Overview and Integration of Cellular Metabolism

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Lecture 33: Metabolism of Ammonia and ammonia toxicity

Hello everyone, welcome back we are continuing with protein metabolism and today's topic is metabolism of ammonia and ammonia toxicity, where we will be covering the following concepts. We will be looking at how ammonia is formed in the body, what are the sources from which we get ammonia, how ammonia is transferred in the body, how ammonia is detoxified and what is the mechanism of ammonia toxicity all right. So, to start with let us summarize the whole thing at the very beginning right, we should all know by the end of the session that ammonia is actually produced by all tissues ok during metabolism of various compounds specially the proteinaceous nitrogenous compound and ultimately it is finally disposed by the formation of urea. So, that is the end goal that will be reaching today. Next ammonia once it is produced it must be readily cleared of the body, the body is maintaining such a mechanism so that it cleans ammonia very fast otherwise it would affect the brain because ammonia is very toxic to central nervous system. So, hyperammonia is a lot of concern all right.

So, there are multiple mechanisms in our body by which ammonia should be rapidly cleared off from the circulation. So, let us look at the sources of ammonia to start with as I always mentioned main source of ammonia is the nitrogenous waste from proteinaceous diet, the more we protein we eat the more ammonia will be produced from our body. So, it is much more concerned specially in western diets which contains much more protein compared to that in Indian diet we have got a more percentage of carbohydrate. So, the amount of ammonia nitrogenous waste that is produced after western diet is much more nevertheless even in Indian diet when the ammonia clearing mechanism is at a problem ammonia level may go high because not only from dietary sources ammonia is also available from other source.

So, let us look at what are the sources of ammonia and how ammonia is formed right. So, number one source of ammonia that we discussed in the last class is from amino acid the amine group of amino acid when it is removed in the form of trans deamination that is all amino acids undergo transamination where the amino group is transferred from the

amino group to the keto acid that leads to the production of glutamate in all tissues and ultimately glutamate is transferred to the labour where it is acted upon by glutamate dehydrogenase it is the reaction of oxidative deamination. So, ultimately deamination of glutamate is the major source of production of ammonia in the body, but we also discussed in the last class that there are minor ways of deamination other than oxidative deamination such as reductive deamination hydrolytic and intramolecular deamination. Since we already discussed them in the last class we are not going in details, but we already know, but and you should know that these are also the reaction that produce ammonia these are also the sources of ammonia. Now, something new today that is one of the main source other source of ammonia other than amino acid that is from glutamate is by the breaking down of glutamine.

So, glutamine is actually acted upon by the enzyme glutaminase glutaminase enzyme breaks down glutamine it is the hydrolytic cleavage that is a water molecule is needed and ammonia comes out it mainly occurs in the kidney this reaction occurs in the kidney where ammonia is directly excreted it is directly excreted how it is excreted actually ammonia is highly soluble in water. So, in the renal tract there is excess water in the form of urine. So, ammonia is dissolved in the urine and excreted in the form of ammonium iron and this is one of the major mechanism of acid excretion from the body that maintains the acid base pH. So, glutaminase is mainly majorly found in the kidneys renal system, but we should also keep in mind that there is also an intestinal variety of glutaminase that can if glutamine is available from minor sources such as the mucosal cells alright or from some digestion of dietary protein if glutamine is there in our diet intestinal glutamine is also there that will also break down glutamine lead to the production of glutamate and release ammonia right. So, number one trans deamination that is from amino acid from all deamination of all amino acid next is glutamine this is one major source of ammonia apart from that what are the other sources of ammonia urea alright.

So, urea present in the gut that is by the bacteria they produce the enzyme urease alright. So, what happens urease acts upon urea to produce ammonia well this ammonia is actually absorbed from the intestine and ultimately this ammonia is transported via various transport mechanism that will be discussing it very soon ammonia is absorbed. So, ammonia is produced in the gut by the action of intestinal bacteria another source of ammonia is amines. So, primary amine by the action of amine oxidase group of enzyme also produce ammonia alright. So, amine oxidases right next from the catabolism of purines and pyrimidines.

So, when purines and pyrimidines are broken down again we will be discussing purines and pyrimidines are basically nucleotide we will be recovering nucleotide metabolism in

detail when they are broken down for example, specially adenine nucleotide this is an example of adenosine monophosphate AMP it is acted upon by the enzyme adenosine deaminase. So, in this case this is adenosine deaminase ADA it produces IMP, inosine monophosphate and ammonia. So, deaminase is a blanket group of enzyme that acts on all type of purines and pyrimidine nucleotide this special enzyme that acts on AMP is known as ADA or adenosine deaminase. Next L amino acid oxidase. So, over here we see generally amino acid when they are converted to keto acid we study that in transamination.

So, generally one amino acid and one keto acid reacts together to transfer the amino group to another amino acid and resulting in the formation of another keto acid that was transamination over here what is happening one amino acid is directly converted to keto acid and the amino group is removed this is done by L amino acid oxidase I already told you what is capital D and capital L this actually requires flavin mononucleotide or FMN as a coenzyme mind it it is very minor this is not common main is the oxidative deamination followed by I mean transamination followed by oxidative deamination. So, it is of little importance, but still it is one minor source of ammonia production it is present in liver and kidney, but in very low amount. Well once FMN is used up in the reaction it is reduced and again it is regenerated and that leads to the production of H2O2 which is further broken down by catalase, but what is important for us to know is L amino acid oxidase is one minor group of enzyme that can also produce ammonia. Well there is also a group of enzyme that is D amino acid oxidase it uses flavin adenine dinucleotide or FAD as coenzyme. However, in natural in body L amino acids are more common.

So, L amino acid oxidase are more common than D amino acid oxidase, but in totality they are very minor compared to other mechanisms of ammonia production. Well looking at all the other minor pathways of ammonia production this is the rest of it for example, histidine to urochemical acid by the enzyme histidase histidase acts on histidine as per genease acts on as per a gene to produce aspartate and ammonia serine and threonine are converted to pyruvate and alpha keto butyrate we studied alpha keto glutarate, but this is alpha keto butyrate, but their respective dehydrated enzyme they also produce ammonia. Cysteine undergoes deamination and simultaneously trans sulfuration by the enzyme desulfurase, desulfhydrase. We actually we showed this part in the last class where we were discussing how am I am the nitrogen skeleton of amino acids are removed. So, these are all very minor ways of production of ammonia the major ways being production of ammonia directly by oxidative deamination that is from glutamate by the enzyme glutamate dehydrogenase or LGDH and by breaking down of glutamine by the enzyme glutaminase alright.

So, till here if all the sources of ammonia are clear to you we can now move into the next part of the lecture which deals with how this ammonia whenever they are produced how they are disposed alright. Now one of the main thing to notice see ammonia generallyis produced from glutamate that is from ion acid I told you this is a glutamate dehydrogenase reaction right. So, this is GDH which produces ammonia, but the problem is this glutamate dehydrogenase reaction happens only in liver peripheral tissues cannot deaminate glutamate. So, there has to be a way where amino acids are actually undergoing trans deamination how this is the help of this glucose alanine cycle or it is also referred to as Cahill cycle ok. So, let us see what happens over here.

So, nitrogen the actually the amino group is actually transferred to pyruvate to form alanine this is a transamination reaction that is happening in the at the level of muscle. This alanine because glutamate cannot be treated over here there is absence of glutamate dehydrogenase. So, glutamate cannot be further metabolized. So, what glutamate does it get rid of its nitrogen by passing the amino group from pyruvate. So, one it passes its amino group to a keto acid and itself it becomes alanine and alpha ketoglutarate.

So, this alanine actually carries the nitrogen group and it goes to the liver liver then picks up the alanine it again undergoes another transamination where alpha ketoglutarate with the help of alanine transaminase it forms glutamate. But in glutamate GDH is present and therefore, it can be now acted upon by glutamate to liberate ammonia and this ammonia then enters the urea cycle and because in liver we have all the other mechanisms by which ammonia can be disposed. So, mind it in periphery the nitrogen is actually travelling via alanine and this pyruvate again undergoes gluconeogenesis to produce glucose glucose is released in the blood glucose reaches the muscle it undergoes glycolysis to produce pyruvate and thus the cycle is completed. We all know by now what is glycolysis and gluconeogenesis and this is the beauty where both carbohydrate metabolism and protein metabolism are integrated together and in this specific case it is acting to clear the nitrogen or the ammonia from the peripheral tissues to the liver all right. Ultimately as the last line says the ammonia is finally, entering into the urea cycle which will be discussing later in much details.

So, first was glucosamine cycle or Cahill cycle what is the next major mechanism of transport of ammonia this is via glutamine formation mainly it happens in the brain and also in muscles. So, what happens glutamate actually traps ammonia to form glutamine ok. So, glutamine is actually the amide form of glutamic acid that produces or that is a forms a storage form as well as a transport form of ammonia ok. So, in brain glutamine is a major mechanism of removal of ammonia while in the liver it is urea formation. So, when it reaches the liver or nitrogenous product reaches liver we can now extract the nitro ammonia to produce urea, but in brain urea cycle does not happen ok.

So, in brain in order to get rid of the ammonia it is trapped in the form of glutamine and it reaches the circulation. So, in the circulation what happens ultimately the glutamine releases I mean reaches the kidney and kidney already read how it can be obtained by renal glutaminase and ammonia can be directly excreted via the urine the same mechanism it is being excreted into the liver kidney and intestine if it is in I mean goes into the intestine there is also intestinal glutaminase from where it will be absorbed in the portal circulation it will go into the liver and finally, in the liver everything can be taken care of. Since glutamine is actually the major form in which the most of the nitrogenous wastes are being shunted out of the brain it has been found out that plasma concentration of glutamine is actually very high much higher than other amino acid which actually first indicated led to the search of the answer which ultimately led to the solution that glutamine is actually the major form of transport of ammonia ok. So, in a very interesting article by Lacey and Wilmore in 1990 they actually referred to glutamine as a Trojan horse because glutamine plays a very important role in removal of ammonia it is the most abundant amino acid in blood it carries it is more than 50 percent of free amino acid is actually glutamine it actually it is a donor group of ammonia will really read next how glutamine is actually donating ammonia we already read by the action of glutaminase glutamine can be broken down into ammonia right that ammonia can participate in multiple reactions as well and it also acts as a fuel for gut kidney and system immune cells in many other ways it can help in transmission I mean development of a neurotransmitter by getting converted to glutamate in the brain. So, actually it has got multiple protective mechanism, but still why it is known as Trojan horse well Trojan horse the references basically it is a hollow wooden horse by hiding in which Greeks actually found a way to enter Troy.

So, a Trojan horse is referred to a mechanism which actually remains hidden in the system it plays some important role, but when tying comes it actually can disrupt the whole system you have must have read the Trojan horse is a type of virus. So, glutamine can also act in a similar way it performs many function, but when the tying comes this glutamine can become a very important culprit which will be studying in detail in the ammonia toxicity in the following slides. Anyway so, this glutamine synthesis is actually done by the enzyme glutamine synthetase right and when again that we read that glutamine can again be broken down by glutaminase to liberate ammonia right in and then ultimately goes into the urea formation. But the thing is glutamine formation is extremely important why? Because glutamine is involved in donating a nitrogen group which can be utilized in formation of various aromatic and several other amino acid it can be used in synthesis of purine, pyrimidine, amino sugar so many things. So, basically whenever we need a nitrogen group ammonia is actually carried in the form of glutamine to multiple peripheral tissues which actually donates the nitrogen group.

So, this synthesis of glutamine is done by the enzyme glutamine synthetase mind it one very fundamental thing let me tell you whenever we are listening to the enzyme synthetase it means there is an active energy that is involved or ATP a synthase enzyme generally ATP is not involved. So, whenever you are listening to this name synthetase mind it there will also be always been ATP or whenever there is an ATP involved the enzyme generally will be synthetase enzyme when there is something formation is occurring right. So, how it is regulated? It is regulated by feedback inhibition simple whenever the excess product is formed we do not need glutamine anymore. So, then it will be feedback inhibited we already know what is feedback inhibition moreover it is also regulated by global cascade. So, generally glucagon it forms c a m p cyclic c a m p formation is is happening cyclic a m p.

So, generally adenylation of tyrosine residue actually blocks the whole thing why any anabolic process will be blocked by glucagon we know this concept and this is how glucagon or glutamine synthetase enzyme is regulated. Now, the question is how do we use ammonia right. So, ultimately ammonia is pushed to the liver by glucosylenin cycle or in the formation of glutamine. So, if we look at what is the use of ammonia in our system we can till at least till now we can answer few questions from this slide ok number one by the enzyme glutamate dehydrogenase. We already know that gluta alpha ketoglutarate can form glutamate with the help of the enzyme glutamate dehydrogenase because that is a reversible enzyme that can utilize both NAD and NADP plus we know that right.

Next glutamate can form glutamine by the enzyme glutamine synthetase that we also read just now and also we read that glutamine can also be converted to glutamate by the enzyme glutaminase right often that is also termed as glutamate synthetase anyway. So, those are very important. So, glutamate synthase excuse me glutamate synthase because that does not use ATP. So, this one is glutamate dehydrogenase this one is glutamine synthetase and this one is glutamate synthetase or glutaminase they are same. We are left with few other option that we have not explored yet number one is synthesis of asparagine and synthesis of carbamoyl phosphate.

These are the bonds these are the mechanisms where ammonia is also used that we have not studied yet. So, let me tell you this is one example where asparagine is synthesized with the help of ammonia or ammonia is utilized or it is transferred. So, there is transfer of nitrogen group how oxaloacetate is transaminated to aspartate all right and aspartate undergoes action with the help of the enzyme asparagine synthetase again it is an active enzyme or active high phosphate bond requiring enzyme. Hence the name synthetase and it forms asparagine this is also one use of ammonia that is it forms the amino acid

asparagine with the help of the enzyme asparagine synthetase. And lastly one of the most important reaction where ammonia is used that is formation of carbamoyl phosphate.

So, how carbamoyl phosphate is formed well again formation of carbamoyl phosphate is an energy dependent process it needs the enzyme or it needs an ATP hence the enzyme is known as carbamoyl phosphate synthetase. So, carbamoyl phosphate synthetase are also present in two location number one it is present in mitochondria where a CO_2 in the form of bicarbonate and ammonia in the form of NH_4 is acted on by CPS1 or carbamoyl phosphate synthetase one to form carbamoyl phosphate. Carbamoyl phosphate can also be formed from glutamine and bicarbonate leading to the formation of carbamoyl phosphate and glutamate and this happens in cytosol. Well both the reaction actually are involved in ammonia I mean involvement of ammonia in the form of a nitrogen group, but the reaction that directly involves ammonia utilization is this one carbamoyl phosphate synthetase one because this is a reaction which is very much needed for urea cycle or disposal of ammonia. Whereas this reaction the one that happens in cytosol it is used for pyrimidine biosynthesis pyrimidine synthesis we will be discussing this one CPS2 in later classes when we are discussing nucleotide metabolism, but for now we should know that the CPS1 enzyme is extremely important because it helps in urea formation.

So the ultimately when we are dealing with metabolic disposal of ammonia one and one answer for human is urea. So formation of urea in liver is actually quantitatively the most important route for ammonia disposal because ammonia from all sources are ultimately shunted to liver and ultimately this urea can for once formed in the liver it can is soluble in waters it can go to the kidney and it can be excreted by the glomerular filtrate in form of urine. So this is a diagram which shows how we are getting glutamate from various sources whether it is breaking down of glutamine or whether it is transamination and glutamine is entering into the mitochondria. So ultimately we are getting ammonia and this ammonia is combining with a bicarbonate and ATP to form carbamoyl phosphate and this carbamoyl phosphate is actually what the end product or the compound which enters into the urea cycle. So ornithine to citrulline is actually urea cycle which we will be discussing later in details because this is the urea cycle there are many steps involved there are multiple enzymes that are involved and we have a dedicated class for urea cycle multiple classes for urea cycle where we will be showing all learning all how the what are the steps what are the cycles what are the enzyme deficiencies what are the problems but for now we should know that carbamoyl phosphate synthase synthase one when it forms carbamoyl phosphate it is actually targeting the nitrogenous waste towards formation of urea because when this urea is formed it is actually excreted by the kidneys alright.

So this is the crux of the ammonia metabolism where it is basically showing the same thing in a different form where ultimately via trans deamination for transamination we are getting glutamate, glutamate dehydrogenase, oxidative deionization we are getting ammonia it can also be obtained from multiple minor sources for example glutamine if glutaminase is acting in the kidney it can be directly restricted into urine but in other cases it can either be utilized again in the formation of glutamine or it can be I mean accepted or it can be produced from glutamine by the action of glutaminase and ultimately it goes to the urea liver where it undergoes urea cycle in production of urea and ultimately it is in the form of urea where it is excreted in urine because higher animals are actually ureotelic mammals are ureotelic we excrete urea birds are uricotelic and fishes are ammonotelic they can directly excrete ammonia from the system alright. So now finally we are into hyperammonemia a condition where there is excess amount of ammonia. So generally it does not happen why it does not happen because hepatic urea cycle generally that is the amount of utilization or conversion of ammonia to urea it exceeds the amount of ammonia production. So generally the when the excretion is more than the production naturally the value or the amount of serum ammonia is usually very very low 5 to 50 but if due to any reason the liver function is compromised liver cannot act there is some problem in the liver then suddenly the ammonia level will go very high it can rise above 1000 micro mole per liter and this will lead to a lot of problem this is basically medical emergency patient will become unconscious they will faint they will be tremor they will be slurring of speech they will be patient will appear sleepy but actually is not sleeping patient will feel a large vomiting they will be cerebral edema raise interventional tension patient cannot see. So these are all the symptoms of ammonia toxicity and ultimately if it is not treated it can lead to coma and death.

So generally two major types of hyperammonemia that is a condition where there is high ammonia have been identified number one is acquired hyperammonemia that is normally I am walking talking fine suddenly I develop hyperammonemia it can happen it means one day or someday due to some reason there I have got a liver problem in the form of hepatitis virus in the form of ischemia there is a loss of blood supply to the liver there is some toxin ok some drug poison that is affecting my liver for example a very common example that is 50 tablets of paracetamol the simple calpol branded or pyrecytic tablet that we take for fever if 50 tablets of paracetamol are taken believe it or not the liver will invariably feel and the patient will die well this is a very dangerous thing to note because we immediately need to provide an antidote that is in the form of N-acetylcysteine but that is beyond the discussion of this class that is a toxicology class anyway so I am just telling you liver can be act upon by multiple toxin the most common toxin in developing country and also developed country is alcohol chronic alcohol can lead to liver failure in the form of liver cirrhosis and if liver cirrhosis is there the problem is liver circulation is hampered liver circulation is also known as portal circulation there

is a blood supply chain inside the liver if it gets hampered what will happen ultimately this portal blood containing all the toxic ammonia will be directly shunted into the blood stream and that is when the amount of ammonia will rise and all the symptoms of ammonia hyperammonia will be evident and this will lead to a situation where detoxification of ammonia is severely hampered right next cause being hereditary hyperammonia means children or baby boys born with problems in ammonia metabolism and this is mainly due to defects in enzyme in relation to the urea cycle which will be detecting I mean studying later when we are discussing about urea cycle and its enzyme defect. So now let us understand why ammonia is toxic to the brain so this is the last part of our discussion where we will be actually learning why why we are so much cautious why you are so much afraid and what problems does ammonia create to our brain well it is very easy to understand if you have the basic concept of biochemistically or you see we have seen that ammonia reacts with alpha ketoglutarate to form glutamate right via the enzyme glutamate dehydrogenase. So if there is excess amount of ammonia that will produce excess glutamate and that will lead to depletion of alpha ketoglutarate alpha ketoglutarate is an important intermediate of TCA cycle if this happens alpha ketoglutarate level will go down and there will be depletion of energy in the neuron TCA cycle will be hampered and there will be depletion of neuronal energy and neuronal metabolism will be severely hampered there will be no ATP production neuron and no neuronal signaling. Next mechanism there are multiple mechanism next glutamate when it is excess it will be used in the formation of glutamine right. So what will happen we already know glutamate is deviated towards glutamine because it is a major mechanism of trapping of ammonia.

So ultimately what will happen the amount of glutamate will decrease glutamate is actually utilized in the formation of one neurotransmitter that is gamma aminobutyric acid all right. So if glutamate is depleted the inhibitory neurotransmitter GABA is depleted GABA actually suppresses the brain action it prevents brain from behaving erratically. So if there is a loss of negative neurotransmitter there will be excitatory activity and pressure will develop tremors flying of speech seizure all those problems all right. So within a situation where glutamine is excess well if glutamine is excess it creates another problem glutamine actually comes out of the brain ok. So it is a brain cell neuronal cell where glutamine will come out and it comes out of an exchange transporter where the amino acid tryptophan comes in so glutamate comes out and tryptophan comes in.

So the now there is a situation when there is excess tryptophan in the brain and tryptophan excess leads to the production of serotonin which is an excitatory neurotransmitter. So in the last mechanism we read there is an inhibition of inhibitory neurotransmitter that is depletion of GABA and here we are seeing there is

overproduction of serotonin. So both will act together to fire the neuron in such a way that there will be excess tremors seizure and all the activities flying of speech. And another problem with glutamine is glutamine is actually a major part in the astrocyte ok because all the brain glutamine is are present in the neuron cells where glutamine synthetase is actually present in the astrocyte. So there is there is more ammonia there will be more and more glutamine formation in the astrocyte.

Glutamine is actually osmotically active so neuronal cell it is a neuronal cell it will imbibe water molecule and ultimately it will lead to swelling of the neurons. I already told in the previous class when there is a swelling in the brain it will exert pressure because the brain cannot expand that means the hard skull and there will be raised intracranial tension which will lead to unconsciousness and coma. Patient will lose consciousness and if not treated patient will die. Last mechanism is ammonia has been seen to I mean is believed to activate the NMDA receptor N methyl diaspartate receptor which actually increases nitric acid NO formation. What is NO does? It actually reduces the activity of glutamine synthetase thus reducing the ammonia from the brain because glutamine as we read was one of the mechanism by which ammonia was being removed from the brain.

But if there is less production of glutamine more and more ammonia will be accommodating and it will be more and more toxic to the brain. So to conclude in today's class we have learnt what are the sources of ammonia, how ammonia is formed in the body, how it is transported for excretion, what are the various reaction that utilizes ammonia and ultimately we have also read what are the mechanism of ammonia toxicity. These are the references in which you can use for further reading and I thank you for your attention. See you in the next class.