Organic Chemistry in Biology and Drug Development
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Lecture - 59
Aromatase Inhibition and Anti-ulcer Drugs

In the last session, we were discussing the enzyme aromatase whose function is to convert the androgens into estrogens. If you can stop the biosynthesis of estrogen you can have an anti-cancer drug. That biology has been discussed. Now, the question is how the androgens are converted into estrogen. The reaction is nothing but removal of a methyl that you have to do in order to aromatize the ring.

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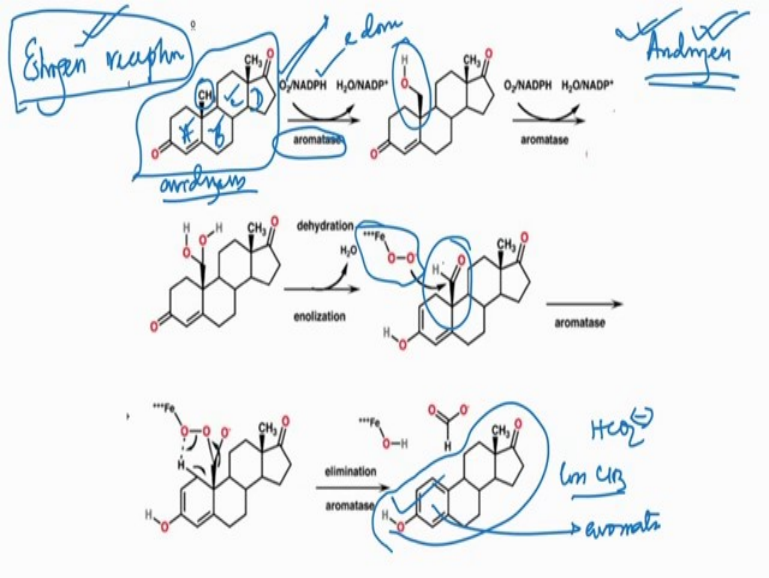

First, the methyl is oxidized to CH_2OH. It is an iron mediated oxidation, an oxygen is the co-substrate and electrons come from the NADPH. NADPH is electron donor, oxygen is a co-substrate and then iron is the mediator of electron transport. So, first it goes to alcohol. Then another round of oxidation takes place. It goes to the aldehyde and from aldehyde we will start.

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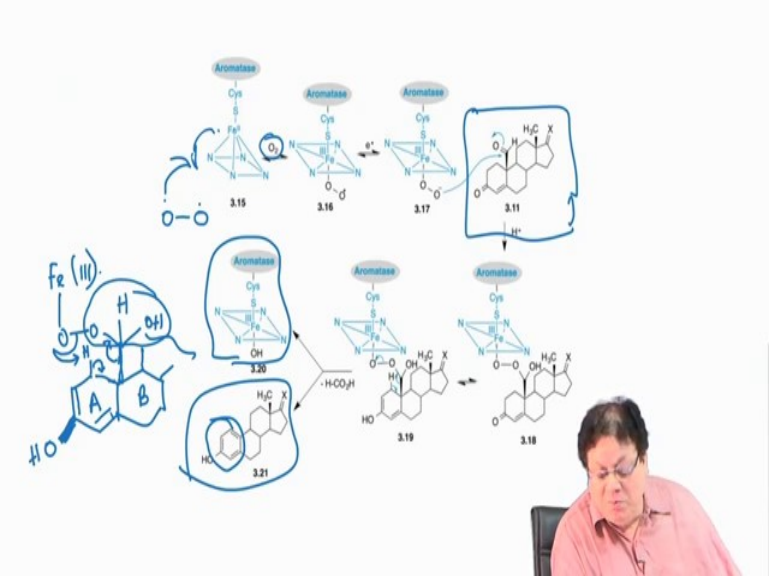

This is the aldehyde. This is aromatase enzyme. It has porphyrin ring where typical 4 nitrogen system is linked to the ferrous. So, the iron is in the ferrous state. Then the aromatase have a cysteine residue and the iron is coordinated to the sulfur.

When the iron is in the ferrous state it is a pyramidal structure. When it becomes the Fe(III) it becomes square planar. It becomes Fe(III) by forming a bond with the oxygen. Oxygen is triplet. Iron gives one electron to the oxygen and this oxygen donates other electron to form this iron oxygen bond.

The NADH transports one electron. So it becomes O minus and this O minus attacks the aldehyde carbonyl. Ferric state is now attached to the oxygen. This is you're a ring and B ring. Now, you have double bond O here and you have one hydrogen.

This is the situation and the C ring is attached. This is the fate of the methyl. There was a double bond. So, you can form this enol now. As you form the enol this hydrogen becomes acidic and that comes here and this bond goes out that comes here that actually basically takes the hydrogen. So, this takes the hydrogen that comes here that goes there.

So, this is basically what? This is nothing but formate and this is the iron. This ultimately will be iron (III) OH that comes out. A double bond is now incorporated to make it an aromatic ring. So, this is the mechanism of your aromatase.

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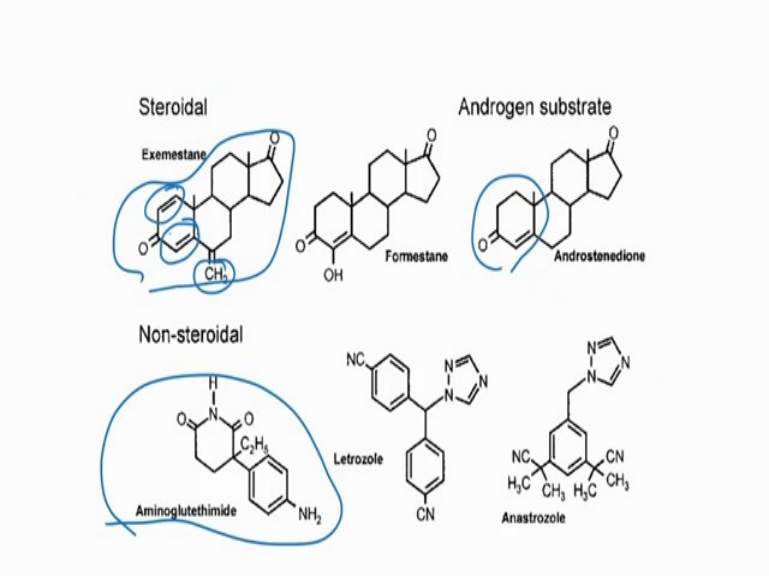

There is these drugs which are acting as aromatase inhibitor. There are non-steroidal drugs also. These drugs have no relation with the steroid but they are inhibiting the aromatase. These are steroid type molecules. How they act as inhibitors?

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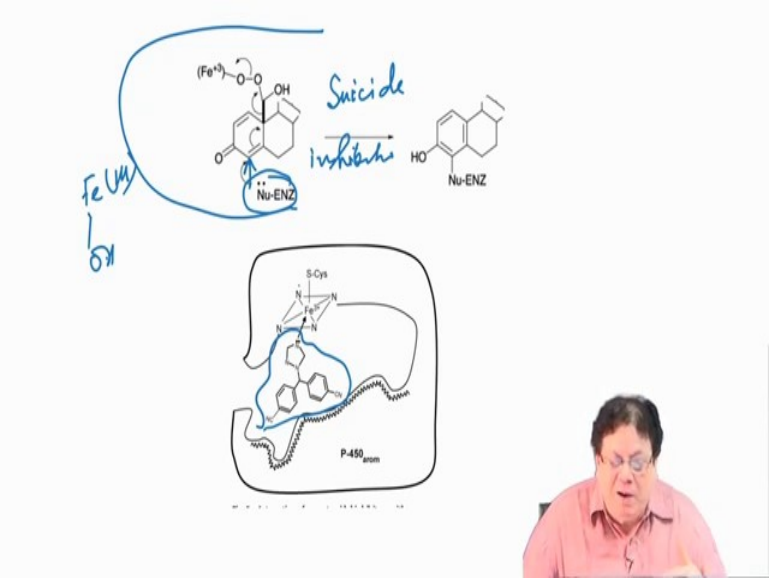

Basically all these molecules have this extra double bond. Here it is a methylene, here it is an extra double bond which is already present in your androgen. If you put double bonds it creates potential sites for attack by any nucleophilic amino acid in the enzyme aromatase.

This enzyme was doing the oxidation of the aldehyde. Remember the last step of this ferryl oxo species is the breakage of this peroxo linkage. That was the intermediate here and then that breaks. The hydrogen is not there. This is a vinyl hydrogen. So, you cannot abstract the hydrogen.

The nucleophile in the form of an amino acid residue attacks here, these goes out here and that breaks. Iron comes as the Fe(III) OH but the aromatase enzyme is now hooked here. So, this is nothing but a case of suicide inhibition.

Similarly for the other compounds, they have no relation with the steroid. They actually kill it to the iron and stop the oxygen to bind to the iron. So, they actually block the oxygen to bind to the iron. Oxygen is the co-substrate. So, by stopping the aromatase enzyme from working the anti-cancer drug is developed.

First, we discussed that in case of cancer, you have a problem with the cell functioning. There is no programed cell death which was called apoptosis.

So, an apoptosis phenomenon is not working in cancer. For preparing the cell to different stages, there are proteins which are called cyclins and there are kinase enzymes which are called CDK. They actually get the signal from outside of the cell and then collect the signals. There is no need for cell growth for the time being.

Once it receives all those signals it activates the growth factor. Then it pushes the cell in the forward direction. Forward direction means from G_1 to S, S to G_2 and G_2 to M, M means the mitotic phase. If there is no requirement of cell cycle it goes to the resting stage that is called G_0 state. If there is some problem with the DNA the cells are destined to die. It is called apoptosis.

When the enzymes cannot repair the problem with the DNA it will ultimately throw that cell into death. So, one good target is this cyclin dependent systems. So, there are many kinase enzymes inhibitors which are now available in the market as anti-cancer drug, although we have not discussed that.

But later on we stated what could be the targets for cancer and we found that the DNA could be a very good target because that is the commander in chief of the cell cycle. So,

you can use alkylating agents, you can use chain cutters, you can use intercalators and we have given a example of all these agents.

Then discussed the HDAC *i.e.* the histone proteins- how can you modify them or part of them not to work efficiently and HDAC inhibitors as simple as SAHA i.e. hydroxamic acid which chelates to zinc. HDAC is a zinc dependent hydrolytic enzyme. So it stops the HDAC from working. That was one new development. Then we talked about the aromatase inhibition. So, estrogen production from androgen can be stopped. There are many drugs that are available in the market today.

So, we finished the anti-cancer drugs. Now we will move to anti-ulcer drugs. We suffer from acidity quite often and today there are very good drugs. Earlier days when people have hyper acidity they used to suffer from what is called peptic ulcer i.e. Ulceration of the GI tract. Now today we have very good drugs to combat the formation of ulcer because we can take care of the acid.

Earlier days, the treatment for acid was to use bases. Very simple that if acid is formed then you have to add base to neutralize it and that is the genesis of development of gelusil or whatever. Very similar types of agents are hydrated aluminum magnesia.

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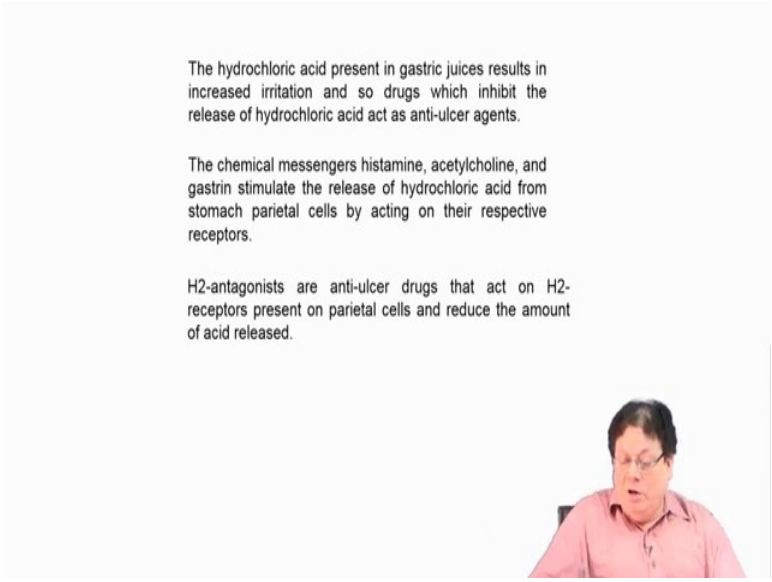

It does not stop the production of HCl in the stomach because simple Le Chatelier's principle says that the more you neutralize the HCl the more HCl will be formed in the

body. So, we should really know what actually is causing this acidity. If we know the reason for having hyper acidity we can develop in a much better antacids which are basically targeting the actual reason for hyper acidity.

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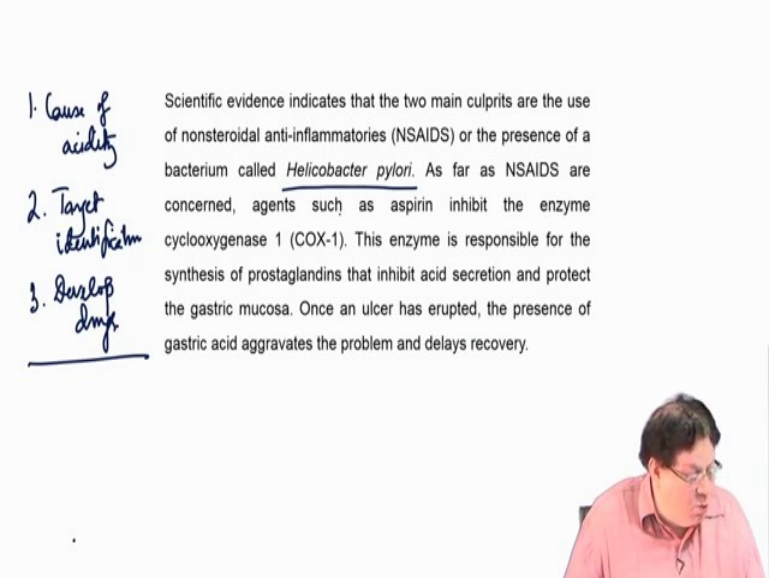

Acidity also can be caused by some infecting agent which is called *H pylori.* It is one microorganism which also can cause hyperacidity. The person who has developed that H pylori is the causative agent ulcer got a Nobel Prize and he is an Australian. He himself took that *H pylori* tablets and then tried to see that whether it develops ulcer or not. So, that is the in the quest of discovery they can even risk their own health.

For *H pylori*, we have anti-biotics. Once it is known that *H pylori* is the causative agent you can tackle by utilizing amoxicillin and an antacid.

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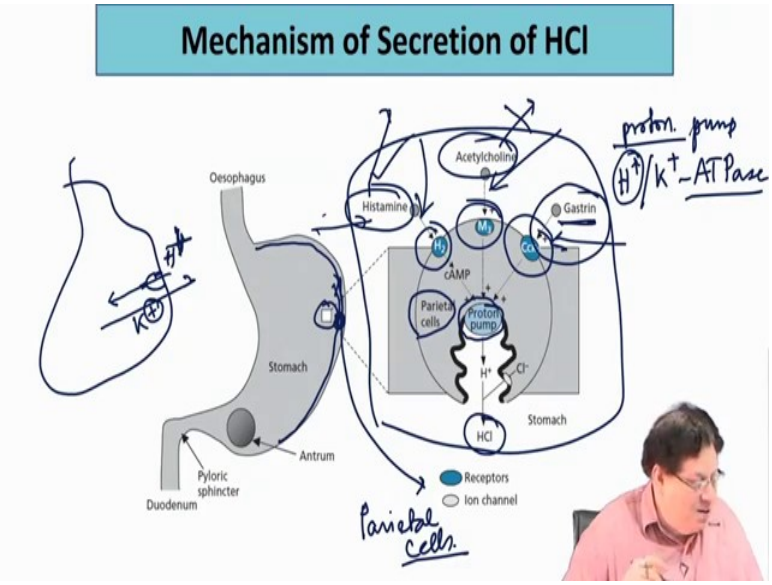

We are talking about the actual reason of having acidity. Acidity is required to maintain a particular pH because some of the enzymes like pepsin works at an acidic pH. You need acidity a perfect pH to digest food. Pepsin breaks the peptide bond and it works in the acidic pH. So, to maintain acidity HCl production is needed. This is the rough cartoon of the stomach. In the outer lining of the stomach, there are some cells called parietal cells.

The parietal cells basically is a connection between the outside of the stomach whatever is flowing here and inside. It can absorb something and there is a gap here. Through the gap it can push the ions or other small molecules whatever is required. It has the proton pump which pumps in protons.

So, basically you have this stomach. There is a parietal cell which has opening here. Its job is to pump proton inside the stomach. Where from the chloride ions come? Actually, before that there an enzyme called carbonic anhydrase.

The carbonic anhydrases form a family of enzymes that catalyze the interconversion between carbon dioxide and water and the dissociated ions of carbonic acid. That is the source of H plus. Bicarbonate does not enter the parietal cells, the proton enters along with chloride and to maintain balance bicarbonate is pumped out of the system. Ultimately H and Cl remains. So, that makes HCl.

There must be some control mechanism by which this proton pump works. Proton pump puts proton inside but some counter ion also should be taken. This is positively charged, your stomach cannot be positively charged. You have to take out some positive ions and it takes out the potassium ion out of the stomach. So, this is called H plus K plus proton pump. The first cation means it is going inside and the second cation means it is going outside.

This is an enzyme which hydrolyses ATP and energy comes from hydrolysis of ATP. So, it is a proton pump which is nothing but a H^{+}/ K^{+} ATPase. What regulates the proton pump? 3 things- one is histamine. Histamine is the decarboxylated version of histidine. If you decarboxylate histidine you get histamine. Like dopamine, we have read decarboxylated version of L-DOPA. Similarly histamine has a receptor on the parietal cell.

There is another neurotransmitter called histamine. By the way, you can call it as a hormone because it works at several places. There is a receptor for acetylcholine and there is a receptor for a peptide which is called gastrin. If they bind to this receptor it sends a signal to the proton pump to work and then push the H plus inside the stomach and that causes acidity.

Your strategy is either to stop the acetylcholine from binding here, or to stop the gastrin from binding there, or to stop histamine from binding here. I think this strategy was not successful. You can use inhibitors of acetylcholine but the acetylcholine is a very important neurotransmitter. So it will stop the brain from proper functioning. You cannot stop the acidity problem targeting acetylcholine. So remaining is histamine.

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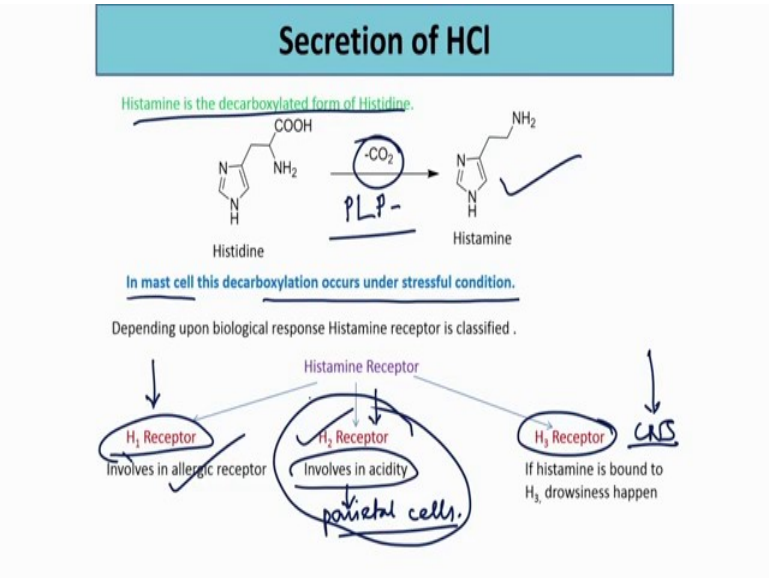

Decarboxylated form of histidine gives histamine. It is a PLP dependent enzyme. There is a cell called mass cell that this decarboxylation occurs. Now, this histamine has 3 types of receptor, one is H_1 receptor.

When it binds to H_1 receptor it causes allergic response. Basically sneezing is caused by an allergen. You get lot of histamine production and that goes and activates the receptor H_1. That is H_1 receptor which is important for allergy. Then there is a H_2 receptor, this H_2 receptor is present in the parietal cells. So, H_2 receptor causes acidity.

There is a third receptor H_3 which is present in the CNS central nervous system. If it binds to H_1 receptor it causes allergy. If it binds to H_2 receptor it causes acidity. If it binds to CNS it causes drowsiness. When you take anti-allergic tablet us you also becomes sleepy because your drug is not very specific. It is an antagonist to H_1. It is also binding with the H_3 receptor. If it binds to the H_3 you get drowsiness.

Now, today we are talking about this H_2 receptor, the acidity.

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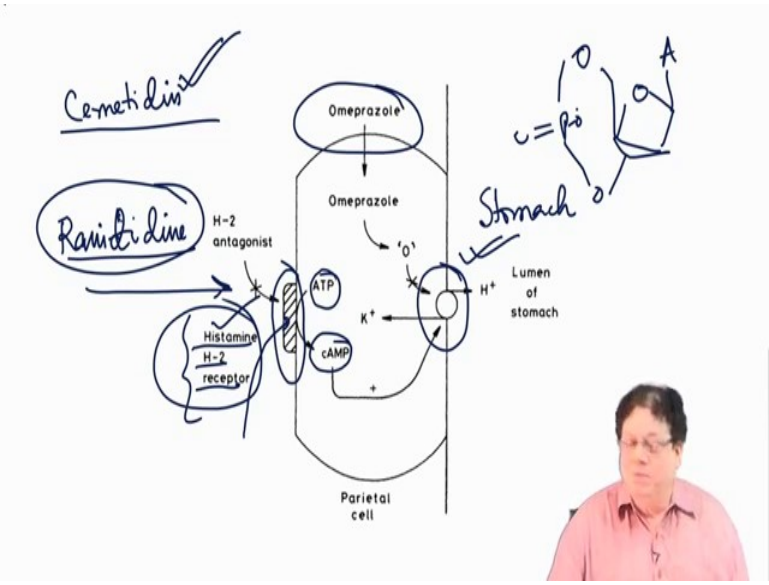

Now, in the parietal cell you have the H_2 receptor. By the way receptors are named according to the hormone or neurotransmitter like adrenergic receptor which are the receptor for adrenalin. This histaminergic receptors are 3 types -H_1, H_2, H_3. We are talking about H_2 which causes acidity.

If the histamine binds here there is a reaction that takes place at the parietal cell. ATP is converted to cyclic AMP. So, this is the primary messenger histamine and this is the secondary messenger cyclic AMP. This is cyclic AMP. Cyclic AMP goes to the pump now and says to start work. So, it now pushes the H plus here and potassium plus inside. You can stop acidity by targeting the histamine receptor. You can also look at the pump. You can stop the pump also because that is also an enzyme. There are very good H_2 antagonist. You have to know the names ranitidine or the first one that that was discovered was cimetidine.

You have ranitidine, you have famotidine. The first generation is now gone out. Ranitidine is still very successful drug for controlling the acidity. So, that works by an antagonist of histamine, H_2 receptor.

The pump is stopped by a well-known drug omeprazole. Now, the anti-ulcer compounds are basically omeprazole, rabecprazole, lansoprazole, then pantoprazole all this compounds. They are actually inhibitors of this proton pump.

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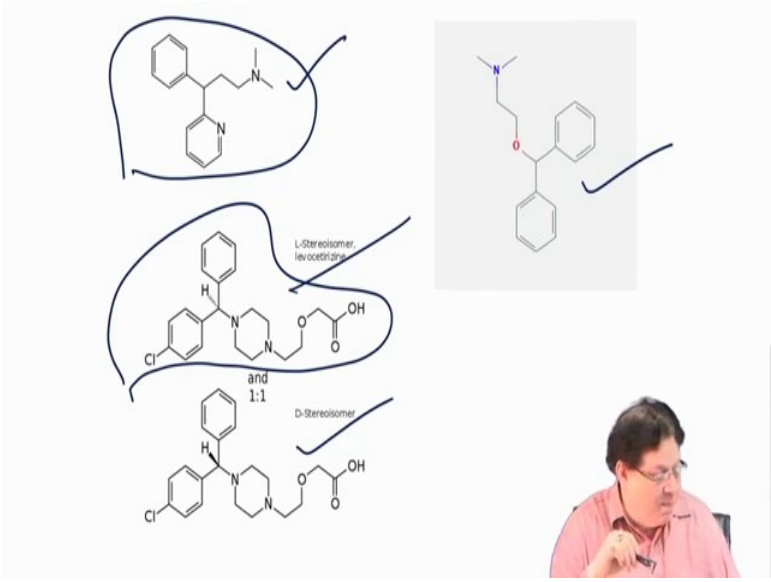

The mechanism of proton pump is actually started from H_1 receptor. This was the H_1 receptor. How this ranitidine was discovered? First they started looking at the receptor. So, initially they develop H_1 receptor. All this anti-allergic drugs were first discovered which H1 receptor antagonist are. Now we have this is pheniramine, this is diphenhydramine. This is a much better one which is called cetirizine and then you have this levocetrizine, the L isomer is what is called levocetrizine. They actually bind to the H_1 receptor. Levocetrizine is even specific which does not interfere with the H_3 receptor. This is so successful, it causes less drowsiness.

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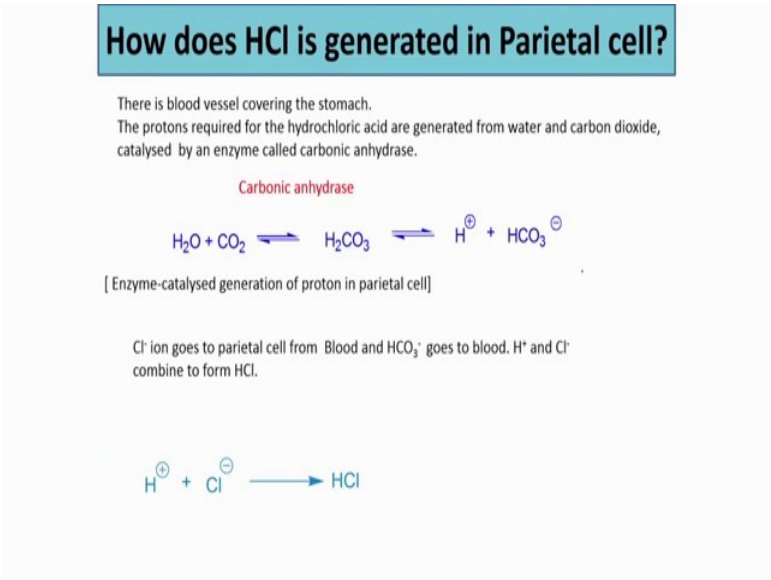

I think I told you about the source of HCl.

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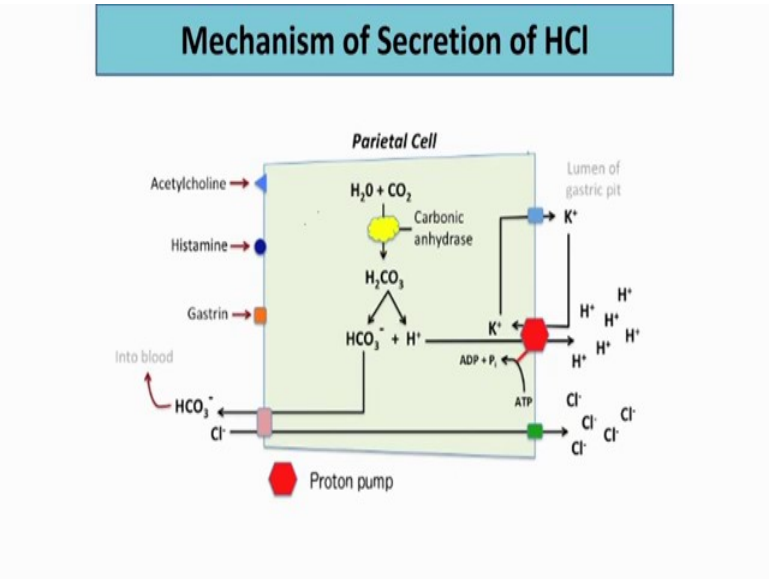

I told you what happens in the parietal cells.

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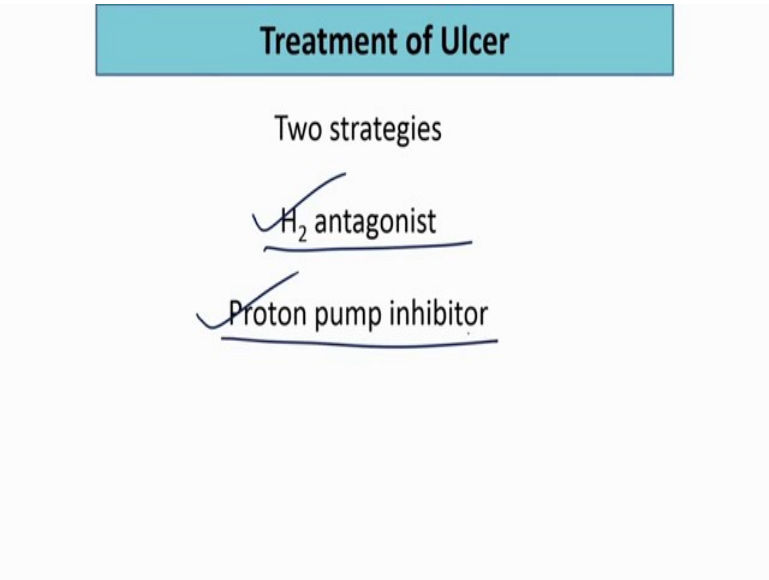

Now, two strategies for an antacid- one is got managing the hyper acidity, another is proton pump inhibitor.

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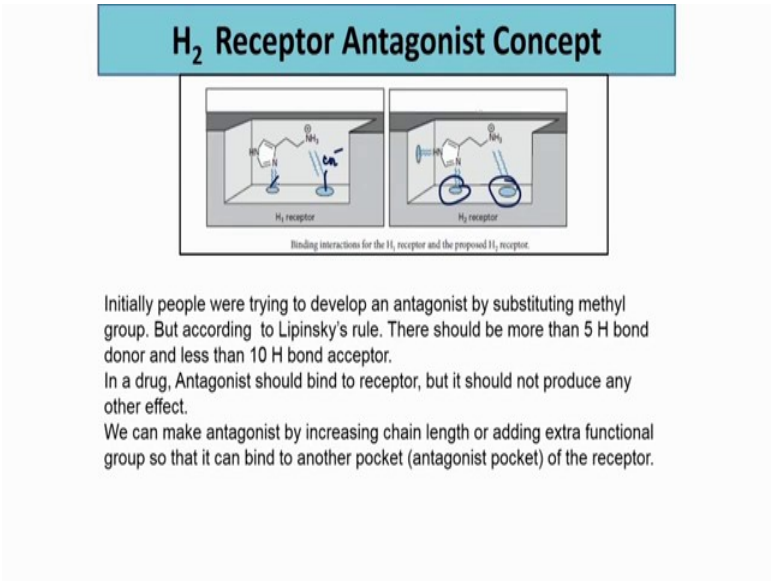

How H_2 antagonist was discovered? Initially nothing was known but they thought that there could be some receptor. If you look the structure of histamine it will be present as NH_3 plus here and this imidazole. There can be some introductions here and then some electrostatic introductions here. Possibly there is a CO_2 minus here and there could be some hydrogen bonding effect which forms the hydrogen bond to nitrogen.

But the problem is how to really develop selective agent that is not an anti-allergic but only goes to the H_2 receptor. We are just making the discussion short. If you try to utilize this type of system i.e. only this type of amine and imidazole you are not satisfying the Lipinski's rule.

Remember Lipinski's rule is basically the initial screening of a drug molecule. There should be-5 hydrogen bond donors, 10 hydrogen bond acceptor. It is Lipinski's rule of 5. Pharmaceutical companies they will work on Lipinski's rule. We academicians think that Lipinski's rule is not correct. It is not the ultimate but it is a way of screening out structures.

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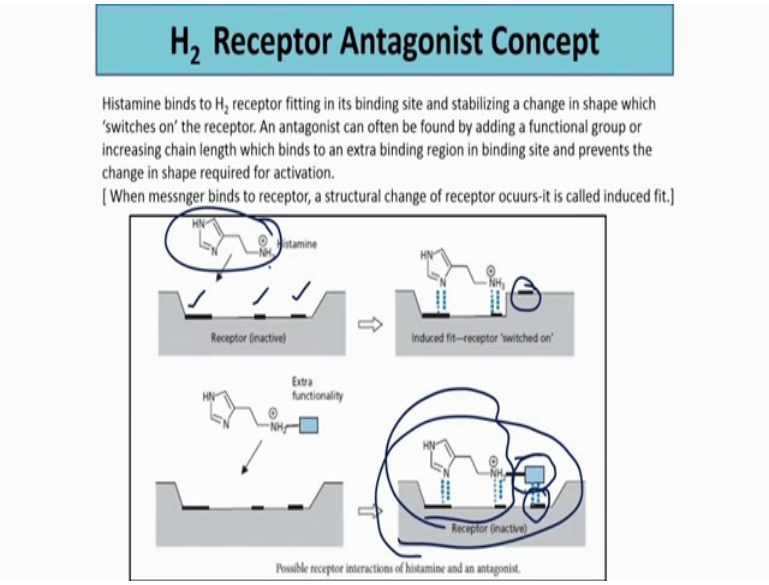

There may be other binding sites in the H_2 receptor. If you attach another binding agent to the histamine they attach something else which can also form hydrogen bond at the other binding side. Although they did not have some idea initially about this binding site, but they have this hypothesis that there are basically 3 sites in the H_2 and two sites in H_1.

If you take only histamine they will go to these two sites. The other third site is remained vacant. If you now attach something which can interact with the third site you will get very specific H_2 receptor binding molecule.

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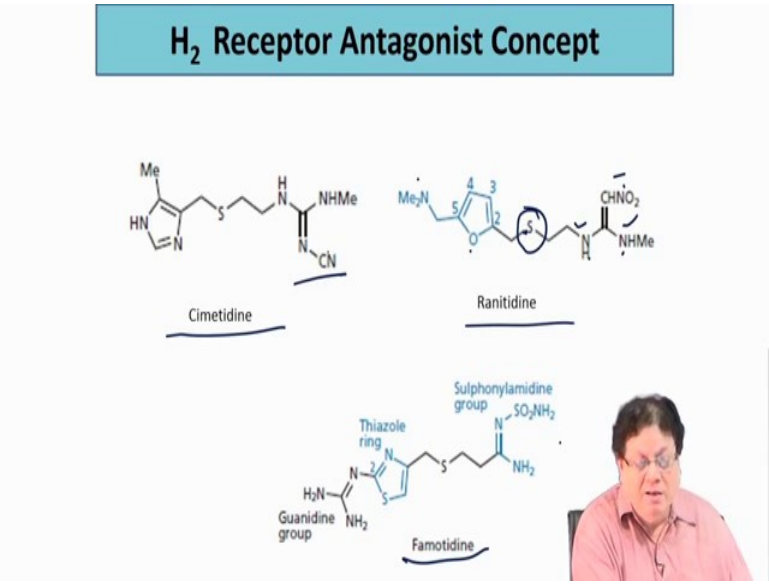

So, ultimately they came out with this type of molecule, cimetidine, then ranitidine, then famotidine. Where they are binding? First they actually try to satisfy the Lipinski's rule- they put the sulfur inside. So, they put lot of nitrogen donors. Then they have acceptors.

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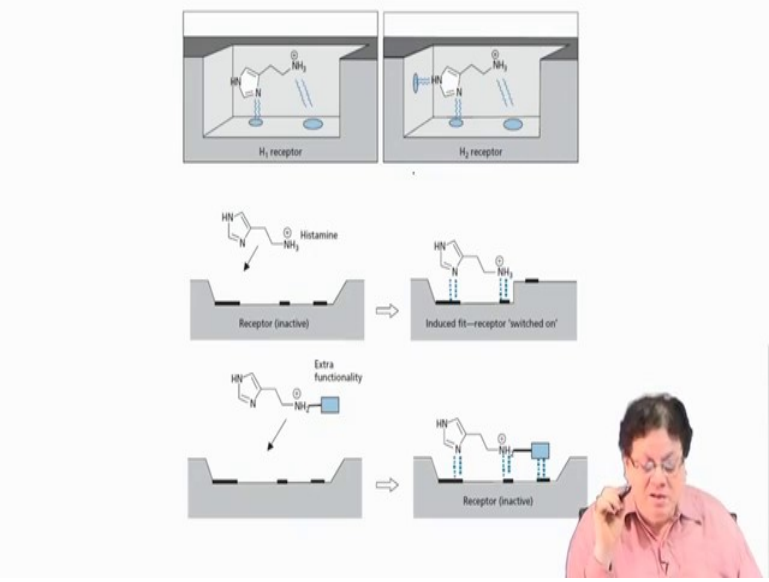

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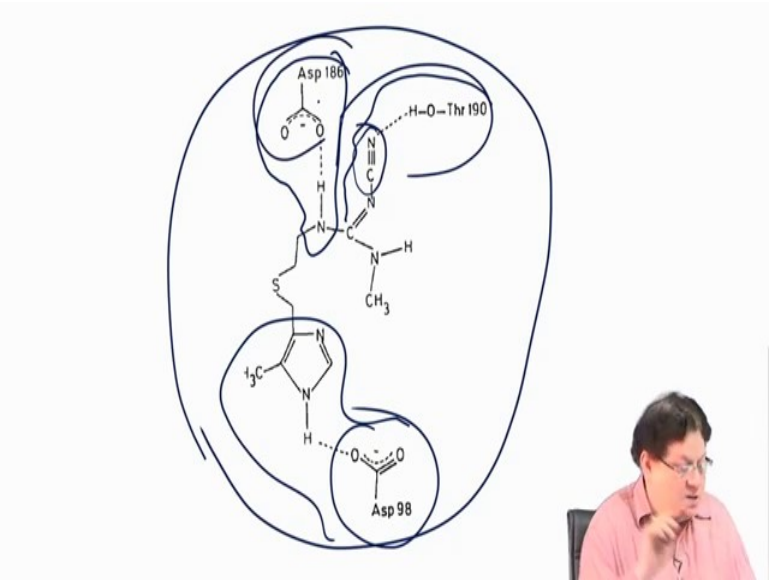

Now, by crystal structure or by modeling studies they found or it is hypothesized that an aspartate is here which forms a hydrogen bond interactions. A threonine in the active site forms hydrogen bond.

In cimetidine, C triple bond N goes to the threonine. This goes to the aspartate and that goes to another aspartate. I told you that there are 3 binding pockets. One binding pocket takes care of the imidazole that you cannot change and this is the threonine, and that is the aspartate.

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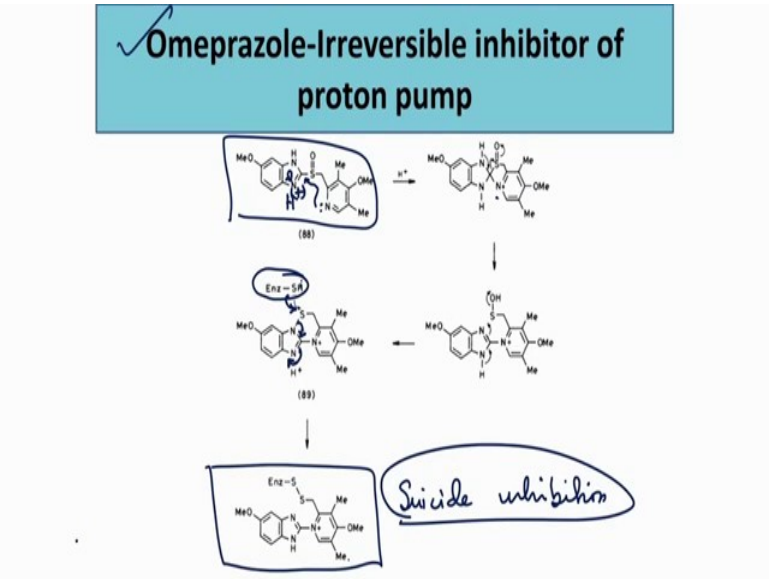

Now, let us come to the final part of this anti-ulcer drugs - the discovery of omeprazole. Remember, omeprazole is the one this type of molecules which are called proton pump inhibitors. There is a series of drugs omeprazole, rabeprazole, pantoprazole. We just talk about the omeprazole. This is the molecule called omeprazole. It has got a sulphoxide, it has got this benzimitazole and a pyridine nucleus.

It exploits the hyper acidity of the stomach. So, first it is the basic nitrogen of the imidazole that gets protonated. So, once it becomes protonated this nitrogen is nucleophilic. Nitrogen from the pyridine attacks here and this comes here. You get a molecule like this. This is a sulphoxide so that it can also abstract a hydrogen which results in the breakage of the carbon sulfur bond.

This is nothing but a Pummerer rearrangement. Pummerer rearrangement is basically a nucleophilic ring opening or by a hydrogen loss ring opening of the carbon sulfur or breakage of the carbon sulfur bond. So, once that is broken it will be SOH. This is NH, this is double bond and that nitrogen is plus. Now, this again comes here, that goes and removes the OH. So, you get a cyclic compound like this.

This is now this sulfur. This carbon nitrogen sulfur bond is very vulnerable because this nitrogen can be protonated again and that makes nucleophilic attack at the sulfur site. Thus proton pump with a active serine SH, cysteine SH attacks this sulfur, this comes here and that goes out, that takes the proton. You get a stable enzyme and transformed substrate. It is a covalent bond. So this is a case of suicide inhibition.

Now to conclude this anti-ulcer chemistry, there are basically two strategies- one could be on the H_2 antagonist, another could be the proton pump H_2 antagonist. Lot of structure activity relationship was done and then they realize that there are 3 binding pockets and they have utilized this 3 binding pockets in order to have very specific H_2 antagonist which does not bind on H_1 or H_3. So, that is the genesis of development of ranitidine, cimetidine, and those type of drugs.

And then the proton pump inhibitors are basically suicide inhibitors and it is catalyzed in presence of high acidity. The first step is basically protonation and that protonation is the driving force for formation of a enzyme transformed substrate by a covalent bond. So, that is called suicide inhibition.

So, I think that is the end of the anti-ulcer chemistry developments and today peptic ulcer is almost gone. People are not suffering from peptic ulcer. In the 30 years back, lot of people were suffering from peptic ulcer. Now this ulcer problem is almost gone these days with the advent of omeprazole, ranitidine.