**Hyperammonemia**

In healthy adults consuming average amounts of protein the degradation of amino acids results in formation of approximately 20 g ammonia daily. The majority (90%) of the waste N is eliminated in the form of urea and only 5% of the nitrogen in the urine is in free ammonia (free amino acids, creatinine, uric acid represent additional minor forms of disposed N). The ornithine cycle that synthesizes urea maintains the ammonia concentration in the range of 10-20 μM in the systemic blood. This function is essential to prevent the toxic effects of ammonia in the brain which develop at blood concentrations of ammonia higher than 50 μM. Since the complete set of enzymes of the ornithine cycle is found only in the liver, the most common cause of hyperammonemia is the liver failure, but in view of the multiple biochemical functions of the liver this symptom is only one of the aspects of the complex metabolic disturbance observed in liver disease, which will be discussed at a later stage of the biochemistry course. The discussion in the present seminar will be limited to isolated forms of hyperammonemia resulting from enzyme defects in the ornithine cycle. After this seminar the participant should be able:

1) To appreciate the relative contribution of various physiologically relevant sources of ammonia for the ornithine cycle
2) To interpret the physiologically relevant regulatory mechanisms in the ornithine cycle
3) To explain the degree of hyperammonemia and the specific metabolic abnormalities observed in various enzyme defects of the ornithine cycle
4) To interpret the toxic effects of ammonia
5) To understand the metabolic background of various therapeutic strategies applied in hyperammonemia.

**Background**

1) **Sources of ammonia**
   The sources providing ammonia for the ornithine cycle are potential targets of medical intervention in the treatment of hyperammonemia. Thus, it is essential to consider their relative contribution:
   - 35% of the ammonia incorporated in urea comes from free ammonia in the portal blood (including the 25% recycled ammonia derived from the degradation of urea by bacterial urease in the large intestine);
   - 10% comes from degradation of portal glutamine;
   - 20% is the product of the mitochondrial glutamate dehydrogenase (in transamination the amino group of many amino acids is transferred to α-ketoglutarate and the formed glutamate is the immediate donor of ammonia for the ornithine cycle);
   - 35% originates from deamidation of glutamine and asparagine, direct deamination of amino acids (threonine, glycine, serine, histidine, methionine) or indirect deamination of aspartate in the purine nucleotide cycle in the liver.

   In the intestine the neutral NH₃ is absorbed, but the positively charged NH₄ is not. Thus, a decrease in the pH restricts the absorption of ammonia.

   Glutamine represents 25% of the total amino acids circulating in systemic blood. Its source is primarily the skeletal muscle and this overwhelming abundance is based on the routes of amino acid metabolism specific for the muscle. The figure below helps the understanding of the preferential formation of glutamine in muscle.
Degradation of branched chain amino acids and glutamine synthesis in skeletal muscle.

Purine nucleotide cycle as a source of ammonia for glutamine synthesis in muscle.

2) Regulation of the ornithine cycle
The reaction catalyzed by carbamoyl-phosphate synthetase I (CPSI) is rate limiting in the ornithine cycle. The relevance of its regulation can be interpreted in physiological terms if the role of its two substrates is considered.

i) To prevent the toxic effects of ammonia increased CPSI activity is necessary both after a meal (because of intensive degradation of newly absorbed amino acids) and in long-term fasting (when muscle proteins are degraded to provide substrates for gluconeogenesis). Two key mechanisms can be distinguished in the acute regulation of CPSI: allosteric control by N-acetyl glutamate and covalent modification through acetylation/deacetylation. The mitochondrial level of N-acetyl glutamate depends on the relative rates of its synthesis and export to the cytosol. The synthesis is activated by arginine acting as allosteric activator of N-acetyl glutamate synthase, whereas the
cytosolic efflux is inhibited in the presence of glucagon. Thus, when following a meal the extrahepatic arginine synthesis (see figure below) supplies more arginine or when in fasting the glucagon levels are high, the increase in the mitochondrial concentration of the allosteric activator of CPSI maintains its adequate activity.

Following absorption several amino acids (glutamine, glutamate, prolin) are converted to arginine, thus raising its blood concentration.

The figure below shows the NAD-dependent deacylation reaction catalyzed by sirtuins (SIRT), which helps the interpretation of the covalent modification of CPSI.

The figure below summarizes a recently described mechanism for CPSI activation through a SIRT5-catalyzed deacetylation, the rate of which depends on the NAD availability (NAD concentrations rise after a meal because of dietary tryptophan load and in long-term fasting because of induction of Nampt, nicotinamide phosphoribosyltransferase (Nakagawa T, et al, Cell 2009; 137:560).
ii) Bicarbonate is not only a substrate for CPSI, but also a component of the blood buffer system. In acidosis lower CPSI activity supports the compensation of the pH, because bicarbonate is conserved to raise the buffering capacity of blood. The suppressed rate of urea synthesis in acidosis is based on the following mechanisms:
- slower transport of amino acids into hepatocytes at lower pH
- decreased activity of liver glutaminase at lower pH
- the neutral NH$_3$ and not the positively charged NH$_4^+$ is the substrate of CPSI
- the Km value of N-acetyl glutamate synthase for glutamate increases at lower pH
- the activity of mitochondrial carbonic acid anhydrase decreases at lower pH.

These mechanisms result in retention of bicarbonate in acidosis, but at the same time they necessitate alternative routes for elimination of ammonia. In acidosis liver is turned from glutamine consumer to net glutamine producer. For the interpretation of this function the zonal metabolic differentiation of hepatocytes should be considered, which is illustrated in the figure below.

The glutamine formed under such conditions is degraded in the kidney and these reactions also contribute to the compensation of acidosis according to the scheme shown below.


3) Additional specific symptoms in various enzyme defects of the ornithine cycle
Hyperammonemia develops in the case of deficiency of any enzyme of the ornithine cycle, but its severity depends on the site of the defect. The most pronounced hyperammonemia is observed in deficiency of the mitochondrial enzymes, because in this state the reactions that directly use ammonia are affected. If the defect is in the cytosolic branch of the process, ammonia can be incorporated in citrulline that is excreted in the urine. However this form of excretion results in loss of C-atoms too, which limits the N-elimination capacity (hyperammonemia develops, but it is milder). Additional laboratory findings come from the accumulation of intermediates from reactions preceding the site of the defect in the ornithine cycle with most expressed elevation in the concentration of the substrate of the affected enzyme (e.g. citrulline in argininosuccinate synthetase deficiency). The ornithine transcarbamoylase deficiency deserves special attention, because following accumulation in the mitochondria carbamoyl phosphate, the substrate of the deficient enzyme leaks into the cytosol, where bypassing the rate limiting step it enters the pyrimidine nucleotide synthetic pathway. Thus, orotic acid appears in the urine of these patients (for interpretation of this symptom see the chapter on pyrimidine metabolism in the recommended textbook). In the case of deficiency of the citrulline/ornithine transporter the HHH syndrome develops (hyperornithinemia, hyperammonemia, homocitrullinemia), because in this state the ornithine transcarbamoylase uses lysine instead of ornithine producing homocitrulline.

4) Neurotoxicity of ammonia
Most of the toxic effects of ammonia in the central nervous system are related to disturbances in the normal glutamate/glutamine metabolic cycle operating between astrocytes and neurons. Glutamate is the most abundant excitatory neurotransmitter in brain and this cycle maintains the normal glutamate levels in the presynaptic neurons. At the same time it serves as a shuttle to transfer the 4C-atom intermediates of the citric acid cycle produced by pyruvate carboxylase in the astrocytes for utilization in the neurons that do not express this enzyme. The physiological pathway of glutamate/glutamine interconversion is illustrated in the figure below.
In hyperammonemia the neural glutaminase is inhibited and the glutamine synthesis is enhanced. The outcome is accumulation of glutamine in the astrocyte and osmotic swelling.


The membrane permeability of the swollen astrocytes increases and consequently the ion gradients on the two sides of the plasma membrane are dissipated resulting in decreased glutamate uptake. The extracellular accumulation of glutamate initiates neurotoxic effects through sustained activation of glutamate receptors. Further aspects of the excitotoxicity of glutamate will be discussed at a later stage of the biochemistry course.
Hypermmonemia results in energy deficit in brain which can be traced back to mitochondrial dysfunction (inhibition of $\alpha$-ketoglutarate dehydrogenase, opening of the mitochondrial permeability transition pore) (Felipo V, Butterworth RF, Neurochem Intern 2002; 40: 487).

5) Basic principles for treatment of hyperammonemia
The following metabolic principles guide the therapeutic management of hyperammonemia
1. Reduction of ammonia generation
   - low-protein diet with high relative content of essential amino acids
   - oral administration of lactulose (its fermentation in the intestine reduces the pH and thus the absorption of ammonia)
   - administration of non-absorbing antibiotics (neomycin) that decrease the bacterial degradation of urea in the intestine
2. Enhancement of the elimination of ammonia
   - hemofiltration, hemodialysis, peritoneal dialysis
   - administration of amino acid conjugating agents (benzoate, phenylacetate, phenylbutyrate), the action of which is illustrated in the scheme below (which amino acids could be the source of the N-atoms in the conjugating glycine and glutamine?)
   - supplementation of the diet with intermediates that can enter the intact part of the ornithine cycle and drive the N-excretion in the form of alternative end-products with much higher C/N atom ratio than urea (e.g. administration of citrulline in CPSI deficiency or supplementation with arginine in argininosuccinate synthatase deficiency)
   - administration of branched-chain $\alpha$-keto acids
3. Acute symptomatic treatment: osmotically active agents (mannitol) for reduction of brain edema.
Recommended literature


Rose C. Effect of ammonia on astrocytic glutamate uptake/release mechanisms. J Neurochem 2006; 97 (Suppl. 1): 11–15


Clinical case

Hereditary hyperammonemia

A 6-month-old infant began to vomit occasionally and ceased to gain weight. At age 8.5 months he was readmitted to the hospital. Routine examination and laboratory tests were normal, but after 1 week he became habitually drowsy, his temperature rose to 39-41 °C, his pulse was elevated, and his liver was enlarged. The electroencephalogram was grossly abnormal. Since the infant could not retain milk given by gavage feeding, intravenous glucose was administered. He improved rapidly and came out of the coma in 24 h. Analysis of his urine showed abnormally high amounts of glutamine and orotic acid, which suggested a high blood ammonium concentration. This was confirmed by the laboratory.

Biochemical questions
1. Hereditary hyperammonemia can result from defects in genes of ornithine cycle enzymes. Which enzymes might be affected?
2. Considering the data, which enzyme may be defective in this patient?
3. Why was the urine glutamine concentration elevated?
4. Why is orotic acid excretion increased in the patient described?