Organic Chemistry In Biology And Drug Development
Prof. Amit Basak
Department of Chemistry
Indian Institute of Technology, Kharagpur

Lecture – 45
Catechol Amine based and GABA Neurotransmitters

Welcome back to this course on Organic Chemistry in Biology and Drug Development. We have already completed the first part of this course that dealt with organic chemistry in biology and we have read the amount of biology which is required for understanding the drug discovery and development process. And we have started the drug discovery or development processes by pointing out that the practice nowadays is to ultimately come up with a drug and there are several steps that include identification of the target, validation of the target, hit identification, and lead identification.

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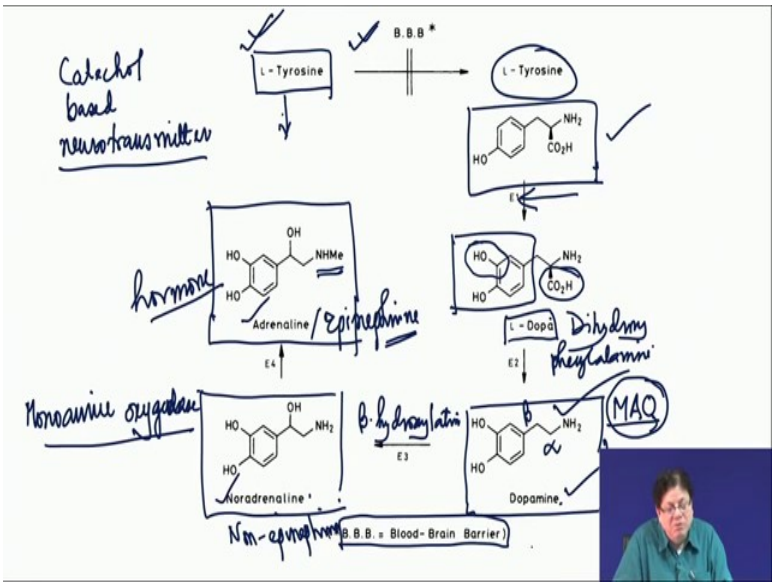

Then lead optimization followed by PK PD studies (pharmacokinetics and pharmacodynamics studies), then the toxicological studies and the preclinical which also includes the studies on animals or sometimes other *in-vivo* studies; it could be with cells, you can determine the toxicity, cytotoxicity etcetera.

And once that is done, then the drug goes for the human trials that is the clinical trials and there are different phases- phase 1, phase 2, phase 3 and once it is approved then for

the subsequent years, the pharmaceutical companies also look at the activity or any side effect that the drug can have as a long term side effect. So, after its introduction to the market, in the subsequent years, they also follow the effect and that is called phase 4 clinical trial.

Then we started and completed the combinatorial chemistry; that means, how to arrive at quick hit compound by the process which is basically generation of a large library of compounds and then *via* high throughput screening, you can pin down the hit compound quite rapidly, that is what the domain of combinatorial chemistry is.

And then we started the actual medicinal chemistry; that means, the target oriented discovery of compounds which are acting as hits. So, the first one we talked about is the neurotransmitters and we have covered acetylcholine as the first neurotransmitter. We have seen that the acetylcholine can have two receptors, muscarinic receptors and nicotinic receptors.

And they are entirely different; one is ligand gated ion channel and the other is the G protein coupled receptors. And there is an enzyme called acetylcholine esterase which is very important, because that controls the concentration of free acetylcholine versus the bound acetylcholine. Bound means bound to the receptor; thus the optimum concentration is maintained.

So, if there is a problem with your acetylcholine esterase, if it is blocked, then you have problems. So, apart from the drugs, there are anti-doses to generate acetylcholine esterase especially when there are poisoning by the nerve gas type of compounds or if somebody has consumed any organophosphorus which are present in insecticide compounds. Now, today we will discuss another two neurotransmitters, one is catechol based neurotransmitter; that means, they have a catechol moiety. What is the catechol moiety? That is *ortho* dihydroxy aromatic ring.

Now, the first class belong to this dopamine that is dihydroxy phenyl ethyl amine and also you have noradrenaline and the third one is adrenaline. By the way adrenaline is a hormone, but these are neurotransmitters. You know the difference between the hormone and the neurotransmitter that basically neurotransmitters are acting at a very short distance; noradrenaline and dopamine also belong to the class of a neurotransmitters.

So, whenever we study neurotransmitters like this and if there is a problem in the concentration these neurotransmitters, then we have diseases. Now, to keep the concentration of the dopamine or noradrenaline or even adrenaline at the optimum level, you have to know the biochemistry and the biosynthesis of these molecules.

Once you know the biosynthesis then there is the question of intervening in the process of production or destruction of these neurotransmitters, because the disease arises due to imbalance between the destruction and the formation. Now, the question is that what these neurotransmitters do in the body?

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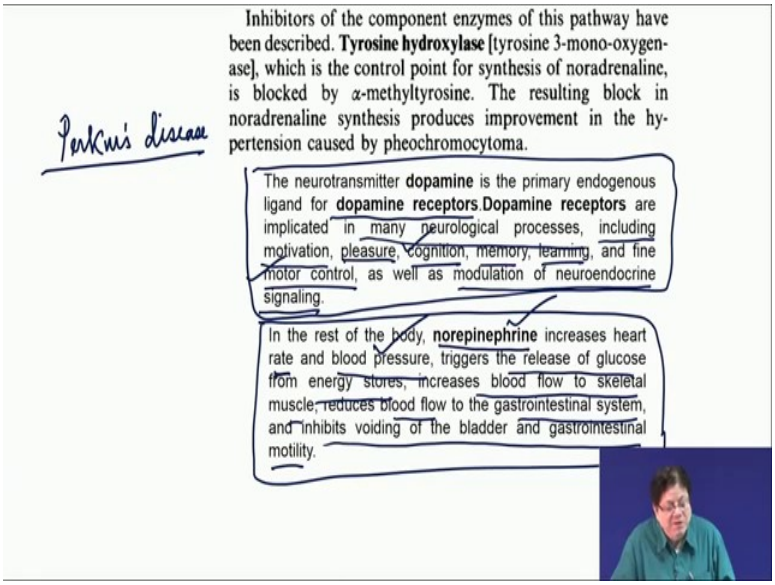

So, the neurotransmitter dopamine is written here is a primary endogenous; that means, inside endogenous ligand for dopamine receptors; all neurotransmitters work by binding to a receptor, and these receptors are very characteristic of the type of neurotransmitter that you have. Like acetylcholine, we know that cholinergic receptors and then you have for dopamine, which will be called dopaminergic receptors. So, dopamine is a primary endogenous ligand for dopamine receptors.

Dopamine receptors are implicated in many neurological processes; that means, when dopamine binds to these receptors, lot of signal transduction processes take place and that ultimately results in motivation, pleasure, cognition, memory, learning, fine motor control; that means, the way we move our hands and limbs as well as the modulation of endocrine signaling, neuroendocrine signaling, that is basically the modulation of

signaling by the hormones generated from the endocrine glands, because the hormones are usually secreted from the glands.

So, that is why; so, you see that there are so many effects that the neurotransmitters like dopamine have. And then the other one is what is called norepinephrine or in earlier days, it was known as noradrenaline. So, norepinephrine increases the heart rate, blood pressure, triggers the release of glucose from the energy stores, increases blood flow to skeletal muscle, reduces blood flow to the gastrointestinal system and inhibits voiding the bladder and gastrointestinal motility.

So, here the very important part is that it increases the heart rate and blood pressure. So, it must be the causative agent behind hypertension, of course, and hypertension has different mechanism by which the blood pressure can rise, but one of the mechanism is related to norepinephrine related.

Now, let us see how this dopamine is generated, dopamine and norepinephrine is generated inside the in the neurons, ok. Now, what happens? The starting point is a simple amino acid which is called L-Tyrosine, ok. Now, tyrosine is you know its a protein building amino acids. So, apart from the amino acid function, this has another role very important role in generating the dopamine and norepinephrine and finally, adrenaline or that is called epinephrine only.

So, when we get excited like during an exam we have the more of the adrenaline secretion there, so that you sustain to the stressed conditions.

So, whenever there is any stress, then you have this more secretion of adrenaline and basically; that means, you have more secretion of noradrenaline. Now, L-tyrosine is the starting point. Now, L-tyrosine is an amino acid which is shown here. Now, what happens? The brain cells; that means, the neurons get this metabolite like L-tyrosine, if I take L-tyrosine orally then the L-tyrosine will be absorbed from the gastrointestinal track and then it will be circulated in the blood. It goes into the blood and that circulates.

Now, the question is when something is in the blood, that does not mean that it goes to the brain, but the drug may not be transferred to the neuronal cell directly. There is a barrier from blood to brain and that is called the blood brain barrier. Not all molecules

reach the brain from the blood. And L-tyrosine crosses the blood brain barrier and then reaches the brain.

Now, the process by which this crossing takes place is usually *via* a career molecule which takes L-tyrosine and crosses the blood brain barrier and puts the tyrosine inside the brain cells. So, this is your tyrosine. As it reaches the brain, there is an enzyme which is called tyrosine hydroxylase, which actually puts a hydroxyl group in the aromatic ring and that is basically the catechol moiety.

See, tyrosine you can call as hydroxy phenylalanine and this will be dihydroxy phenylalanine. So, that is what is abbreviated as L-Dopa; that means, di hydroxy phenyl alanine, L-Dopa.

Now, L-Dopa then undergoes a decarboxylation; obviously, this will be a PLP mediated decarboxylation because we know alpha amino acids undergo decarboxylation *via* enzyme which is dependent on the pyridoxal phosphate. So, after it loses CO_2 it generates dopamine. And then dopamine undergoes beta hydroxylation. This is your alpha carbon, from the starting amino acid this is your beta carbon, so you get beta hydroxylation.

So, once you get beta hydroxylation, which gives you what is called noradrenaline or norepinephrine. And then that amine gets methylation and it becomes a NHMe, that is what is called adrenaline. So, that is adrenaline, this is noradrenaline, then you have dopamine, it is a backwards L-Dopa; but the starting point is L-tyrosine crossing the blood brain barrier.

Some diseases may arise due to less production of this of dopamine; in many of these say depression, because dopamine gives you pleasure, cognitive behavior, and then motor functions etcetera. So, maintaining a concentration of dopamine is very important. Now, how the concentration can be low?

First of all, either it may not be produced; so, enzymes may not be very active. So, it is not produced in large amount. The other way is there are enzymes which are called MAO enzymes, monoamine oxidase. What it does? It destroys the primary amines if they are generated. This is called monoamine oxidase.

So, there are two ways by which dopamine concentration can be low; one is that the biosynthetic machinery is not functioning properly that is number 1 and number 2 is that the monoamine oxidase maybe overactive and that is causing the destruction of the primary amine which is there in dopamine which is there in nor-epinephrine. So, these are the two processes, ok.

What we are discussing is that if you see that there is a problem in the biosynthetic machinery, then how to increase the concentration of these catechol amines?. One way is that you take more of tyrosine, suppose the tyrosine is not present in large amount. So, if you take more of tyrosine then tyrosine crosses the blood brain barrier and you maintain a higher concentration of tyrosine here.

So, that will be converted into the dopamine that is one way. But the problem is that if somebody takes tyrosine from outside, then tyrosine being a protein amino acid, it will be consumed to make the proteins that are required in the body.

So, by the time everything reaches the brain, that will be very less because most of the tyrosine will be utilized to make the proteins. So, that is not a viable treatment that if you take too much tyrosine. The next way is that you can have a very direct approach that dopamine is a very simple compound; you can make it in large quantities.

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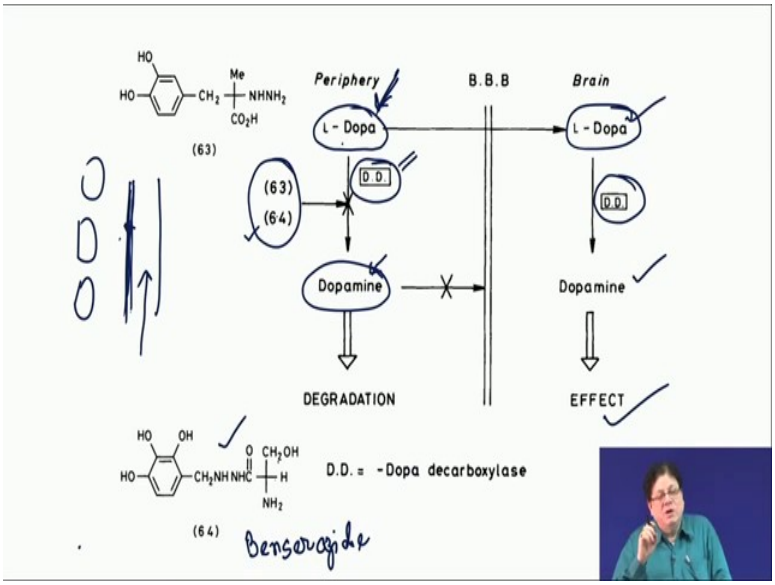

So, this is what basically we are talking about. Periphery is basically just outside the boundary of the blood vessel. So, in L-Dopa, you see here it is written that tyrosine crosses I told you, but tyrosine is not the answer to treat this type of diseases because tyrosine will be utilized mostly in its primary role that is to make the proteins. L-Dopa definitely is not used here, it is not protein amino acids, so you can take large doses of L-Dopa and that can cross the blood brain barrier. So, that has the ability to cross the blood brain barrier.

So, this is a viable alternative for treatment of low dopamine concentration that you take L-Dopa, so that should cross the blood brain barrier and then the decarboxylase enzyme (Dopa decarboxylase) should converted that to the dopamine, and then dopamine produces the effect that is required.

But the problem is that L-Dopa is also a an amino acid and again here by the time it reaches, near the blood brain barrier there is this decarboxylase enzymes which decarboxylates Dopa into dopamine. What I am saying that if we supposed it is the blood vessel and the neuronal cells are somewhere near here, and this is the peripheral region.

And what happens? That L-Dopa goes in the bloodstream and L-Dopa is mostly decarboxylated into dopamine before it reaches the brain cells. So, if it is already converted to dopamine, problem is dopamine cannot cross the blood brain barrier. So, dopamine will not enter the brain cells if we use the L-Dopa, which is a problem. Of course, you can say that some of it definitely goes into the brain, but that may not be of very optimum concentration.

Now, we have learnt three things; L-tyrosine is not the answer, L-Dopa is a partial answer that some portion of it goes into the brain by crossing the blood brain barrier and then formation of dopamine, but a majority of it is decarboxylated. You have dopamine before it if it crosses blood brain barrier. So, dopamine cannot cross it. So, most of it is remaining outside the brain cells. And this dopamine undergoes degradation, by many monoamine oxidases that will degrade the dopamine.

So, to bypass this, you need to utilize inhibitor of this Dopa decarboxylase. This is peripheral Dopa decarboxylase and this is not the peripheral which is in the brain cells, on this side is brain on this side is the periphery, so it is the peripheral Dopa

decarboxylase that is the problem. So, what you do? Along with L-Dopa, you add inhibitors of this Dopa decarboxylase and this does not cross blood brain barrier.

So, it can only inhibit the peripheral L-Dopa decarboxylase. So, you stop this decarboxylation, and then most of the L-Dopa will be on this side. The equilibrium is shifted now. So, that will create a high concentration of L-Dopa and then that will be converted into dopamine and you get that desired effect.

So, these are some of the compounds which act as inhibitors; Benserazide is a Dopa decarboxylase inhibitor. And then, you have a very similar kind of inhibitor which is called Carbidopa.

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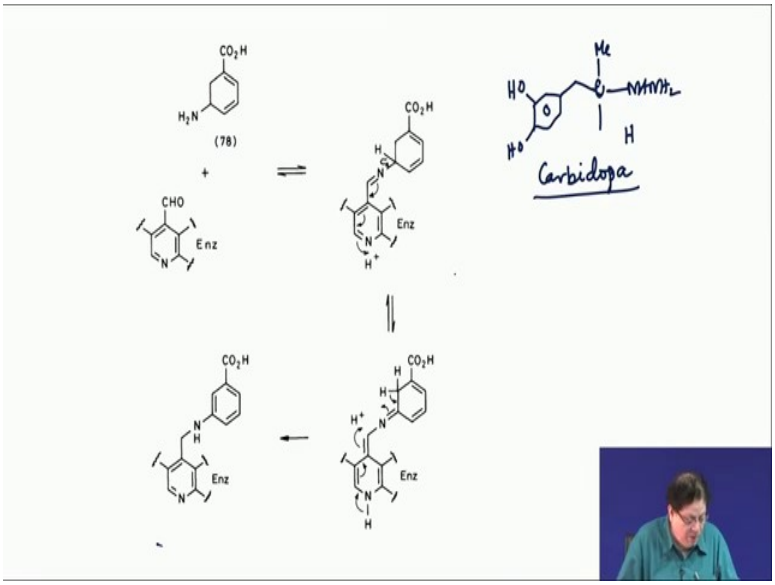

Methyldopa is also another compound which can regulate the blood pressure, because methyldopa got a methyl group at the alpha position and that creates different, that creates an antagonists type of effects, so that the blood pressure which is associated with norepinephrine and adrenaline or epinephrine. So, that blood pressure can be controlled. In fact, in the earlier days, one of the good medicines was this M Dopa for controlling the blood pressure.

I told you about these motor functions that is controlled by dopamine. This is what is there in the case of Parkinson's disease. Parkinson's disease is a very bad disease where people lose the motor functions, they cannot even move their hands or limbs, also they

cannot remember things because their cognition behavior also goes off, and memory also is not there.

And the only way the Parkinson's disease can be treated is to utilize this L-Dopa along with the peripheral Dopa decarboxylase remember. Then you can use a lesser dose of L-Dopa and that creates the concentration of dopamine to some optimum level.

However, you can slow down the Parkinson's disease, but it is not possible to cure the Parkinson's disease because whatever genetic machinery is defective, that you cannot repair. What you are doing? Just from the external source, trying to maintain the concentration of dopamine, it is not a cure, but it is a way to slow down the progress of the Parkinson's disease.

So, this is very important. These catechol amines are very important neurotransmitters. These are associated with Parkinson's disease and also with the mood fluctuations because it gives pleasure, it controls your mood. So, that is also very important. So, that is what is all about the dopamine chemistry.

Now, let us talk about one more neurotransmitter which we have already told you; earlier it came into our discussion when we said that many of the amino acids that are present in the body are not alpha amino acids. They are not part of the protein that we make, but they have a distinct function, mainly as neurotransmitters, like glutamic acid. Glutamic acid is itself a neurotransmitter.

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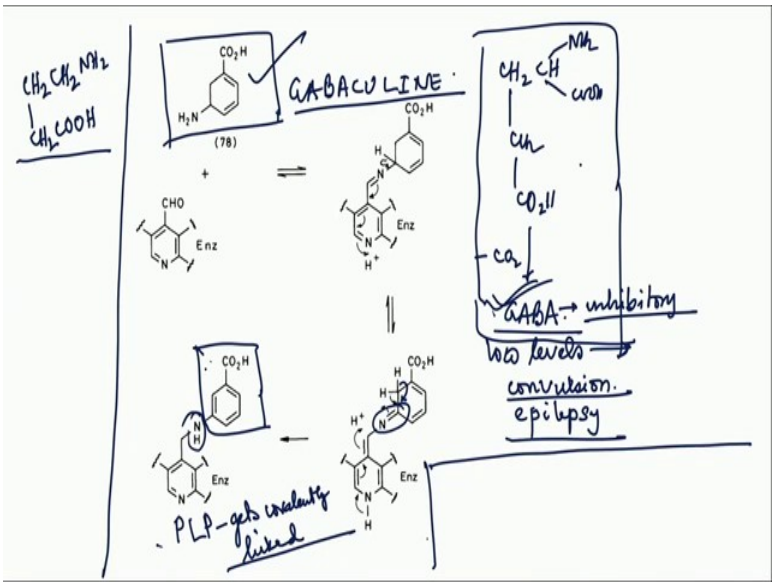

And then from glutamic acid. There are amino acids which are generated; one of them is called the GABA or the gamma amino butyric acid. Remember that we have discussed that when you were talking about the chemistry of PLP, so we have gamma amino butyric acid. It is a very simple compound.

And its biosynthesis is obviously, from the glutamic acid. So, if you have L-glutamic acid, CH_2, then $CHNH_2CO_2H$ and then CH_2, so it is CO_2H. So, that undergoes decarboxylation. So, this is called glutamate decarboxylase because it is decarboxylating a glutamic acid. So, what you get is what is called GABA. Now, GABA is called an inhibitory neurotransmitter.

See we have both types of neurotransmitter one is excitory and the other is inhibitory; and we have to have both because all the time we cannot be excited, that is not good, because your blood pressure goes up, norepinephrine will be more. So, there must be something which is inhibitory. So, it controls the effect of neuro excitation. So, this is an inhibitory neurotransmitter.

Now, low levels of GABA are implicated in diseases like epilepsy; it causes convulsion; that means, the person loses his sense and that is what is convulsion. Some people have this problem that occasionally they lose their sense and it appears that the person is almost dying.

Now, epilepsy is related to the lowering of the concentration of GABA, the gama amino butyric acid. Now, I already told you the biosynthesis of GABA; that means, it comes from glutamic acid. So, glutamic acid crosses the blood brain barrier and then it undergoes decarboxylation by the glutamate decarboxylase and GABA is formed.

How the concentration of GABA is maintained? See, this is very important. You need to know not only how it is generated, you also need to know how it is degraded. Like acetylcholine is generated and then its degradation was by the enzyme acetyl choline esterase. Dopamine is generated from L-tyrosine, its degradation is by monoamine oxidase.

Then you have this GABA. Its formation is controlled by the glutamate decarboxylase, but the question is how it is degraded. If you can increase the concentration of GABA that is going to act as an anti-epileptic agent. Question is that how GABA is a degraded.

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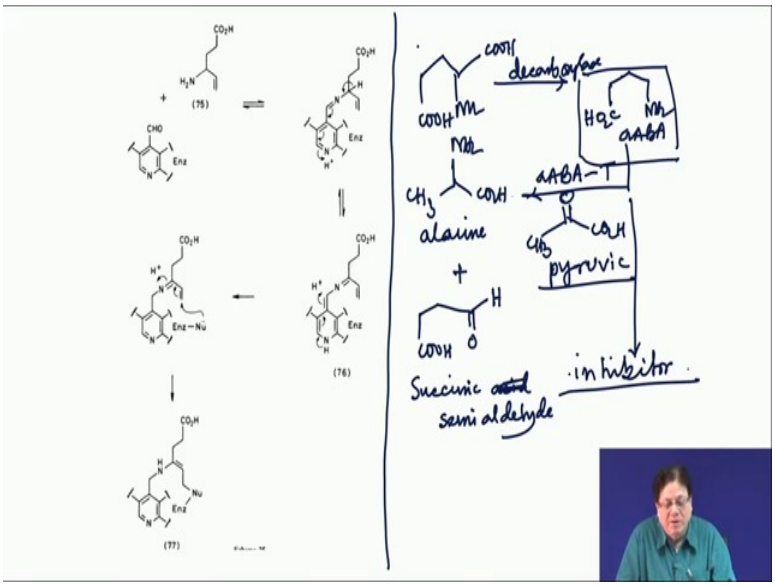

So, you have glutamic acid like this and that undergoes decarboxylation and you form the gamma amino butyric acid. So, this is GABA and then this GABA undergoes transamination that occurs by the enzyme GABA-T. GABA-T means gamma amino butyric acid transaminase. That means, what is transamination?

That is also a pyridoxal system; you have read already pyridoxal mediated reaction where amine from one compound is transferred to the alpha keto acid removing the

carbonyl and replacing that with amine and in the process the original compound which is an amine donor that is converted into a carbonyl compound. So, here for this GABA, the corresponding alpha keto acid is the pyruvic acid.

So, any transaminase will have two components, one is an alpha keto acid, another is the amino acid. It is not necessary that it will be alpha amino acid; here it is a GABA (Gama amino butyric acid). So, what will be the reaction? The keto acid will be converted into what? So, this is nothing, but alanine. And what will happen to the GABA? That will be CO_2H and then, instead of NH_2 you will have a carbonyl.

So, this is the compound; that means, remove the NH_2 put a carbonyl, but remember there is a hydrogen here also. So, that is called succinic semialdehyde. So, one of the carboxy in succinic acid is converted into the aldehyde. So, this is how the GABA is degraded.

If there is a low concentration of GABA, you have two choices, either you have to add more glutamic acid, so that you get more concentration of GABA or you can stop this GABA transaminase by using an inhibitor, so that GABA is not degraded rapidly into alanine and succinic semialdehyde. So, the first one will not work like L-tyrosine. If you give L-tyrosine you are not going to get any high concentration of dopamine because tyrosine is a part of the protein amino acids. So, its major function will be to utilize in the formation of the protein.

Similarly, glutamic acid is like that. Glutamic acid is a part of your protein amino acid. So, that will also be mostly consumed in making the protein amino acid, so that part will not work. So, what can work is that if you can form an inhibitor, so the treatment is basically finding an inhibitor of GABA transaminase. So, now you see your earlier knowledge about biochemistry is becoming relevant in understanding the medicinal chemistry, the genesis of different drugs and the strategies that you have to adopt to make those.

Now, let us talk about this GABA transaminase inhibitors. So, one compound is what is called gamma vinyl GABA.

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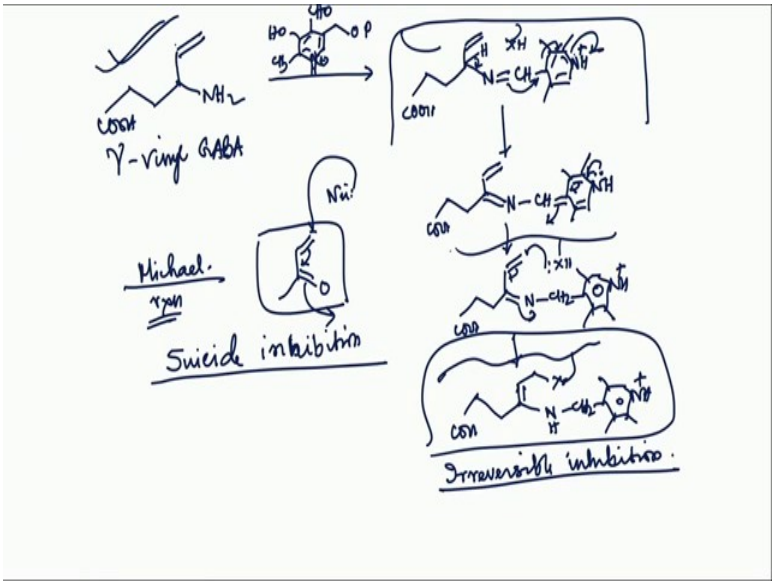

A very similar compound to GABA, but you put a double bond here. So, this is called gamma vinyl GABA. Remember the transamination reaction involves reaction with pyridoxal phosphate. You should remember the structure. This is the CH_2OP and this is OH and that is a methyl. So, methyl, this is OH and this is OP that is called pyridoxal phosphate and in the biological system, it is present in the protonated form.

Free amines will definitely react and form the imine, so that forms the imine with pyridoxal. So, that will form the imine. So, CH and then the pyridine nucleus NH plus and then double bond and we are not writing the substituents, we are writing this because it is trisubstituted system. So, once that is formed, you know that this is an electron sink because of the positive charge. So, what will happen?

You know that the chemistry is dictated by either loss of hydrogen or by loss of carbon dioxide; these are the two reactions. So, here there is no CO_2H, so the alpha-hydrogen will be lost and this goes here, goes up to the nitrogen, so you have CO_2H and then you have this double bond, you have a double bond here N and you have CH and then the pyridine is no longer aromatic, so it will be like this.

Now, it regains the aromaticity by pushing back again, but the hydrogen that is taken up is at the CH_2. So, you will get a CO_2H in CH_2 and then the pyridinium system NH plus in these three places and this is a double bond. Now, this is an iminium ion. So, this is

nothing, but this is resembling an alpha beta unsaturated carbonyl system where the oxygen is replaced by nitrogen.

The usual reaction of this alpha beta unsaturated systems attached to an electron withdrawing group is called Michael reaction. Now, the nucleophile can attack here to have 1,4-addition called Michael addition. So, now, this molecule is well placed act as an acceptor of a nucleophile *via* Michael addition.

So, remember these reactions are actually going on in the active site of an enzyme. So, if the enzyme has an electron rich group XH, it could be SH, it could be OH, could be a NH also. So, what the XH can do? It can add to this double bond and form a covalent connection.

So, basically CO_2H and then X will add here and will be double bond here and this NH and then CH_2, and you have this pyridinium thing, NH plus. Now, this will remain like that. So, basically it is a case of irreversible inhibition, because you have a covalent bond connecting to the substrate, the substrate is hooked to the cofactor, see everything is now blocked.

The question is whether it is an active site directed or is it a suicide inhibition? You see this is a suicide inhibition because the normal reactions proceeded up to certain extent and that creates a reactive system. So, this is nothing but what is called suicide inhibition. I just remind you that suicide inhibition is the one where the substrate is processed to some extent and then it generates a very reactive compound and to which the enzyme side chain reacts.

So, that is the case here, so it is not only irreversible inhibition, it is a suicide inhibition. So, this is approved as a drug to treat the epilepsy because you are inhibiting that transamination enzyme. Similarly, you have another compound which is this compound. It is not an aromatic compound; it is a dihydro aromatic compound, because that is saturated.

So, it will have a great tendency if there is a scope of forming a double bond there. Let us see how it works. So, you have this compound; you see this is almost like a GABA amino acid. But it is hooked up to this diene. Now, what will happen? I can show it here.

First this pyridoxal phosphate reacts forming the imine and very similarly. it acts as an electron sink and the electron flows here up to this point.

So, we have the imine here and then as soon as the imine is formed here, like vinyl GABA, you get a alpha beta unsaturated imine. So, there will be addition of the active site amino acid *via* Michael addition. Here what happens? This hydrogen will now be lost and this double bond will come here, so that means, this is NH now and there is a double bond here. So, now, it has become an aromatic compound.

So, it has become aromatic compound. Now, there is no way that this pyridoxal phosphate can be freed from the molecule. So, this PLP gets covalently linked to this compound. This is the commercial name is GABACULINE. These two compounds are used as drugs to treat the epilepsy. And if somebody asks what the mechanism is, you can say that the mechanism is basically irreversibly inhibiting the transaminase enzyme.

Now, just there is a slight difference in the mechanism of action of the two. Vinyl GABA is covalently linked to the enzymes as well as to the cofactor, GABACULINE is linked to the cofactor, but if you can neutralize the cofactor then the enzymes cannot act; because the enzymes need this cofactor to function as a transaminase enzyme. So, that is all about the three types of neurotransmitters.

We have covered the acetylcholine, we covered the dopamine, and we have covered GABA and its associated diseases, and how to treat the diseases.

Thank you.