**Organic Chemistry In Biology And Drug Development**
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**Lecture – 46**
**Hypertension: Humoral Mechanism and Renin/ Angiotensin System**

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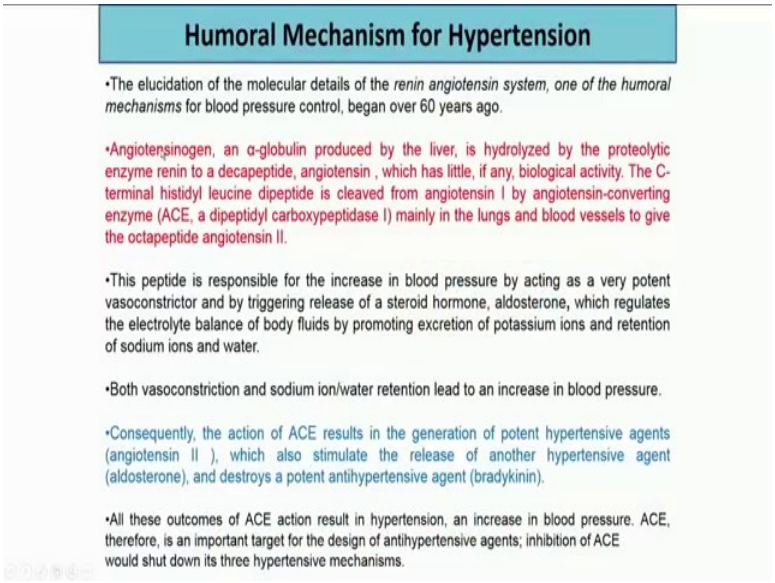


Welcome back, in this session we are going to discuss a very important lifestyle disease which is called Hypertension.

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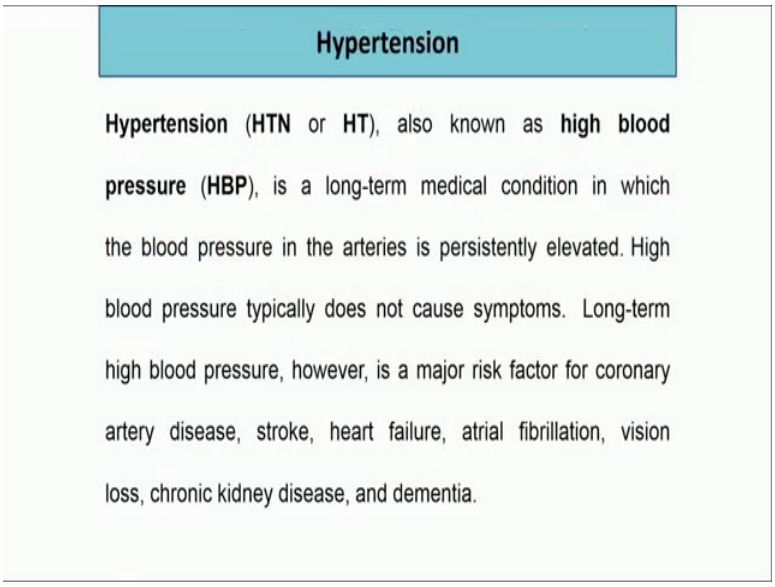

Lifestyle disease because the major reason for hypertension is stress that is associated with our daily lives and then we are going to study what is the chemistry that goes on which elevates the blood pressure. Now before I go into the details, I can say that there are many mechanisms by which the blood pressure can be elevated.

One simple example is that if somebody takes a lot of salt in his diet, sodium chloride, then the amount of sodium in the blood is high and that is one way to increase the blood pressure. And there are also movements of calcium through the channels and that is also an important component of mechanism of increasing the blood pressure. So, you can have calcium channel blockers for the lowering of the blood pressure.

There are many other methods of lowering the hypertension; another feature which is very common for higher blood pressure is the narrowing of the arteries. If the artery diameter becomes less then to push same amount of blood through that narrow passage, the pressure goes high. And the whole thing is narrowed down because of the deposition of mainly cholesterol type substances and that is what is called arteriosclerosis, where there is this deposition of cholesterol within the walls of the artery and narrowing the artery.

Higher cholesterol means at the some point of time, you are going to have this deposition of cholesterol and that will also be a causative agent of high blood pressure. But today we are not discussing all these 3; what we are discussing is another way which is called humoral way of elevating blood pressure, humoral means the body fluid, humoral mechanism of hypertension.

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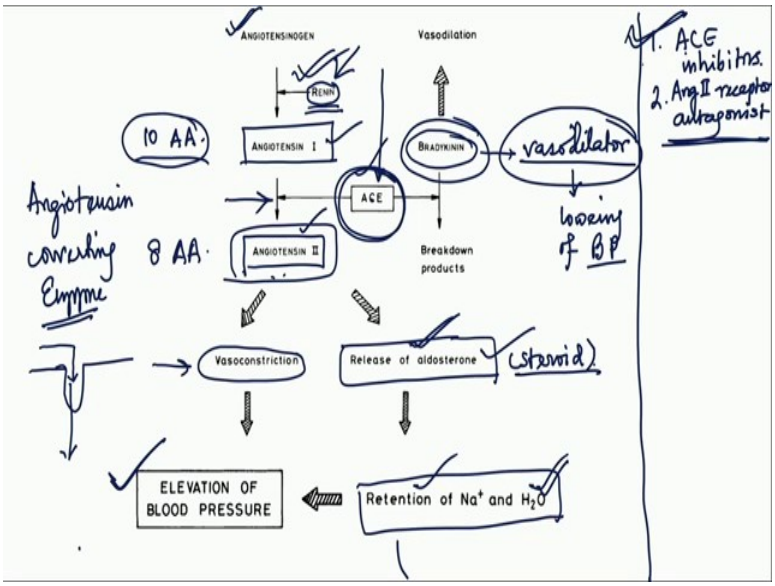


Humoral means through body fluid, which how can increase the blood pressure. If the body fluid means the blood contains more sodium, and then you will have a higher blood pressure. Now, sodium is a little bit easier to control because you can cut down the intake of the salt, but if there are breakdown of other metabolic processes, then that you have to be very careful; you can target those metabolic processes and then see how the blood pressure can be controlled.

Now one such humoral mechanism of increasing the blood pressure is what is called the rennin angiotensin system; I will come back to that rennin angiotensin system, before that just give a broad overview of hypertension; how this blood pressure is commonly known that if some people say that I am suffering from hypertension. Hyper means high. So, higher tension means higher blood pressure, this is also abbreviated as HTN or even just higher tension also known as high blood pressure HBP.

It is a long term medical condition in which blood pressure of the arteries persistently elevated. High blood pressure typically does not cause symptoms, but long term high blood pressure is a major risk factor for coronary artery diseases, it is found that blood pressure has a connection with higher cholesterol level also. There is a layer of cholesterol which is solid. So, your arteries get narrower and whatever inside the cholesterol crust is detached from inside of the arteries. So, that ultimately flows through

the blood and blocks your heart valve or it can go to the brain and cause what is known as the cerebral attack.

So, it is a major factor of coronary artery diseases, stroke, heart failure, vision loss, chronic kidney disease and dementia. For people suffering from high blood pressure, their brain function also slowly decreases and then ultimately it will cause what is called dementia; which means, you forget things. Kidney disease is another one caused by high blood pressure, kidney faces lot of trouble in flushing out the unwanted things and so, the long term effect is a chronic kidney disease.

Now I told you that I will be talking about the renin angiotensin system, the renin angiotensin system is one of the humoral mechanisms of blood pressure control. It was known about 60 years ago that what the biochemistry of renin angiotensin system is. In the body, there is a protein which is called angiotensinogen, which is a bigger protein.

This angiotensinogen is hydrolyzed by an enzyme which is called renin and when it is hydrolyzed, it makes a smaller peptide that contains 10 amino acids. This word gen like pepsinogen, chymotrypsinogen means a precursor of the actual compound. So, chymotrypsinogen is the precursor of chymotrypsin, then pepsinogen is the precursor of pepsin, similarly angiotensinogen is a precursor of angiotensin, but this angiotensin is called angiotensin 1; that means, this decapeptide 10 amino acid containing peptide is obtained from angiotensinogen by action of the renin.

So, what is Renin? Renin must be a protease, because it is hydrolyzing this big protein into a very small peptide of ten amino acids, this is angiotensin 1. Then angiotensin is converted into angiotensin 2, angiotensin 2 is 8 amino acid containing peptide. So; that means, now this is what is called angiotensin converting enzyme, the enzyme that is here which is also a protease by the by way because you are removing amino acids by hydrolyzing the peptide bond.

So, that is called angiotensin converting enzyme, ACE. Now this angiotensin 2 which is obtained from angiotensin 1 is an 8 amino acid containing peptide. It is basically a hormone, it has got receptors and it binds to the receptors and then it has its own receptors, it binds there, and the signal that it creates, when it binds to the receptor, it causes vasoconstriction. Vasoconstriction means your blood vessels become narrower, it

is not by any deposit, it is by the action of the of this hormone angiotensin 2. So, the blood vessel contracts. So, that is what is called vasoconstriction.

So, if there is vasoconstriction; that means, the blood pressure is going to increase; that means angiotensin 2 binds with the receptor and sends a signal which causes vasoconstriction. So, there is elevation of blood pressure so, angiotensin 2 has been implicated in the elevation of blood pressure. But in addition to that, there are other potentially harmful effects of angiotensin 2, one is that there is a protein or a small peptide which is called bradykinin, that is another hormone, but the bradykinin is just the opposite of angiotensin 2, bradykinin is a vasodilator.

Vasodilator means it increases the diameter of the blood vessels. So, if that is the case; that means, you get vasodilation, vasodilation means lowering of blood pressure and vasoconstriction means elevation of blood pressure. So, *via* vasoconstriction, this elevates the blood pressure because angiotensin 2 works in that fashion, it binds to the receptor causing the elevation of blood pressure. The other important thing is that this angiotensin converting enzyme which hydrolyzes angiotensin 1 and making angiotensin 2, it also hydrolyzes bradykinin and destroys it.

So, if you destroy the bradykinin; that means, your vasodilation activity by bradykinin that also is lowered so; that means, this combined effect will even further increase the blood pressure and that is not the end of it; this angiotensin 2 when it binds to a receptor, it not only does vasoconstriction, but it also sends signal which then leads to the release of another hormone which is called aldosterone, that is a steroidal hormone.

Now, if it is a hormone then it has got it is own receptor. So, aldosterone now binds to this receptor and the signal that it sends is that do not lose the sodium, better retain the sodium and remove or excrete the potassium, and also you retain the water. So, that means, there are 3 kinds of effects, one is just direct vasoconstriction by angiotensin 2. Another is this enzyme angiotensin converting enzyme destroys bradykinin which is a vasodilator. And third is this that it also enhances the release of another steroidal hormone that is called aldosterone and this aldosterone enhances the retention of sodium and water in the blood.

So, all these combined together has a disastrous effect on elevation of blood pressure. So, that is the chemistry behind the humoral mechanism of how the renin angiotensin system

works. Now you can say that I want to have an antihypertensive drug. So, what will be my best strategy? I can use inhibitor of angiotensin converting enzyme, because if I inhibit angiotensin converting enzyme; that means, that inhibits the production of angiotensin 2, that inhibits the breakdown of bradykinin, if angiotensin 2 is not produced; that means, vasoconstriction will be less and that means, the release of aldosterone will be less and release of aldosterone less means less retention of sodium and water.

So, you can also target renin, renin is the first enzyme in the process and then I can target renin and then stop angiotensin 1 from producing. Even if there is ACE; that means, the angiotensin converting enzyme is active, but your lack of substrate concentration will tell you that angiotensin 2 will be produced in less amount. So, renin also is a target and angiotensin converting enzyme is also a target.

Now let us consider these two enzymes, but we will not talk about the renin, but; obviously, renin is also a target, but most of the successes that have come is basically the inhibition of the angiotensin converting enzyme, that is the major research that was done during the 80s and then the first set of drugs based on ACE inhibitors came into the market and today they are also sold in large quantity in the market.

But beside that there is another one which we should not overlook; angiotensin 2 has receptors; it binds to the receptor and then sends the signal which creates vasoconstriction.

So, what you can do, you can make a receptor antagonist of angiotensin 2. Remember an antagonist means it binds here and does not produce any effect.

So, the angiotensin 2 cannot bind and so, the vasoconstriction effect of angiotensin 2 is gone and if you can block the other receptor which also sends the release of the aldosterone then that will also be stopped. You are not inhibiting the enzyme; you are inhibiting the receptor sites utilizing antagonists.

So, there are two kinds of drugs based on this angiotensin renin system, one is ACE inhibitors and the other is angiotensin 2 angiotensin 2 I can just simply (Ang 2) receptor antagonist. So, these are the 2 strategies you can take, we will talk about them in this medicinal chemistry section. We will talk about the ACE inhibitors, how they are

designed and how the companies ultimately were able to find drugs which are occupying a large section of the antihypertensive drugs today in the market.

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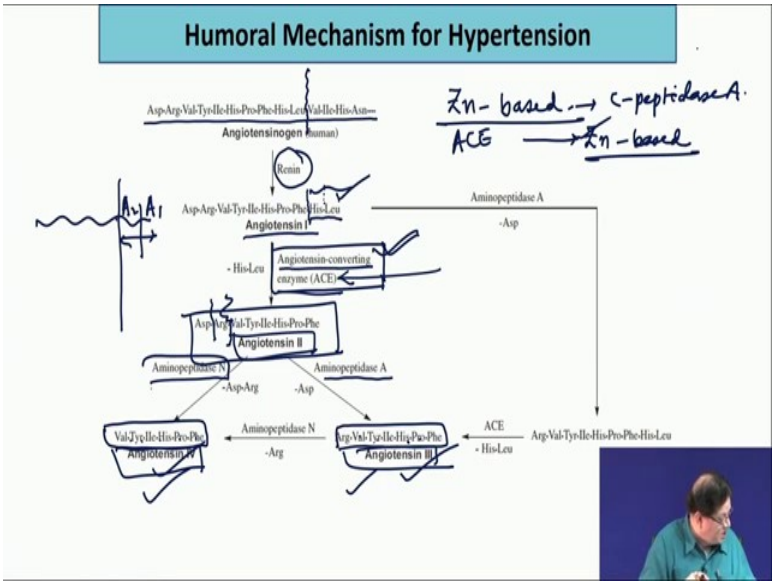


I have not said the amino acid sequences of this angiotensinogen or what is angiotensin 1; some partial sequence of angiotensinogen is shown here, you do not have to remember this so, this is a big protein. So, using renin, angiotensinogen is converted into angiotensin 1 which is a decapeptide. So, this renin actually cleaves the bond between leucine and valine; it cleaves the bond between leucine and valine and then forms this decapeptide which is called angiotensin 1, then angiotensin converting enzyme comes and converts the 1 into 2.

So, what it does, it breaks now the bond between phenylalanine and histidine and removes that dipeptide and this is the octapeptide which is the angiotensin 2. So, this is our compound, we are mostly concerned with this compound, and we mostly concerned with this enzyme, because we have seen the disastrous effect that these things can have.

However, just to extrapolate it a little bit see angiotensin 2 also can be degraded further by an amino peptidase; amino peptidase means which cleaves from the amine side the nitrogen side. So, amino peptidase takes the aspartate off and forms what is called angiotensin 3 and another aminopeptidase N, which cleaves the dipeptide from the N terminus and that means, it is a 6 amino acid containing peptide and here it is 7 amino acid containing peptide. So, this is angiotensin 3, this is angiotensin 4.

Now, if you stop the production of angiotensin 2 by ACE, which will also lower the production of angiotensin 4 and angiotensin 3. The question is whether they have any beneficial effect or if they are also part of this blood pressure elevating process. Interestingly what has been found, that angiotensin 3 and angiotensin 4 are also responsible partly for the elevation of the blood pressure.

So, that is good that if you can stop the production of ACE, the angiotensin 2; that means, you lower the concentration of angiotensin 3, you lower the concentration of angiotensin 4. So, basically with one strategy you can stop the production of so many blood pressure elevating agents, ok.

Now, let us see what type of enzyme angiotensin this ACE is. ACE takes out the dipeptide from the C terminus. Now you know if something is hydrolyzing from the C terminus that is called carboxypeptidase, but carboxypeptidase usually causes the breakage of the last amide bond like carboxypeptidase A which takes up all amino acids except the basic amino acids here. So, this is called a dicarboxy peptidase A because it actually hydrolyzes not the last one, but the penultimate one, because it is releasing a dipeptide.

So, carboxypeptidase A breaks here; that means, there is one particular amino acid here, but this dicarboxy peptidase is basically breaking the penultimate, penultimate means the one before that the last one. So, that is the penultimate amide bond $A_1$ $A_2$. So, it is a dicarboxy peptidase.

Now, if you want to make an inhibitor of angiotensin converting enzyme, you need to know the target. The known target is angiotensin converting enzyme, but then it is better that you note the structure of this compound. That means, a crystal structure, the 3 dimensional structure of it.

So, the question is, when this research started, whether angiotensin converting enzyme structure was known or not? It was not known at that time; but what they found that there is a metal involved in the breakdown of this peptide bond and there is the well-known enzyme whose crystal structure was known those days was carboxypeptidase A.

So, carboxypeptidase was a known to be zinc based enzyme and what they can do, what they knew at that time that, this is carboxypeptidase, I can write C-peptidase A and this

ACE angiotensin converting enzyme, they found that this is also a zinc based dicarboxypeptidase.

Mark laboratories or some big pharmaceutical company they started this research and they first assumed that the carboxypeptidase A has the similar structure, because its crystal structure was known, the structure of ACE is very similar it resembles the carboxypeptidase A. So, they took carboxypeptidase as a model for designing the inhibitor for ACE.

So, that was the starting point, now you can say that if this was wrong then what would have been the case, but there are analogies because it is a zinc based enzyme that is also a zinc based enzyme. So, that was the major analogy. So, it must be having a similar mechanism like the carboxypeptidase A.

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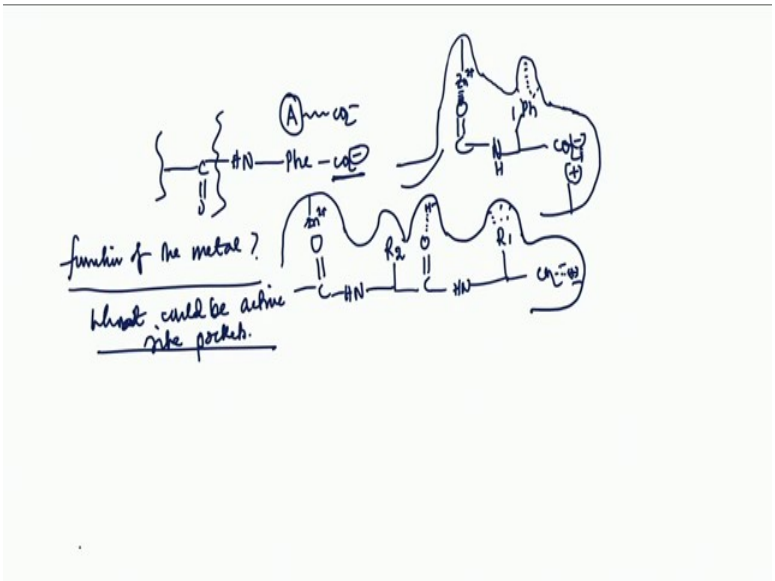


Now, carboxypeptidase A has got an amino acid it has got an amino acid $CO_2$ minus and except the basic amino acids, it accepts other amino acids like phenylalanine, say phenylalanine carboxylic acid and then phenylalanine is connected *via* a peptide bond to other amino acids. So, now the carboxypeptidase is hydrolyzing this bond that we know and it is a metalloenzymes. So, what is the function of the metal?

The next question is that what could be the active site pocket? Since it is a carboxypeptidase and we know that the C terminus ends with a $CO_2$ minus. So, definitely

we can roughly predict even if we do not know the X-ray crystal structure or see what it can happen that, at the end there must be some pocket which is having a positive charge like $NH_3$ plus because if that be the case then only the $CO_2$ minus can go and bind here.

Similarly, you can say for amine terminus, hydrolysis at the amine terminus the amine is usually present as $NH_3$ plus. So, the binding takes place at a $CO_2$ minus. So, they can form a salt-bridge or electrostatic interaction. So, then you have suppose a phenylalanine. So, $CH_2Ph$ and then you have a NH you have a CO, actually NH is in anti-disposition. So, that is NH that is CO and then other amino acids.

So, I can say definitely there must be zinc here which is attached because it is zinc mediated hydrolysis. So, this zinc plus two is acting as a Lewis acid. So, the carbonyl oxygen is chelated to the zinc, which activates the oxygen and then ultimately in turn that activates the carbon for attack by a nucleophile and this phenyl which is there must be some pocket here which is hydrophobic pocket that stabilizes the phenyl. So, I can without going into any details, say that this is the situation for carboxypeptidase.

Now remember that ACE hydrolyzes the dipeptide; that means the penultimate peptide bond. So, definitely there is a $CO_2$ minus and then whatever amino acids are here; suppose $R_1$, then you have NH, then you have CO, but the zinc must not be here, because if zinc is here then the terminal peptide bond would have been hydrolyzed. So, then there is some compound here; suppose this is $R_2$ and then you have this NH then you have this CO.

So, now you can draw a kind of active site structure so, there must be zinc here, then there must be some group which stabilizes this $R_2$, then there must be some group, but that is not zinc, some hydrogen bond donor would stabilize the carbonyl oxygen *via* hydrogen bond and then another stabilizing factor here and a plus charge somewhere here so, that the carboxy is stabilized.

So, based of these, you can say that this could be the rough sketch of the active site of angiotensin converting enzyme. So, from here we will proceed to the next session because it will take some time, it cannot be completed at this point there are lot of discussions after that and very interesting chemistry evolved, specifically the design strategy came out of this angiotensin converting enzyme inhibitors.

And today when we talk about there is a common terminology that rational drug design, some courses are called rational drug design, but I have deliberately excluded that word rational, I have said organic chemistry in biology and drug development; I did not say rational drug development.

Because although the drugs are developed by a logic, the logic is that from comparing with different enzymes, finding a target, which forms a part of the basis of the drug discovery process, but always try to remember that nothing is *ab initio*, *ab initio* means from scratch; it will be very difficult to really develop a drug like that. The case of aspirin, I told you that there are some willow trees, you get this salicin which is an glycoside of salicyl alcohol and from that ultimately aspirin was discovered. Similarly there are dicoumarol that we will discuss that it is a vitamin K, I think we have discussed already dicoumarol.

Dicoumarol that is also a serendipitous discovery from something people observed and then they tried to modify whatever the observation was. So, *ab initio* drug discovery is all most impossible, there must be some clue somewhere from where we can build up our or build on our rational to design the drugs.

So, lot of people criticize that the phrase rational drug discovery may not be appropriate, because you already have some preliminary knowledge of something and then from there you start discovering the drugs, but anyway I do not mind keeping the rational word, what I mind is that if you say *ab initio* drug discovery, that is a big problem.

*Ab initio* drug discovery means from scratch without knowing anything that is very difficult that is usually not possible, some type of clue or hint has to be there in drug discovery. So, the next session we will come back to this aspect again.

Thank you.