HYPERAMMONEMIA

Background information

Ammonium ion metabolism

Virtually all tissues produce ammonia, which is present predominantly as ammonium ions. These ammonium ions arise primarily from the catabolism of amino acids. Ordinarily the ammonium ion concentration in the peripheral blood is maintained at a very low level. Normal concentrations of ammonium ions in plasma are 25-50 μmol/L. Much higher levels are often found in severe hepatic disease. Regardless of their source, ammonium ions (as ammonia) are exceedingly toxic to the central nervous system; consequently, they must be detoxified and eliminated.

What is the role of gut in the metabolism of ammonium ions?
Is ammonia produced by the muscles as well? What is the mechanism of ammonia production in the muscles? Is there any connection between the intensity of physical exercise and muscle ammonia production?

Detoxification in the brain

The brain detoxifies ammonium ions by converting them to glutamine. The brain is a rich source of glutamine synthetase.

Which type of cells has the highest glutamine synthetase activity in the brain?

Other tissues also contain glutamine synthetase, and the high levels of glutamine found in the blood after ingestion of foods rich in protein may represent a storage and transport form of ammonia. Much of the circulating glutamine is eventually hydrolyzed to glutamate and ammonium ions by a glutaminase in the kidney.

What is the importance of the glutaminase reaction in the kidney?
What is the localization of glutaminase in the kidney?
How is glutaminase regulated in the kidney?

Transport of ammonium ions to the liver as glutamine

Alternatively the glutamine of the blood may be hydrolyzed by liver glutaminase to yield ammonium ions which can be used by that organ for urea synthesis. Plasma concentration of glutamine is almost twice that of any other single amino acid (approx. 0.5-0.7 mmol/L).

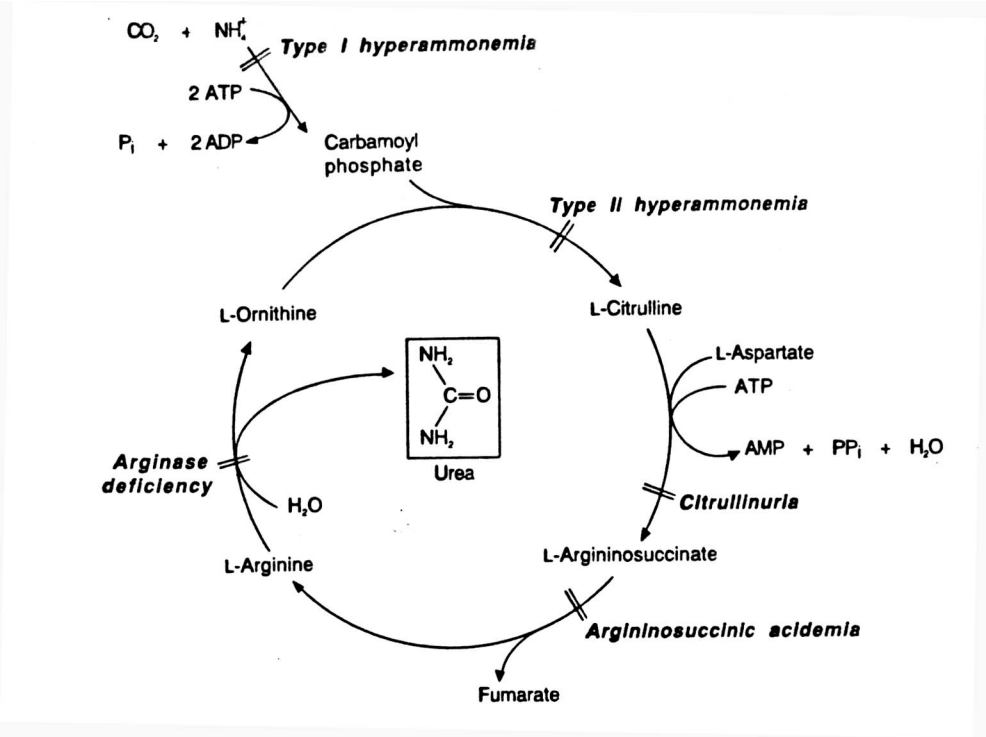
Detoxification in the liver

Most ammonium ions in the portal blood are detoxified in the liver. There they are converted to urea, a form of nitrogen much less toxic for the central nervous system. If the urea-synthesizing system should fail as a result of a malfunctioning liver or because of portal obstruction, ammonium ions would

pass into the systemic circulation and ammonia intoxication results. Ammonia intoxication produces blurred vision, tremors, slurred speech, and ultimately coma and death. However, this condition, called hepatic coma, is complex and may be precipitated by many other factors.

Inherited disease of the urea cycle

Hyperammonemia is often associated with inherited abnormalities of urea cycle enzymes. Fig. 1. shows the location in the urea cycle of the reactions associated with each of these diseases.



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Case 1.

Mary M., aged 9 months, had developed normally until she was weaned at 6 months. She was put on a mixed diet and promptly became irritable and less alert and she began to vomit. She was taken to hospital where she had episodes of screaming, listlessness and ataxia and failed to recognize her parents. Her

condition deteriorated rapidly, her lethargy often progressing to coma, especially after a protein meal. Her head circumference was small for her height and her liver was enlarged.
Liver function tests showed slightly raised serum transaminase, indicating minor liver damage. The urine was persistently neutral or alkaline, which proved to be due to excessive NH_3 excretion. The presence of glutamine in the urine led to the finding of raised serum levels of that amino acid. The blood NH4+ was extremely high (Table I.) but fell to normal levels when the protein intake was reduced. Analysis of her urine showed reduced amount of urea.
When Mary was put on a low-protein diet, her condition improved. The liver regressed to its normal size, the serum transaminase level returned to normal, and Mary recognized her parents once again.
Because an abnormality of the urea cycle enzymes was suspected, a biopsy specimen of liver was taken at laparotomy. Shortly afterwards Mary's condition worsened, with vomiting, coma and convulsions, and excessive amounts of NH4+ in the blood. She died a few days later. All enzymes of the urea cycle were determined and found to be present at the normal levels, except ornithine transcarbamylase, which could not be detected.
Table I.

Metabolite	Patient	Normal range
Ammonia μmol/L	290-700	25-40
Glutamine mmol/L	1.9	0.55-0.70

Why did the patient's condition did not manifest itself until she was weaned?

Case 2 Hereditary hyperammonemia
A 6-month-old infant began to vomit occasionally and ceased to gain weight. At age 8.5 mo he was readmitted to the hospital. Routine examination and laboratory tests were normal, but after l wk 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 hr. Analysis of his urine showed abnormally high amounts of glutamine and uracil, which suggested a high blood ammonium concentration. This was confirmed by the laboratory.

Biochemical questions
1. Hereditary hyperammonemia can result from defects in genes for urea cycle enzymes. Which enzymes might be affected?
2. Considering the data, which enzyme may be defective in this patient?
Explain your rationale.
3. Why was the urine glutamine concentration elevated?

4. Why is orotic acid excretion increased in the patient described?
5. Offer a genetic explanation for the observation that this disease is usually lethal in males but not in affected females.
6. What is the significance of the observation that the mother of the patient has an aversion to eating meat?
7. This patient was treated using procedures available at the time (see Goldstein AS et al: Pediatr Res 8:5, 1974). He was given a daily diet of 1.5 g of protein/kg body weight. After 2 years on this diet, his height and weight were judged to be normal for his age. What is the effect of diet on a growing child in terms of nitrogen balance?
8. How would you treat a similar patient today?

References

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Case 3 Defective urea cycle

A newborn infant was found to have hyperammonemia with respiratory alkalosis. An inherited defect in the urea cycle or transient hyperammonemia was suspected.

Biochemical questions

1. Plasma citrulline levels were checked and found to be 5 μmol/L; normal is 50 μmol/L. This indicated a defect in the urea cycle rather than transient hyperammonemia. It also ruled out the involvement of certain urea cycle enzymes. Which ones were ruled out?
2. Urinary orotate concentration was measured and found to be normal. This eliminates the possibility that another urea cycle enzyme was defective. Which one is ruled out and why?
3. The patient was treated with sodium benzoate and sodium phenylacetate. What is the rationale for this treatment?

4. Extracts of a liver biopsy were assayed for N-acetylglutamate synthase. No activity was found. Is this enzyme cytosolic or mitochondrial? What relationship does this enzyme have to the urea cycle?
5. With this information at hand, the patient was maintained on a diet that contained arginine and carbamoylglutamate, 2 g/kg/day. What reasoning is behind the use of each of these substances?

References

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Additional questions

1. What is the origin of the nitrogen atoms of urea?
2. The keto forms of valine, isoleucine, and leucine were administered to a patient with hyperammonemia. Explain why the plasma ammonia level fell twofold.
3. What metabolite or substrate will accumulate in each of the urea cycle enzymopathies? Based on this information propose a series of tests that could be used to differentially diagnose each of the possible enzyme defects.
4. Provide a biochemical explanation for the clinical finding that a deficiency of arginase is less severe than a deficiency of any of four other enzymes of the urea cycle?
5. A deficiency in AL (argininosuccinate lyase) can be treated effectively by dietary restriction and arginine therapy. Explain why this therapeutic regime is not sufficient for the management of patient described in the case study.
6. Describe the possible effects on the metabolism of valine, isoleucine, threonine, and methionine in an infant who is breast-fed by a strict vegetarian mother. (Hint: see Figure below or Heaton D: N Engl J Med 300:202, 1979.

Discussion of case 2.

l. Defective enzymes of the urea cycle.

Hyperammonemia in a newborn or very young infant is the characteristic sign of an inherited defect in a gene for an urea cycle enzyme. Genetic diseases have been found associated with deficiencies in all the urea cycle enzymes, but the most frequent abnormality is in ornithine transcarbamoylase, a mitochondrial enzyme encoded by a nuclear gene on the X chromosome.

2. Enzyme defect

Other clinical signs of urea cycle diseases are a low to normal blood urea nitrogen (BUN), lethargy, irritability, hypotonia, and later, convulsions and coma. Death may quickly follow if the patient is left untreated. The enzyme affected in this patient is ornithine transcarbamoylase because of the excretion of uracil. Excessive excretion of uracil, or its precursor orotic acid, results from an accumulation of carbamoyl phosphate in the mitochondria. In the absence of omithine transcarbamoylase, carbamoyl phosphate accumulates and leaks out into the cytoplasm, where it can be used to make carbamoyl aspartic acid, the first intermediate in the pathway to pyrimidine synthesis.

This case is unusual in that the symptoms took so long to appear. In most patients, extracts made from needle biopsies of the liver reveal a total lack of ornithine transcarbamoylase; however, in a few males (DiMagno et al, 1986), and in many females, enzyme activity can range from 0% to 30% of normal in males and even higher in females. The disease is described as X-linked dominant because most females are somewhat affected. Females usually respond well to dietary treatment.

3. Glutamine excretion

Urine glutamine concentration increases because it exceeds the kidney's ability to hydrolyze glutamine to glutamate and ammonia. Glutamine is another carrier for ammonium ion in the blood. It is excreted in compensation for the inoperative urea cycle.

4. Inheritance

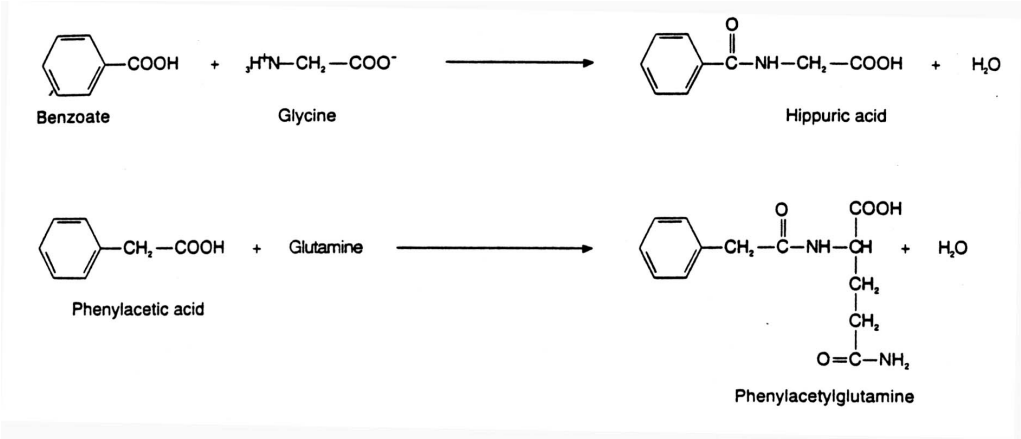
Because the disease is X linked and males have only one X chromosome and females have two, one would expect that the disease would be much more severe in males than in females. Unfavorable Lyonization however, can produce female patients that are almost as severely affected as males.

5. Nitrogen balance

An adult, who is not growing, requires comparatively little dietary protein to maintain good health. A growing child, however, requires considerably more. The load on the urea cycle is directly proportional to the amount of protein in the diet. Consequently, the growing child of 0.5 to 1 yr of age must have 2 g of high-quality protein/kg/day to maintain good growth, that is, to stay in positive nitrogen balance. In this case the patient was given somewhat less protein to ensure that large amounts of ammonium ions would not accumulate. For this patient the low-protein diet prevented ammonia intoxication but allowed normal growth. This treatment is not sufficient for most patients with a defect in the gene for ornithine transcarbamoylase.

6. Modern treatment

Therapy today involves hemodialysis and transfusion as soon as possible to avoid the irreversible brain damage that can result if the plasma ammonium concentration remains high for a long period. This is followed by intravenous treatment with sodium benzoate and phenylacetate (phenylbutyrate orally). These substances act to trap ammonium ions, as glycine and glutamine, and convert them to forms that are readily excreted by the kidney. The enzymatic reactions involved are:



Both glycine and glutamine are nonessential amino acids that can be synthesized from non-protein metabolites. Glycine eliminates one nitrogen molecule per mole, but glutamine disposes of two per mole. These two substances have proved to be very useful in the treatment of hyperammonemia. Long-term treatment involves a low-protein diet with arginine or citrulline supplementation. In patients with a defective urea cycle, arginine becomes an essential amino acid required in larger amounts than normal.