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WAYS OF NEUTRALIZING AMMONIA IN THE HUMAN BODY, URINARY SYNTHESIS. ORNITHINE CYCLE

[1]Ayyubova Sevinch Murodjon kizi, [2]Hakimova Fayyoza Jahongir kizi, [3]Mukhamedova Sevara Nigmatulla kizi

[1]The student of Tashkent Pediatric Medical Institute, Department of Biochemistry
[2]The student of Tashkent Pediatric Medical Institute, Department of Biochemistry
[3]Research advisor, The assistant of Tashkent Pediatric Medical Institute, Department of Biochemistry

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Abstract*. Ammonia is a key metabolite derived from amino acid metabolism and breakdown of nitrogenous substances in the gut,1,2 and present hypotheses suggest that it is of central importance in the pathogenesis of hepatic encephalopathy. Current therapeutic strategies are directed towards reducing circulating concentrations of ammonia. The two main pathways of ammonia detoxification are synthesis of urea and glutamine. In the physiological state, the liver is central in urea synthesis and this forms the basis of ammonia removal.3 The other organs capable of metabolising ammonia are the brain and skeletal muscle through synthesis of glutamine. In the presence of liver disease however, loss of functional liver mass and portocaval shunting may contribute to increased ammonia levels in plasma, thus predisposing to hepatic encephalopathy. In cirrhotic patients, skeletal muscles play an important role in the detoxification of ammonia to glutamine.4 Glutamine synthesis is however only a temporary method of ammonia detoxification. To remove ammonia from the body in a situation when urea synthesis is compromised (as happens in liver disease) requires transport of glutamine to the kidney, where it is metabolised to release ammonia, which is then excreted in urine.*

Keywords*: ammonia, metabolism, transport of glutamine, liver, kidney, neurohormones, ATP, Urea Cycle.*

Relevance.

Ammonia is a toxic product of nitrogen metabolism which should be removed from our body. The urea cycle or ornithine cycle converts excess ammonia into urea in the mitochondria of liver cells. The urea forms, then enters the blood stream, is filtered by the kidneys and is ultimately excreted in the urine.The overall reaction for urea formation from ammonia is as follows:

Ammonia + CO[2] + 3ATP ---> urea + water + 3 ADP

Steps in the Urea Cycle

The urea cycle is a series of five reactions catalyzed by several key enzymes. The first two steps in the cycle take place in the mitochondrial matrix and the rest of the steps take place in the cytosol. Thus the urea cycle spans two cellular compartments of the liver cell.In the first step of the Krebs-Henseleit cycle, ammonia produced in the mitochondria is converted to carbamoyl phosphate by an enzyme called carbamoyl phosphate synthetase I. The reaction can be given as follows:

NH[3] + CO[2] + 2ATP → carbamoyl phosphate + 2ADP + Pi

The second step involves the transfer of a carbamoyl group from carbamoyl phosphate to ornithine to form citrulline. This step is catalyzed by the enzyme ornithine transcarbamoylase (OTC) . The reaction is given as follows:

Carbamoyl phosphate + ornithine → citrulline + Pi

Citrulline thus formed is released into the cytosol for use in the rest of the steps of the cycle.

The third step is catalyzed by an enzyme called argininosuccinate synthetase, which uses citrulline and ATP to form a citrullyl-AMP intermediate, which reacts with an amino group from aspartate to produce argininosuccinate. This reaction can be given as follows:

Citrulline + ATP + aspartate → argininosuccinate + AMP + PPi

The fourth step involves the cleavage of argininosuccinate to form fumarate and arginine. Argininosuccinate lyase is the enzyme catalyzing this reaction, which can be represented as follows:

Argininosuccinate → arginine + fumarate

In the fifth and last step of the urea cycle, arginine is hydrolyzed to form urea and ornithine. This is catalyzed by arginase and can be given as follows:

Arginine → urea + ornithine

The overall reaction can be given as follows:

2NH[3]+ CO[2]+ 3ATP g urea + 2ADP + AMP + PPi + 2Pi

Significance of the Urea Cycle

The main purpose of the urea cycle is to eliminate toxic ammonia from the body. About 10 to 20 g of ammonia is removed from the body of a healthy adult every day. A dysfunctional urea cycle would mean excess amount of ammonia in the body, which can lead to hyperammonemia and related diseases. The deficiency of one or more of the key enzymes catalyzing various reactions in the urea cycle can cause disorders related to the cycle. Defects in the urea cycle can cause vomiting, coma and convulsions in new born babies. This is often misdiagnosed as septicemia and treated with antibiotics in vain. Even 1mm of excess ammonia can cause severe and irreversible damages.

Materials and methods of research

A blood aminogram is routinely used in the diagnosis of urea cycle disorders. The concentration of the nitrogen-carrying amino acids, glutamine and alanine, in plasma is elevated in the case of OTC deficiency. In babies, elevated levels of orotic acid in the urine may be an indicator of OTC deficiency. Increased levels of blood citrulline and argininosuccinate are also seen in cases of citrullinemia.In older children, these disorders may present in the form of growth failure, psychomotor retardation and behavioral abnormalities. Hence, blood ammonia and urinary orotic acid monitoring and quantitation are crucial in patients with unexplained neurological symptoms.

Liver cirrhosis is characterised by progressive changes in systemic and renal haemodynamics. In the initial compensated state, patients have splanchnic vasodilatation and increased cardiac output but no clinical signs of fluid retention. During the decompensated phase, there is sodium retention, oedema, and ascites. These changes are accompanied by alterations in renal haemodynamics and the ability of the kidneys to excrete water and solute load, resulting in disturbances of sodium, potassium, and acid-base homeostasis. Such renal derangements are likely to have an impact on other excretory functions of the kidney such as excretion of ammonia.

In the physiological state, approximately 70% of ammonia generated by the kidney is secreted into the renal vein and 30% excreted in urine.5 This ratio is reversed during hyperammonaemia.6 Renal handling of ammonia is also altered during metabolic acidosis and hypokalaemia.7–9 Other potent stimuli modulating ammonia synthesis, transport, and excretion

by the kidneys include renal blood flow, acid-base status of the tubular cell and pH of the tubular lumen, luminal flow rate, and plasma angiotensin II (ANG II) levels.10,11

We have previously shown in well compensated patients with cirrhosis that intravenous infusion of saline over one hour results in a reduction in plasma renin activity (PRA) and ANG II.12 Although patients with cirrhosis manifest the most significant disturbance in ammonia metabolism in the decompensated state, it is difficult to determine the contribution of the kidneys and in particular neurohormones such as ANG II to this disturbed metabolism because most patients are receiving treatment with diuretics and the ethical problems with saline infusion. Therefore, we chose to study patients with well compensated cirrhosis. The present study was performed to test the hypothesis that volume expansion would result in suppression of ANG II levels and result in enhanced urinary excretion of ammonia.

Research results and discussion.

Studies were undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki (1989) of the World Medical Association and after obtaining written informed consent from each patient.

Patients Sixteen well compensated patients with biopsy proven cirrhosis were studied after an overnight fast and the studies were started between 0700 h and 0800 h. Ten of 16 patients had a functioning transjugular intrahepatic portosystemic shunt stent (TIPS) in situ, which had been placed for variceal haemorrhage 6 (±1.2) months prior to enrolment into the current study. Shunt patency and efficacy was established by portography, with all 10 patients showing normal portal pressure gradients (8.1 (0.8) mm Hg). Patients with alcoholic liver disease were required to be abstinent from alcohol for at least one month prior to enrolment in the study. Patients were excluded if they had ascites (demonstrable on ultrasonography), pitting peripheral oedema, hepatic encephalopathy, clinically evident cardiovascular disease, renal dysfunction (decreased renal size on ultrasonography, proteinuria, or creatinine concentration greater than 100 μmol/l), or were receiving any diuretics or vasoactive medications. Patients were maintained on a diet with restriction of sodium intake to 150 mmol per day for one week prior to the study. Patients were maintained in a supine posture throughout the study, adopting the erect posture only for voiding. Prior to the study, two venous cannulae were inserted, one in each arm (one for infusions and the other for sampling). Blood pressure (Dynamap, Critikon, Australia) and pulse were measured at 30 minute intervals throughout the study. Primed continuous infusion of inulin (Inutest, 25%; Laevosan-Gesellschaft, Linz, Austria) and para-amino hippuric acid (PAH) (Merck Sharpe and Dohme, Sydney, Australia) were started as indicated and continued throughout the study. After two hours of PAH and inulin infusion, 1 litre of sodium chloride was infused over a one hour period. The timing of the infusions and various samples during the study are outlined.

Study protocol. ANG II, angiotensin II; PRA, plasma renin activity; PAH, para-amino hippuric acid.

Healthy volunteers Plasma was collected from 27 healthy volunteers with a mean age of 49 (SEM 4.1) years (16 males and 11 females) for measurement of PRA and ANG II.

Blood was collected from a peripheral vein into pre-cooled tubes. Plasma was separated and samples stored at −70°C for analysis at a later date. Inulin concentration was measured using spectrophotometry, and PAH using high performance liquid chromatography.12,13

Measurement of plasma renin activity Radioimmunoassay for measurement of PRA was based on the principle that angiotensin I is generated by the action of renin on its substrate

angiotensinogen. An inhouse antibody for angiotensin I was used. The coefficient of variation for the assay was 5.2%.13

Measurement of angiotensin II Samples of blood were obtained in ANG II inhibitor. ANG II values were measured by radioimmunoassay with an inhouse rabbit antibody R6B4. The coefficient of variation for the assay was 3.2%.12

Measurement of plasma and urinary ammonia Blood samples were collected on ice from a cannula inserted into a peripheral vein. Plasma was obtained by centrifugation and deproteinised with trichloroacetic acid (50% w/v) for ammonia determination and stored at −80°C for spectrophotometric determination of ammonia (CobasMiraS, Hoffman-LaRoche, Switzerland).14 Urine was collected in a pre- cooled bottle which was maintained acidified with 2 ml of 6.0 N HCl.14 The coefficient of variation for all determinations was <4%.

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