, beta, gamma, there is G protein traversing in the membrane and you have this muscarinic receptor. Acetylcholine comes and binds to the to its receptor pocket and it opens up another pocket in the same membrane protein, but when it opens up this alpha subunit binds to this open part.

And as soon as it binds, the GDP falls off and it is replaced by GTP, and then as a result the whole thing dissociates from here and then this acetylcholine also goes off. So, that closes this pocket and so, this whole thing dissociates off, but they dissociate off in such a way that the beta, gamma unit remain intact and the alpha is separated and it is attached to the GTP. Now this goes and activates an enzyme called adenylyl cyclise, now what is the reaction that it catalyses? It catalyses this this conversion of ATP to cAMP.

This is called a secondary messenger and it carries out the signal like activation of adenylyl cyclase. So, you get cAMP and then that ultimately creates a lot of biochemical reactions which is responsible for cell growth and motility. There are other parts here, but we are only talking about this activation of adenylyl cyclase.

I repeat that first it is the membrane protein the acetylcholine sits upon and activates it, the G protein binds, the alpha subunit binds, the GDP falls off and then it is bound to GTP, then the whole thing dissociates and then the alpha subunit remains alone along with the attached to GTP and that goes and activates adenylyl cyclase.

So, you get this cAMP which is a second messenger because this is not the primary one. The primary one is the acetylcholine, because of the acetylcholine you are releasing cAMP and this cAMP now goes and takes out the signal and creates a cascade of processes like activation of PKA which ultimately results in cell growth and motility; that means, the survival of the cell. So, these are the 2 receptors; muscarinic receptors is a GPCR and the other is nicotinic acetylcholine receptors and that is ligand gated ion channels.

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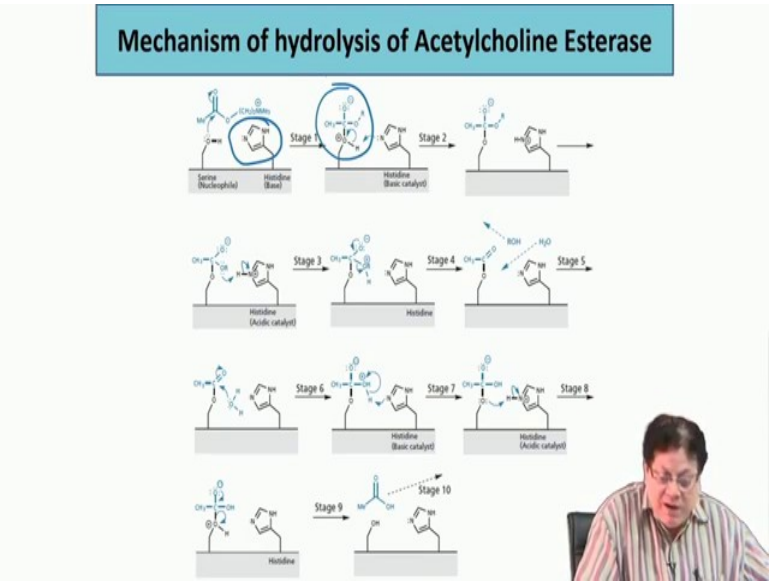

Now, the next question is how to control or who controls the concentration of acetylcholine because that is very important, because that will decide how long this signal will sustain. So, I told you about one enzyme that is called acetylcholine esterase, which hydrolyses the acetylcholine and disturbs the equilibrium to the left side; which means, the resting state of the membrane protein. So, this enzyme is very important acetylcholine esterase.

Now suppose in some cases that the acetylcholine is hydrolysed more rapidly than is expected; that means, you have more concentration of acetylcholine esterase and that is hydrolysing the acetylcholine much before the expected contact time of the acetylcholine with it is receptor. Then what will happen? You have a problem with the signal processing, the signal transduction, and the amount of signal that will be generated. So, there you talk about the disease condition; that means, the acetylcholine concentration

has to be properly balanced. So, that the optimum level of signal transduction takes place.

If the acetylcholine esterase is overactive then you will have less concentration of acetylcholine because you are hydrolysing that and that means, you have less amount of signal that will be generated out of this binding to the receptor. So, this is a very important enzyme and as a consequence. There are two ways of regulating this. Suppose the acetylcholine esterase activity is completely shut off, then that will also be catastrophic because the acetylcholine will be bound to the receptor for a greater length of time and that also causes a lot of neuronal diseases.

We will have a quick look at the mechanism of hydrolysis of acetylcholine esterase. Now we know that it is a serine esterase; serine based esterase we have read in the first part of this course that how serine proteases work. It is very similar serine esterases work where you have this histidine base and then histidine activates the serine and the serine goes and attacks the carbonyl resulting in hydrolysis forming first the tetrahedral intermediate and then later on hydrolysed by water. And that is the mechanism of acetylcholine; it is very similar to what we have read earlier.

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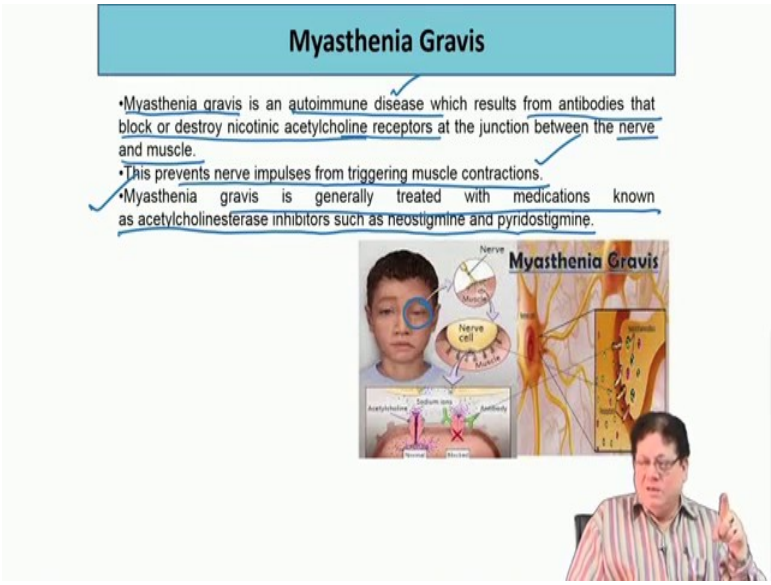

Now see, the diseases that are associated with this balance of the acetylcholine, one is called myasthenia gravis; it is an autoimmune disease which results from antibodies that block or destroy nicotinic acetylcholine receptors. Destruction of the nicotinic

acetylcholine receptors at the junction between the nerve and the muscle occurs. The muscle cells at the junction where you have this nicotinic acetylcholine receptor basically control the muscle contraction and expansion processes. It is an autoimmune disease which destroys the receptors.

Destruction of the receptors implies that you are no longer having the ligand gated ion channels and so your muscle contraction will not take place. This prevents now impulses from triggering muscle contractions. So, that results in this type of eyelids covering most of the eye which is exposed to outside; that means, that portion through which we see, that is very tiny in size. So, most of the time it is almost closed condition, so that is called myasthenia.

That means there is a problem of opening it; that means, because to open and close you have to have a muscular contraction and expansion. So, that is controlled by this nicotinic receptors means nicotinic receptors which are part of the cholinergic receptors. So, this is one disease which is dependent on acetylcholine receptors. Myasthenia gravis is generally treated with medications known as acetylcholine esterase inhibitors.

There are drugs which can treat this and these are inhibitors of acetylcholine esterase. So, you want that acetylcholine should be there as much as possible so, that most of the receptors can interact with the acetylcholine. That means, you have to inhibit the acetylcholine esterase. So, that the acetylcholine concentration is more there.

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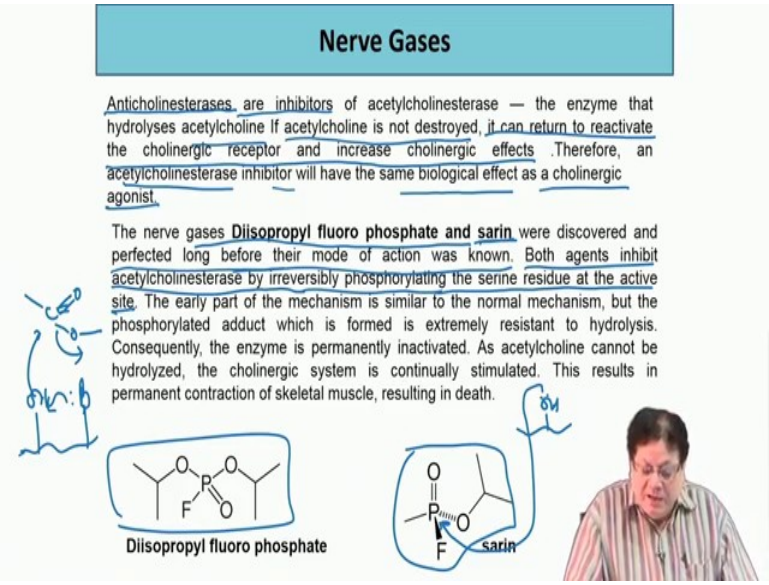

Now anti choline esterases are inhibitors of acetylcholine esterase. The enzyme that hydrolyses acetylcholine; if acetylcholine is non-hydrolysed it can return to reactivate the cholinergic receptor and increase cholinergic effects. Therefore, an acetylcholine esterase inhibitor will have same biological effect as a cholinergic agonist, as if you are not producing acetylcholine in the body.

So, what you can do, you can take muscarine and muscarine is G protein coupled receptors. And if there is a problem with that you can take muscarine and that will activate here the receptor site GPCR. And if you have problem with this muscle; then you can take nicotine because nicotine will also act as a receptor and produce the same kind of signals so; which means, they are agonist. What are agonist, I will telling in agonist are basically go to the receptor and then produces the same type of effect like the natural ligand, in this case the natural ligand is acetylcholine.

The nerve gases, diisopropyl fluorophosphate and sarin; these are very dangerous compounds; they were discovered and perfected long before their mode of action was known. Basically what happened that, you produce something and then you see that it has got some biological effect and you do not know why it is producing the biological effect. So, basically these were first made and then showed that they have very disastrous effect on the on living systems, but the mechanism was not known, but later on, the mechanism was discovered. And what have been found that both agents inhibit acetylcholine esterase irreversibly phosphorylating the serine residue at the active site.

So, if you look at these structures diisopropyl fluoro phosphate and sarin, sarin is again a fluoro phosphonate. So, what happens, it is serine and then you have a base which is imidazole, the mechanism is that the base abstracts the hydrogen, the serine attacks the carbonyl group of the ester and subsequently the ester gets hydrolysed. But instead of acetylcholine what happens; now this fluoro phosphate goes so, now, this serine is here. So, that attacks the fluoro phosphate.

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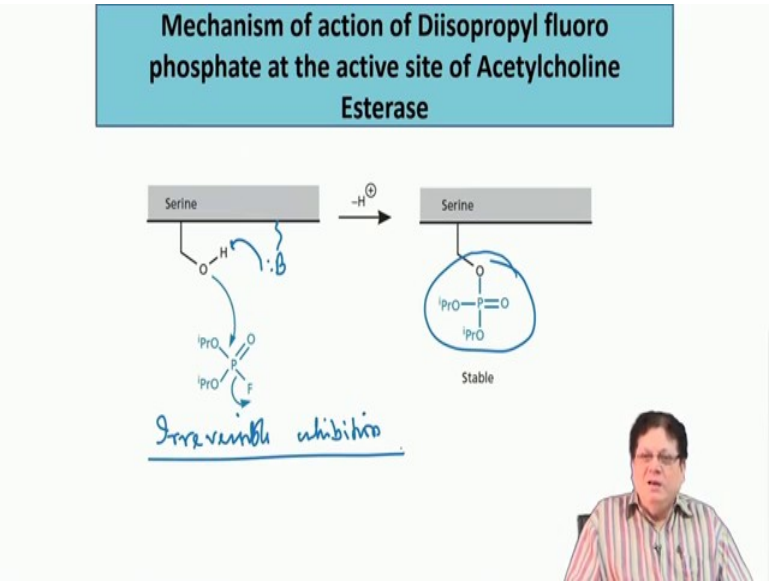

Here it is shown that serine attacking the fluoro phosphate of course, there is a base here that is the imidazole that assists this nucleophilic substitution. But now the fluorine will leave because it is a good leaving group. So, F minus will leave and you get a stable phosphate that will be difficult to hydrolyse. So, basically is an irreversible inhibition of acetylcholine esterase. If you are exposed to this sarin which is a gas, that is even problematic because gas can be circulated from one place to another very quickly.

So, if you are exposed to sarin, you are going to die very quickly because they are very potent irreversible inhibitor of acetylcholine esterase. So; that means, huge amount of acetylcholine is generated in the synaptic cleft and that is not good, because that sends constant signal which ultimately paralyzes the living system.

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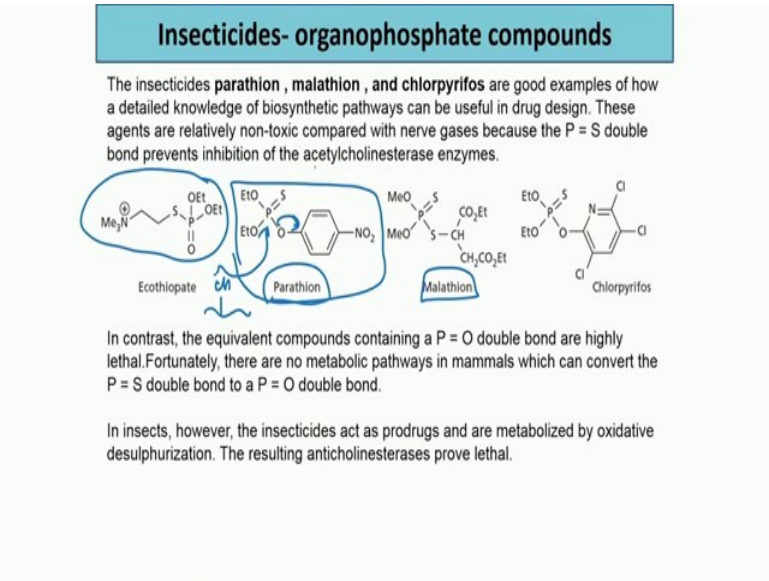

Now, sarin is very bad if it is used for the destruction of the human being, but you can also have acetylcholine esterase inhibitors to kill the insect or pests; the insects also have a acetylcholine esterase in their body.

So, you can actually develop compounds which are extremely important agro chemicals, they are the ones which are called insecticides because they kill the insects. What is the mechanism of their action? There are well known compounds Malathion, parathion and then chlorpyrifos; these are basically all insecticide. They are very similar having this thiophosphate groups. So, your serine is going to attack here and this goes out and then it stays there which in this case is thiophosphate. So, the mechanism is very similar and they are not hydrolysed quickly and in the process the pests is killed the insect it gets destroyed.

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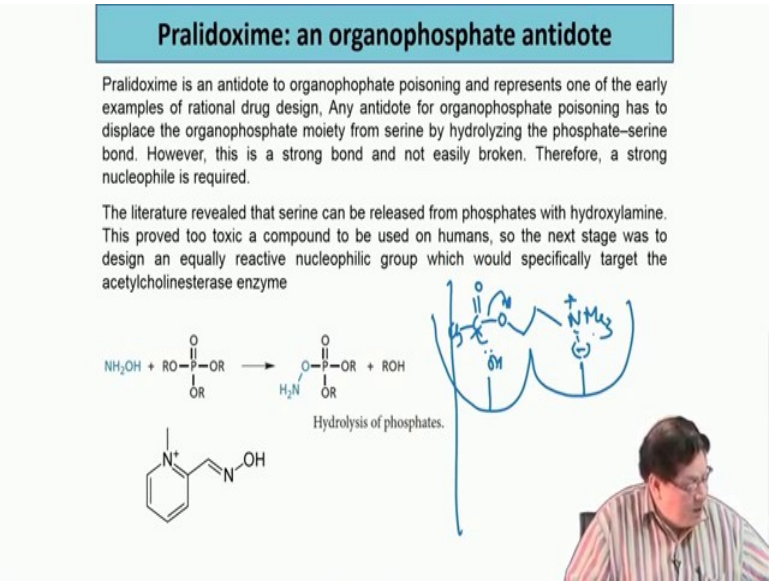

Now the question is suppose somebody is exposed to this sarin gas, and then is there any antidote for it? What will be the antidote? When the serine is hooked up as the phosphate or the phosphonate, the fluorine leaves. Now the question is it is an irreversible inhibition, but can this be again reversed back to the acetylcholine and acetylcholine esterase can be freed from that irreversible complex?

Yes it is possible, but before that I forgot to mention one thing that in acetylcholine, the structure is this O CO CH_3. Now what has been found because this is a substrate so, enzyme is having two sites, in one site there must be a negative charge. So, that this NMe_3 can form a weak bond and in the other site you have this active serine. So, the reactive serine hydrolyses it and this actually offers the binding pocket for the NMe_3 plus.

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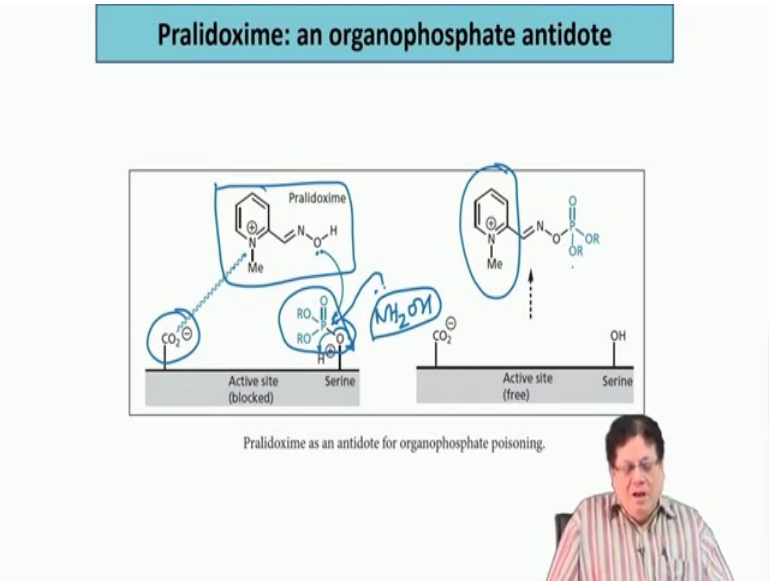

Suppose you want to regenerate e the enzyme from the serine which is now blocked as a phosphate. You make a compound like this pyridinium based compound where there is a positive charge that acts like the trimethyl ammonium salt of acetylcholine and this is the nucleophile, now you do not have any ester group here or a phosphate group here, instead you have a nucleophile. So, you get this type of phosphate if you are exposed to sarin then it was found that this enzyme can be freed by using hydroxylamine.

So, hydroxylamine can attack and then free the serine moiety; but hydroxylamine is very nonspecific; it is a very small compound; it may be quite toxic also because it indiscriminately attacks at different places which is not good. So, what you want is a very specific one, hydroxylamine does not possess the part which participates in the binding process that has the positively charged nitrogen. So, you take an oxime based on a pyridinium moiety.

So, you have the pyridinium moiety which forms the salt bridge here between the carboxylate which is the anion and then the hydroxyl amine is rightly placed so, that it can attack the phosphate and regenerate the serine. So, if somebody is exposed to sarin or any type of nerve gas then the antidote is this one pralidoxime and the mechanism of action is regeneration *via* nucleophilic attack.

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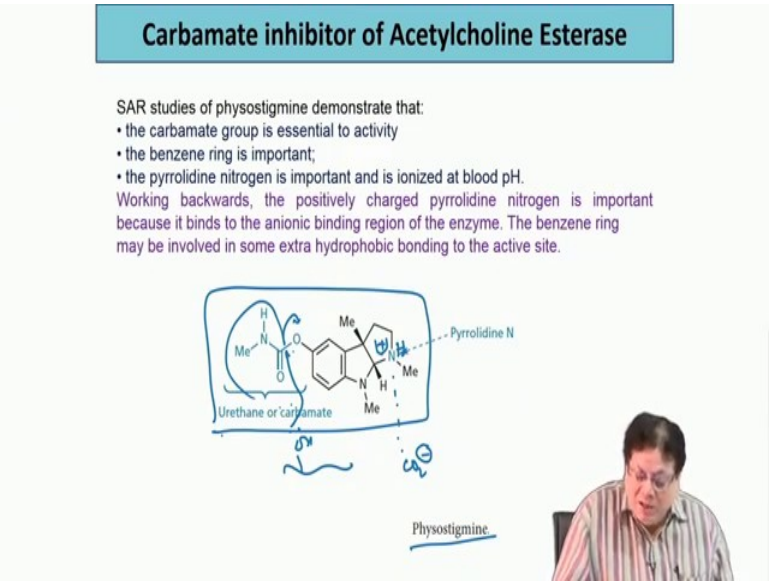

So, for myasthenia gravis physostigmine is used, here this is protonated in the pH that we have in the biological medium. So, it creates the salt bridge and this is an inhibitor. So, here it is the serine and then serine attacks and releases this part and the serine is blocked as this carbamate. Physostigmine is a natural product that works against acetylcholine.

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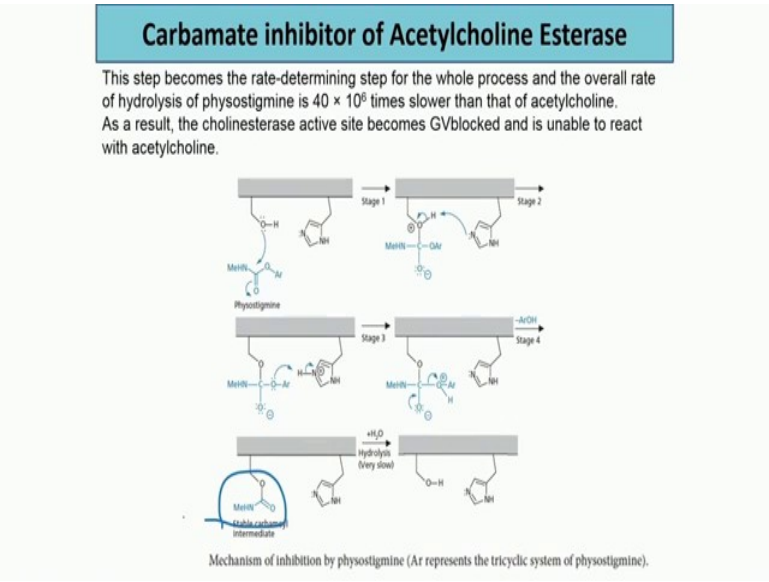

This is the mechanism of physostigmine; just here it is written as Ar, but the mechanism is same as I said that ultimately you will get a carbamate. So, that is quite stable. So, just to summarize now, acetylcholine is a neurotransmitter; it has got two types of receptors,

nicotinic receptors and muscarinic receptors, one is your the GPCR that is muscarinic, another is your ligand gated ion channel.

There is an enzyme which maintains the optimum concentration of the acetylcholine and the enzyme is called acetylcholine esterase and if you can inhibit this, you can have different kinds of drugs and depending on the different type of use. If you are using this eye problem myasthenia gravis, then you use it in human and that actually can solve some of the problems associated with myasthenia gravis. You can use it also to kill humans; these are very reactive compounds which are called sarin or fluoro phosphate and if you want to utilize it for benefit of mankind, then, you actually kill the insects by using it on the agro field where the insects are killed by inhibition of the acetylcholine esterase in the insect.

And the last point is that how can one recover if somebody is exposed to sarin? You can use oxime based pyridinium salt which can recover, it can release the serine which is the blocked as the phosphate and then thereby the person may be saved. So, that is all for acetylcholine.

Thank you.