Human Physiology
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Lecture – 57
Hormones of adrenal cortex - Part : 2

To compare the, why do I call this particular hormonal glucocorticoid? I have already told you because it has a certain action on the liver, on the liver it can mobilize the, it can mobilize the carbohydrate from the cell and it can release glucose. And one of the most, the hormone that has a very strong glucocorticoid activity we have already seen is cortisol. But does it also have a mineralocorticoid activity? And what do I mean when I ask a question, what do I mean by the cell having a mineralocorticoid activity? Basic question. So, find you what is glucocorticoid activity? That it can mobilize glucose from a liver cell. What is a mineralocorticoid activity? Mineralocorticoid activity is simply the capability of the hormone to act on the kidney and to make sure that the sodium does not go away.

I want to retain the sodium. Principal cell, hello. Hello, are you with me? Principal cell, the late distal tubule, intercalated cell and the distal cell. Now I am talking of what, when I talk about aldosterone I am going to talk about, I am going to talk about principal cell about which you have already spoken 100 times.

Principal cell, principal cell. So far, good. Now, so does cortisol also have mineralocorticoid activity? That is where biology starts getting fuzzy, it does. It does. It has predominantly what? Glucocorticoid.

Glucocorticoid, but it has some. Altosturon, does altosturon, well it is a very very powerful mineralocorticoid hormone and I am going to state it because it just acts on the principal cells and brings about the reabsorption of sodium ions. Does it have any glucocorticoid? Well, it has a little glucocorticoid activity. But then question comes as to how are you going to standardize? Therefore, to standardize there is a method. We say that let us talk about cortisol, average plasma concentration is about 12, whatever it is micrograms per 100 ml of blood, average almost secreted about so much and whatever its glucocorticoid activity is 1 and mineralocorticoid activity is 1, is it just a standard I am using, whatever activity, so I will give cortisol, give cortisol, find out how much of glucose is being released, I will call it as activity 1.

I will give cortisol on the kidney, see find how much of sodium it absorbs, I will call it as 1. That is my standard. Ok, so far. And then I am going to compare it with others and what I find is whereas the cortisol is, let us say glucocorticoid activity is 1, cortisone is 0.

3, 0.3, aldosterone is 0.2. Are you getting the standard? Means does aldosterone have glucocorticoid activity? Yes, how much it is? It is one fifth that of? Hello, are you with me? I am not talking Greek and Latin, ok. It is very simple. We are just comparing the activities of different molecules.

But what is amazing is, let us talk about the aldosterone, aldosterone, aldosterone, what is striking here? The mineralocorticoid activity of aldosterone is how much? Times that of what? That of cortisol. Huge amount of, therefore we say that it is very powerful. Then this slide is very interesting for yet another reason. See this is actually a very old story, almost 1950 onwards, the organic chemistry people have done miracles. They have literally, literally synthesized thousands and thousands of molecules with a steroid, with a steroid, put OH there, put this, they put that, put that, put, n number of molecules they have.

And what do you call them? We call them and then, and then do what? And then look for its biological activity, ok. And some of the molecules have emerged such a powerful molecule that one of them is called as, called as what? Dexamethasone. The chances are that if you go to a pharmacy and buy an anti-inflammatory agent or a topically applying cream which is anti-inflammatory, most probably it will contain dexamethasone. Is it a naturally occurring steroid? Yes or no? It is not a naturally occurring steroid, ok. But then you will find that it is a, it has, it has, it is much more powerful than the naturally occurring steroids, ok.

So we have to, so we have, we have a large number of prednisolone, methylprednisolone, dixamethasone, 9-alpha-fluorocortisone, n number of, only some of them which are, which are in clinical practice have been here. But you cannot really learn about steroids without knowing their profound impact on the pharmaceutical industry. Have I conveyed my point? See they are, they are, they are, they are widely people, the chemistry people have done miracles here in coming up with little variation here and there and, ok. It goes in the blood by, like thyroid hormone, this also, there is a, there is a large protein molecule, ok, large protein molecule and we call it as, what do we call it as? Corticosteroid binding globulin. Are you immediately reminded of that, that what? The thyroidic case of, in the case of thyroid, we had a large protein molecule, that protein molecule would bind to the, and it would have the same function.

What do I mean by same function? What do I mean by that? Stabilizing. Stabilizing, ok, do not, do not, do not try to increase the biological half-life of the molecule, ok. And so here we have the, so here we are, so here talking about very large molecule, it is a protein molecule, it has molecular weight of about, how much is there for, can you read for me? Molecular weight of about how much? 58,000 is the molecular weight of the large protein molecule, ok. And then it can keep, and this molecule, and there are several of them, not one of them, there are several of them and all of them are put together and they are also called as the trans-cortains, they are called as what? Corticosteroid binding globulin or they are also

called as trans-cortains, they are huge proteins, they are all synthesized by the liver and they serve, they serve to bind and these proteins have far more affinity for cortisol, they also have affinity for aldosterone, but the affinity for aldosterone is much less. Look, look, what are we talking about, these proteins, different proteins are there, where are they in the blood, where are they coming from? They are coming from the liver.

What do they do? They take a lot of lead to these steroid hormones, good, good, good. And cortisol has a larger, therefore because cortisol binds more tightly to that protein, ok, therefore it can be degraded, it is degraded much more slowly, are you ok so far? And therefore it is biological, what is the biological half-life of cortisol for, please read for me, please how much? 1 and a half hour to 2 hours, ok. And aldosterone has, which binds loosely with less affinity to the trans-cortain molecule, therefore it has a shorter biological half-life, about how much? So please tell me, please tell me the biological half-life of cortisol, fill in the blank and that of the aldosterone, fill in the blank? Greater. Greater.

greater. Cortisol is how much? 1.5 to 2 hours. And aldosterone is how much? 15 minutes. 15 minutes, how much it is? It is 15 minutes, ok. These animal, these I am sorry, these molecules are again degraded, how are they degraded? Very interesting, we know this molecule, very familiar, this cortisol molecule, ok so far, as it is passing through the liver, ok the liver has enzymes, those enzymes will add this particular moiety to the, can somebody tell me what is this position? 3 position.

3 position, at 3 position it adds another molecule which is called as, it is called as conjugation with glucoronic acid, this is called as glucoronic acid. The moment glucoronic moiety is added to the position third of the molecule, either glucoronic or sulphate, one of the two, two things happen, the molecule becomes more soluble, most important and secondly this molecule now cannot combine with its receptor. What have you really done? Yeah, you have completely inactivated the molecule, ok and because it is more soluble now, please remember, steroids solubility is very very low. Are they insoluble? No, they are extremely, solubility is very low. But when you add this, it becomes more soluble, therefore it can be readily filtered at the level, it can be readily filtered at the level of the kidney, it can be thrown out of the body.

Now, this has a very interesting implication, I will tell you how. V vertebrates do not have an enzyme, do not have an enzyme to break the steroid nucleus. This will remain as that and it will get out of your body as the steroid nucleus. Are you with me so far? Ok, yes or no? So whereas some of it goes by way of urine, some of it actually also goes by way of feces, by way of bile, ok. What is the application of this observation? SCAT analysis, the analysis of the stools of the tiger and the panther and the wild animals, ok, to find out about their endocrine system, ok, you can just collect the samples of the droppings of those animals, ok, because they still have these steroid nucleus, ok, and from that, from the chemistry of this you can work out as to whether what is the sexual status of that female or what, you can

work out a lot, there is a huge amount of branch of endocrinology there where you can do the SCAT analysis and find out the physiology of the animal without even drawing a drop of blood.

Hello, are you with me? Are you getting the argument? You see you can't go and draw the blood of a tiger, ok, you can't do that, ok. So collecting these samples, ok, fecal samples and the urine samples if you can, you can do the analysis and you can find out about. I want you to remember it, because recognisable hormonal products can be identified in the urine, it is possible to estimate the daily secretory rate of steroid hormones by non-invasive technique of analysing urinary secretory, ok. Now let us talk about the importance of aldosterone. I told you once, I will tell you once again.

Just open a mouse or a rat, remove both the adrenal glands, switcher of the animal and the animal generally will dry in about 6 to 7 days, ok. What did I say? It will die, absolutely sure. But you see this is the animal is in the cage and in the cage you provide a bottle, you know, so that the animal can drink water. Through that water if you just give saline, the guys are wives. Are you getting the argument? There is a cage, in the animal house there is a cage, the water bottle and the animal comes and licks routinely and you just have water, you can just give one tap water will do for the animal, ok.

If you just give tap water the animal will die. But then what you do is you remove the adrenal cortex, both, both, ok, it will die. But if you in the bottle, if you provide what? Salt, salt solution, slightly concentrated, maybe one spoon, one or two spoons in that bottle the guy lives. Why? Because the salt x.

Because you are right. The adrenal cortex are not there. So what is happening? Sodium is being lost. How? It is being lost. It is being lost how? It is not sinking. It is going, it is getting lost how? Through urine, I mean come on.

It is getting lost how? Through urine. So and what are you really doing? Supplementing, the right word is supplementing, you are just supplementing, ok. As long as the animal is not normal, ok, animal is still very sick but it does not die, ok. This simple experiment, ok, proves the profound importance of aldosterone, ok, aldosterone. You give aldosterone, it is able to say, ok.

So let us see now how, why aldosterone or not having aldosterone kills, ok. So this is what I am showing, I mean just showing you cells and there is a blood vessel, there is intracellular fluid, there is extracellular fluid. Are you ok so far? Done. So now you have removed both the adrenal glands and as a result of that the animal has started losing sodium ions through the urine. Actually you know something, if you start collecting the urine of the animal whose

adrenals have been removed, huge amount of sodium, all you have to do is to, the sham operated, what do I mean by sham operated? What is sham operated? Sham operated means, sham is a control animal, in the control animal the poor guy again you give a cut, ok, on the back, ok, and do everything but do not remove the adrenal glands.

Now what is this, this is a sham operated, sham is false, is it a real surgery or false surgery, false surgery. And why do you do that? Because you want to make sure that you are comparing the experimental animal in which the adrenals have been really removed with an animal which, not with just a normal, no, with the animal which has been sham treated. Means what, which has been subjected to surgery, you have to anesthetize the animal, you have to open the animal, you have to suture the animal, you have to do everything. But do not remove the adrenals. What will I call that animal as? Sham surgery.

And I am going to do what? I am going to compare the urine of the sham surgery animal where I find that urine is almost normal in terms of sodium ion content and then I collect the urine from the other fellows whose adrenal glands have been, corticose have been really removed and what do I find? There is enormous increase in the amount of sodium ions in the urine. Is that point taken? Now as a result of, ok, ok, ok, I already know, I already know that in the blood and in the extracellular fluid sodium ion concentration is about 140 milliequivalents per litre. Hello, sodium ion concentration 140 about something like that, ok. Now as a result of the surgery, ok, as a result now the guy has started losing lot of sodium in its urine, it has fallen to about 120.

How much? 120. So, the sodium ion concentration here, here is fallen. As a result of that osmolality has fallen, ok. Now the osmolality of the cells is still normal, ok. As a result of that the cells will start drawing water from the extracellular fluid, outside it is dilute, they will draw water. Are you with me? As a result of that there is lot of water transfer from the extracellular medium into the cell.

As a result of that the extracellular volume which has to be homeostatically standard, ok, that is reduced. And then the animal knows from what you call as, read here, hypovolemia. Hypovolemia means the total amount of fluids that we have, certain amount intracellular, certain amount of extracellular. As a result of surgery the extracellular volume has started reducing as a result of that. Actually death is because that greatly contributes to the, whereas the aldosterone is necessary for maintaining sodium ion concentration.

It also for, it does exactly opposite with reference to potassium ions, means what? Extracellular potassium ion concentration is extremely low about 4 milliequilons per liter. Extracellular potassium is very low, we have done that 100 times, ok. When you remove the adenyl glands then that potassium goes up. Potassium goes up how much, how much? Potassium goes up from where to where, from where to where, where to where? 4 to 10, 4 to

8, almost double. Are you ok so far? Now that is a very serious condition because, it is a very serious condition because you remember, forget about everything else, let us focus on the heart.

The first the, first the sodium ions open, as a result of that depolarization and then the position and then of course the calcium, the plateau, but then it goes down, it goes down because the potassium ions, potassium ions go out. Are you with me? Potassium ions go out and therefore the curve goes down. Now the potassium ions go down because there is a gradient, there is huge potassium ions inside and there is very low potassium outside, there is a gradient. With that gradient the potassium ions go. Are you with ok so far? Potassium ions go because there is a gradient and that gradient is because potassium is about 4 milli ocrels per liter.

If that 4 becomes 8 what happens to the gradient? Gradient is greatly reduced. When the gradient is reduced the potassium ions do not go that far. When they do not go that far they accumulate some of them accumulate on inside. When they accumulate on inside they partially depolarize. Which is not a great idea as a result of that the whole heart functioning of the heart becomes very weak.

Now let us talk about the, so I am sure you understand this figure we are talking about what? We are talking about the principal cells in the distal, in the distal convoluted tubule we talked about the intercalated cells and we talked about the, we have done this image. I am showing it to you for the maybe third or fourth time. So what have we done? We have taken one cell from there and we know what that cell is. What is that cell? It is a principal cell.

And what does, and what does, okay. And on the basal side what are you going to have? A pump. And on the apical side what are you going to have? ENAC. What is the full form of ENAC? Epithelial sodium channel.

Very good. Epithelial sodium channel. Now the proteins which are, the proteins which make up this channel and the proteins which make the sodium potassium and also the proteins which make up yet other, look, look, look, look. There are about 100 different kinds of potassium ion channels available in the animal and plant world. 100 to my knowledge, they may be more, more, okay. Now here author talks about, about one Maxi-K, ROM-KK, whatever, whatever.

All these, the, listen to this. So, the receptor for aldosterone has action, has genomic action which keeps on activating large number of genes and those genes they have their mRNA, they have their proteins and some of the proteins I am talking of are here and they are here. Are you getting the entire story now? So it is because those proteins are there in the principal

cells and they are functioning because in the principal cell there is a receptor for aldosterone which takes aldosterone, that receptor goes, how that mechanized action will take you. It has a genomic action and you have all those proteins, so huge number of, there is under the influence of aldosterone you have huge number of the copies of this protein system, your this protein system and as a result of the pump you can imagine there is very low sodium ion concentration. Done? Hello? Yes or no? And then as a result of this channel here because there is low sodium, the sodium will be absorbed from the lumen, so whatever sodium that is made out of, huge amount will be absorbed here. Are you okay? Is everybody okay with the story? So remember epithelial sodium channel proteins are aldosterone increases, aldosterone increases sodium potassium pump and facilitates the insertion of epithelial.

Can somebody please tell me what the author is trying to tell us, insertion of epithelial sodium channel proteins? Why is the author using the word insertion? Vesicles, there are vesicles, we have done this, arginine vasopressin, there are vesicles, those vesicles will have those proteins, ENAC proteins and they are inside, so they are pretty useless. Under the influence of aldosterone they will go, they will insert on the apical membrane, and once in the apical membrane they will facilitate the influx of sodium ions from the tubular lumen into the cells. We have done this again where the author is telling us about the structure of the proteins of the ENAC and let us try to understand very briefly what is the mechanism of action of the aldosterone on the target cell. The moment I use the word target cell what am I talking about? Principles, what am I talking about? Principles, okay, what about the principle cell? So here is the aldosterone molecule, where does it come from? It comes from the blood. Is it possible that yes it is bound to the large protein molecule? Yeah, it is possible that it has been roughly found that about 60 percent of the aldosterone in the blood is bound whereas 40 percent is free.

So some of the free molecule can come out of the capillary, no problem? Then it can, it is a steroid molecule, okay, okay. Then one other thing I have to very clearly mention. The steroid molecules have one advantage. What is it? Because they are lipid soluble they can go anywhere. So do you need, you do not need a receptor in the plasma membrane, are you okay? You do not need it.

So where is the receptor located? The receptor is located, the receptor is located in the cytoplasm, okay. And the author is calling that receptor is MR, why is author calling that my aldosterone receptor is MR? Mineral corticoid. It is a mineral corticoid receptor, okay so far. Now this, so and this mineral corticoid receptor is normally under resting conditions it is attached to another chaperon protein, okay, which is here and when that aldosterone comes that chaperon protein is removed, are you with me? And then aldosterone will combine which is a very small molecule, it combines with a huge molecule which is the receptor for mineral corticoid receptor.

That receptor is about 6, 7, 100 amino acid, huge molecule. So receptor is a big molecule, aldosterone is very small molecule. But as a result of the receptor combining with the aldosterone, then it enters into the nucleus, it combines with its promoter or whatever or whatever and then you have a range of, range number of proteins coming in. They include the pump and they include what and they include, a huge number of things are happening as a result of, okay, okay. Let us focus on these two mainly, the pump and ENAC. Are you very clear now? What have we discussed as to how does aldosterone bring about the insertion of the, now this is a very, very, just focus on this, a very interesting phenomena that is operating now.

Listen to this. This is a problem. Let us appreciate the problem first. So you can, you really enjoy the solution. The MR that we have seen in the previous slide, MR, what is MR? It has affinity for aldosterone.

Good, good, good, good, good. That is a problem. The problem is that receptor also has affinity for cortisol. Hey, there is a problem. There is a problem. Because the problem is because the amount of cortisol in the blood may be 50 or 100 times more than that of aldosterone.

Hey, come on, come on, wake up. There is a problem. What is the problem? There is so much of cortisol there and that cortisol has affinity for the same receptor. So it is without fail that cortisol will dominate and the poor aldosterone will not have any, I mean then you will have all the effects of, you will have, if downstream you will have instead of having whatever control of aldosterone you have, you have 100 times more effect. You understand the problem? Very serious problem.

First appreciate the problem. So much, so much cortisol. I mean the nature would have done a wise thing in coming up with the mineralocorticoid receptor that does not have affinity for cortisol. That would have been great, but it has. Now let us see how the nature has come up with the solution. The nature has come up with the solution is that cortisol as it enters into the cell, it is acted upon by another enzyme called as 11 beta hydroxysteroid dehydrogenase, hydroxysteroid dehydrogenase, there are one and two. And this converts the cortisol into another steroid hormone which is called as cortisone.

And that cortisone has no affinity for the receptors. Are you with me? Are you with me? So the cell has, the biology has created a problem and biology has solved the problem. What is the, how it has solved the problem? By not allowing the cortisol to come in contact with the receptor. How to do that? Well, you will be converted into what? Cortisone and does cortisone have affinity for the same mineralocorticoid receptor? No, no. And as a result of that, the mineralocorticoid, so the aldosterone here, here and then you get the rest of the changes.

Are you okay so far? Good. Now listen to this. I am going to use a Hindi word. Let me see how many of you know the word. How many of you have heard the word jashtamad? Raise your hands. How would you describe it in other language? I know the English word licorice, but that does not mean anything. We use it in the kitchen you know, it is, how do you describe? It is a piece of bark of a wood.

You can eat it raw, it is also slightly sweetish. Does it make sense? What do you call it as in English? In English you call it as licorice. What do you call it as? Licorice. I have written here somewhere. It is called as licorice, root of the plant Glycirhiza glabra, also known as what? Another problem, this if you take too much of it, it combines, it blocks the enzyme.

What enzyme? 11 beta hydroxystiride dehydrogenase. Therefore the cortisol cannot be converted into cortisone. Therefore cortisol binds with the receptor for aldosterone. And then you have excessive effect of aldosterone.

Is it the effect of aldosterone? No. It is the effect of cortisol. But it is acting like, it is acting like aldosterone. Downstream, oh look, look, you see there is a receptor. Where is it? It is in the principal cell. If it excites what it will do? It will go and keep on absorbing sodium.

Now you can activate that receptor either by aldosterone or by cortisol. Under natural conditions cortisol is not available because it is converted by the enzyme into what? Cortisone. And if you have eaten too much of lycoris and if you have inhibited that enzyme, then cortisol is available as a cortisol. It will combine with and then you have all the downstream effects which are like aldosterone which means there is too much of aldosterone. Which means you will start absorbing lot of sodium and you will have symptoms of what you called as hyper aldosteronism.

What did I say? Hyper aldosteronism. You will access of, ok. An amazing phenomenon. Ok. Whereas the primary site for the action of aldosteron is what I said just now.

We have been hammering it 100 times. Principal cells. Well there are two more places, sites where it can also act. Several places actually including elementary canal. But some of them clearly are in the salivary glands. Remember salivary glands, the saliva is secreted and saliva has a lot of sodium ions in it.

Primary saliva, primary saliva. Then by the time it passes through the ducts, ok, a lot of sodium is absorbed. That action of reabsorption of sodium is again dependent on what? Aldosteron. And similarly in the sweat glands.

In our skin we have sweat glands and we sweat a lot when it is warm. Ok so far. In the, and we lose a lot of, there is lot of, in the primary sweat there is lot of sodium ions. But it is again absorbed. It is reabsorbed and the sweat that we secrete is minimum sodium ions. But remember something. If there is too much sweat formation and if you lose a lot of sweat in a very short period of time, there is possibility that you are losing a lot of sodium.

Ok. Therefore it is a good idea to replace it with some salt, salt rich drink. So what is the point I am telling you that this is a sweat gland and it is also on the duct of the sweat gland you have, you have what? You have receptors for aldosteron and they play the same role of reabsorption of the sodium ions. Ok. We have done this so we can, I can go very rapidly on this, the sodium.

So let us imagine that there is loss of blood. Ok. Loss of volume. Ok. What will happen? We have done it.

Ok. Renin, renin. Ok. Renin will be released by juxtagemulone cells.

Ok. Angiotensin or gen. Ok. Angiotensin 1. Ok. Angiotensin 2. Ok. And then that angiotensin has many effects. Some of them are directly on the smooth muscles so that angiotensin is what? Constrict the blood vessels and reduce the volume available for the blood, number 1. And number 2, number 2, stimulate aldosteron. Angiotensin 2 stimulates aldosteron and then that aldosteron will absorb sodium. When you absorb sodium it retains water and when it retains water, ok, you are trying to compensate the loss of blood or loss of volume of the biological fluids.

Is it very clear? So this we have already done. This is just to tell you the juxtagemulone cells, angiotensin, angiotensin 1, 2, 3 and then you will have the… So in the… answer my question, answer my question. What have we done? Well go to the adrenal of rat, go to the zona glomerulosa, take one cell and map it here, single cell. Yes. And then ask yourself a question. In the plasma membrane am I going to find a receptor for a peptide hormone? What is the peptide hormone? Angiotensin 2, ok.

And do I find it there? We find there. Ok. Can you see there aii, a2 there, what is it represent? That is the angiotensin 2 receptor. Does it, what kind of receptor is having affinity for angiotensin 2? 7 transmembrane, same G protein coupled system. This is what?

This is G protein coupled system, ok and then it gives rise to and then downstream you have lot of calcium. What do you have? You have lot of calcium, ok. And as a result of that, what are we doing right now? We are trying to study the response of a single cell of the zona glomerulosa and it is responding to the arrival of angiotensin 2.

And what is going to be the end product? The cell is going to release the aldosterone. Very simple, simple cell, zona glomerulosa. We know that these cells are there to release the product, endocrine product and that product is aldosterone. And we are seeing here a very interesting step, ok. As a result of the rise in the calcium and this enzyme etc. there is a, listen to this, a protein and that protein sits just outside the mitochondria, sits on the mitochondria, outer layer, sits on the mitochondria.

And that protein is called as STAR, S T A R, it is called as, please somebody you can read for me, steroid what? Acute regulatory protein. Where is the protein sitting? On the mitochondria. On the mitochondria, ok. What is the function of the protein? It is very interesting.

If the protein is not there, the cholesterol molecule will not enter into the mitochondria. Got the message? Very clear message. STAR protein is necessary for, it is a rate limiting, it is very critical. So as a result of that you have the cholesterol, because of the STAR proteins cholesterol molecule is now inside the mitochondria. In the mitochondria it comes in contact with yet another enzyme which is called as P450.

Now read this carefully. P450 SCC, SCC, can somebody guess what is the meaning of SCC? Side chain cleavage, very good. What is it? It is a cytochrome, the enzyme is called as cytochrome 450, side chain cleavage. As a result of that the chloride, the cholesterol is converted into what? Pregnant alone, we have already seen that. And then it, but this happens outside the mitochondria, then it is converted into progesterone, then it is, so is progesterone there in the adrenal? Yes.

Is it a female hormone? Yes. Is this progesterone molecule being secreted by the adrenal cortex? No, no. It is just, so progesterone here is an intermediary molecule that comes into play, Pregnant progesterone and then it again enters and then, and then it is, progesterone is converted into 11 deoxy what? Deoxy corticosterone, converted to aldosterone, so this biochemical step happens in the mitochondria, it comes out and then it is finally released into the circulation. Have you got the message now? So what have we learnt just now? What have we learnt? The regulation of the secretion of aldosterone under the influence of angiotensin II which is the most important factor that regulates the, ok. Thank you.