Amino acid metabolism II.
Urea cycle
Key points

- AA catabolism generates urea – nontoxic carrier of nitrogen atom.
- Urea synthesis occur in the liver. The amino acids alanine and glutamine carry AA nitrogen from peripheral tissues in the liver.
- Key enzyme involved in nitrogen disposal are transaminases, glutamate dehydrogenase, and glutaminase.
- The urea cycle consist of four steps and incorporates a nitrogen from ammonia and one from aspartate into urea.
- Disorders of urea cycle leads to hyperammonemia condition toxic to nervous system and development.
The amount of nitrogen ingested is balanced by the excretion of an equivalent amount of nitrogen. About 80% of excreted nitrogen is in the form of urea.
Excretory forms of nitrogen

a) Excess NH$_4^+$ is excreted as ammonia (microbes, aquatic vertebrates or larvae of amphibia),
b) Urea (many terrestrial vertebrates)
c) or uric acid (birds and terrestrial reptiles)
Ammonia has to be eliminated

- Ammonia is toxic, especially for the CNS, because it reacts with $\alpha$-ketoglutarate, thus making it limiting for the TCA cycle $\Rightarrow$ decrease in the ATP level.

- Liver damage or metabolic disorders associated with elevated ammonia can lead to tremor, slurred speech, blurred vision, coma, and death.

- Normal conc. of ammonia in blood: 30-60 $\mu$M/L
Sources of NH$_4^+$ for urea cycle

- Ammonia originates in the catabolism of amino acids that are primarily produced by the degradation of proteins – dietary as well as existing within the cell:
  - digestive enzymes
  - proteins released by digestion of cells sloughed-off the walls of the GIT
  - muscle proteins
  - hemoglobin
  - intracellular proteins (damaged, unnecessary)
Overview of amino acid catabolism in mammals

2 CHOICES
1. Reuse
2. Urea cycle
Overview of amino acid catabolism. Interorgan relationships

- **Intestine**
  - dietary amino acids absorbed
  - utilizes glutamine and asparaginase as energy sources
    - releases CO$_2$, ammonium, alanine, citrulline as endproducts
    - utilizes glutamine during fasting for energy
  - dietary amino acids and catabolites released to portal blood.
Overview of amino acid catabolism. Interorgan relationships

- Liver
  - synthesis of liver and plasma proteins
  - catabolism of amino acids
    - gluconeogenesis
    - ketogenesis
    - branched chain amino acids (BCAA) not catabolized
    - urea synthesis
  - amino acids released into general circulation
    - enriched (% of total AA) in BCAA (2-3X)
Overview of amino acid catabolism. Interorgan relationships

**Skeletal Muscle**
- muscle protein synthesis
- catabolism of BCAA
  - amino groups transported away as alanine and glutamine (50% of AA released)
    - alanine to liver for gluconeogenesis
    - glutamine to kidneys

**Kidney**
- glutamine metabolized to $\alpha$-KG + NH$_4$
  - $\alpha$-KG for gluconeogenesis
  - NH$_4$ excreted or used for urea cycle (arginine synthesis)
    - important buffer preventing acidosis
Sources of NH$_4^+$ for the urea cycle
Important reaction for the removal of NH$_4^+$

\[
\begin{align*}
\text{\(\alpha\)-ketoglutarate} & \rightleftharpoons \text{glutamate} \rightleftharpoons \text{glutamine} \\
\text{NH}_4^+ & \quad \text{NH}_4^+ \quad \text{NH}_4^+ \quad \text{NH}_4^+ \\
\end{align*}
\]

A. Glutamate dehydrogenase

\[
\text{Glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O} \rightleftharpoons \text{\(\alpha\)-ketoglutarate} + \text{NH}_4^+ + \text{NAD(P)H}
\]

From transamination reactions

B. Glutamine synthetase (liver)

\[
\text{ATP} \quad \text{ADP} \\
\text{Glutamate} + \text{NH}_4^+ \rightleftharpoons \text{glutamine}
\]

C. Glutaminase (liver, kidney)

\[
\text{Glutamine} + \text{H}_2\text{O} \rightarrow \text{glutamate} + \text{NH}_4^+
\]
Glutamate dehydrogenase

The amino groups from many of the $\alpha$-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules.

Glutamate releases its amino group as ammonia in the liver.

The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD$^+$ or NADP$^+$ as cofactor.

Enzyme is present in mitochondrial matrix.
Combine action of an *transaminase* and *glutamate dehydrogenase* referred to as **transdeamination**.
Nitrogen removal from amino acids

Step 1: removal of amino group
Step 2: transfer of amino group to liver for nitrogen excretion
Step 3: entry of nitrogen into mitochondria
Step 4: preparation of nitrogen to enter urea cycle
Step 5: urea cycle
Step 1: removal of amino group
Aminotransferases have the same prosthetic group

Pyridoxal phosphate (PLP)

Pyridoxamine phosphate
Step 2: transfer of amino group to liver for nitrogen excretion

Excess ammonia is added to glutamate to form glutamine.

Glutamine enters the liver and $\text{NH}_4^+$ is liberated in mitochondria by the enzyme glutaminase.

Ammonia is remove by urea synthesis.
Glucose-alanine cycle

Ala is the carrier of amino nitrogen and of the carbon skeleton of pyruvate from muscle to liver.

The amino nitrogen is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.
Glucose-alanine cycle

- Liver

- Cytosol

- Muscle

- Alanine to liver for transamination to pyruvate prior to gluconeogenesis

- Branched-chain amino acids:
  - Isoleucine
  - Valine
  - Leucine
  - Aspartate

- Branched chain α-ketoacids to liver for further metabolism

- α-Ketoacid (oxaloacetate)

- α-Ketoglutarate

- Glutamate dehydrogenase

- Glutamate

- Pyruvate

- Glucose

- Glycogen

- NH₄⁺

- (i) to intestines for fuel
  (ii) to kidney for acid/base regulation

- From purine nucleotide cycle

- Acetyl CoA + acetoacetate

- Succinyl CoA

- Succinyl CoA + acetyl CoA

- Liver
Source of glutamate and NH$_4$ for urea cycle
Source of glutamate and NH$_4^+$ for urea cycle

Mitochondria, urea cycle
Nitrogen carriers

1. Glutamate
   transfers one amino group WITHIN cells:
   transaminases → make glutamate from $\alpha$-ketoglutarate

2. Glutamine
   transfers two amino group BETWEEN cells → releases its amino group in the liver

3. Alanine
   transfers amino group from tissue (muscle) into the liver
Step 3: entry of nitrogen to mitochondria
Step 4: prepare nitrogen to enter urea cycle
The formation of carbamoyl phosphate
Step 5: urea cycle

- Ornithine transcarbamoylase
- Argininosuccinate synthase
- Arginase 1
- Argininosuccinate lyase
Urea cycle – review
(Sequence of reactions)

- **Carbamoyl phosphate** formation in mitochondria is a prerequisite for the urea cycle
  - *(Carbamoyl phosphate synthetase)*
- **Citrulline** formation from carbamoyl phosphate and ornithine
  - *(Ornithine transcarbamoylase)*
- Aspartate provides the additional nitrogen to form **argininosuccinate** in cytosol
  - *(Argininosuccinate synthase)*
- **Arginine** and **fumarate** formation
  - *(Argininosuccinate lyase)*
- Hydrolysis of arginine to **urea** and ornithine
  - *(Arginase)*
The overall chemical balance of the biosynthesis of urea

\[
\text{NH}_3 + \text{CO}_2 + 2\text{ATP} \rightarrow \text{carbamoyl phosphate} + 2\text{ADP} + \text{Pi}
\]

Carbamoyl phosphate + ornithine → citrulline + Pi

Citrulline + ATP + aspartate → argininosuccinate + AMP + PPi

Argininosuccinate → arginine + fumarate

Arginine → urea + ornithine

Sum: \[2\text{NH}_3 + \text{CO}_2 + 3\text{ATP} \rightarrow \text{urea} + 2\text{ADP} + \text{AMP} + \text{PPi} + 2\text{Pi}\]
The activity of urea cycle is regulated at two levels:

- Dietary intake is primarily proteins → much urea (amino acids are used for fuel)
- Prolonged starvation → breaks down of muscle proteins → much urea also

- The rate of synthesis of four urea cycle enzymes and carbamoyl phosphate synthetase I (CPS-I) in the liver is regulated by changes in demand for urea cycle activity.
Regulation of urea cycle

- Enzymes are synthesized at higher rates in animals during:
  - starvation
  - in very-high-protein diet

- Enzymes are synthesized at lower rates in:
  - well-fed animals with carbohydrate and fat diet
  - animals with protein-free diets
Regulation of urea cycle

N-acetylglutamic acid *allosteric* activator of CPS-I

High concentration of *Arg* → stimulation of N-acetylation of glutamate by acetyl-CoA
Deficiencies of urea cycle enzymes
Ammonia toxicity

**Ammonia encephalopathy**

- Increased concentration of ammonia in the blood and other biological fluids → ammonia diffuses into cells, across blood/brain barrier → increased synthesis of glutamate from \( \alpha \)-ketoglutarate, increased synthesis of glutamine.
  - \( \alpha \)-ketoglutarate is depleted from CNS → inhibition of TCA cycle and production of ATP.
- Neurotransmitters – glutamate (excitatory neurotr.) and GABA (inhibitory neurotr.), may contribute to the CNS effects – bizarre behavior.
Deficiencies of urea cycle enzymes

- Infant born with total deficiency of one or more enzymes survive at least several days.
- Many enzymes deficiencies are partial → enzymes have altered $K_m$ values.
- Case are known of deficiencies of each enzymes.
- Interruption of the cycle at each point affected nitrogen metabolism differently - some of the intermediates can diffuse from hepatocytes → accumulate in the blood → pass into the urine.
- If symptoms are not detected early enough → severe mental retardation → brain damage is irreversible.
N-acetylglutamate synthase deficiency:

- Deficiency or genetic mutation of enzyme (AR) → urea cycle failure.
- A severe neonatal disorder with fatal consequences, if not detected immediately upon birth.
- Hyperammonemia and general hyperaminoacidemia in a newborn (liver contain no detectable ability to synthesize N-acetylglutamate).
- Early symptoms include lethargy, vomiting, and deep coma.
- **Treatment** with structural analog N-carbamoyl-L-glutamate – activates CPS-I, mitigates the intensity of the disorder,
Carbamoyl phosphate synthetase (CPS I) deficiency:

- autosomal recessive metabolic disorder, associated with mental retardation and developmental delay.
- Hyperammonemia has been observed in 0 – 50% of normal level of CPS-I synthesis in the liver.
- Treatment with benzoate and phenylacetate → hippurate and Phe-Ac-Gln are excreted in the urine:
**Ornithine transcarbamoylase (OTC) deficiency**

- The most common urea cycle disorder, resulting in a mutated and ineffective form of the enzyme.
- X-linked recessive disorder caused by a number of different mutations in the OTC gene – males are generally more seriously affected than females (males are asymptomatic as heterozygotes).
- Complications with OTC may include mental retardation and developmental delay.

**Argininosuccinate synthase deficiency – citrullinemia (citrullinuria)**

- Autosomal recessive metabolic disorder, inability to condense citrulline with aspartate.
- Accumulation of citrulline in blood and excretion in the urine.
- Type I citrullinemia - usually becomes evident in the first few days of life.
- Type II citrullinemia - the signs and symptoms usually appear during adulthood and mainly affect the nervous system.
- Therapy – specific supplementation with arginine for protein synthesis and for formation of creatin and ornithin.
Argininosuccinate lyase deficiency (argininosuccinate aciduria)

- Rare autosomal recessive disorder, argininosuccinate is excreted in large amount in urine.
- The severity of symptoms varies greatly, it is hard to evaluate the effect of therapy – useful is dietary restriction of nitrogen.

Arginase deficiency (argininemia)

- Rare autosomal recessive disorder that cause many abnormalities in development and function of CNS.
- Accumulation and excretion of arginine in urine and arginine precursors and products of arginine metabolism.
- Therapy – low nitrogen compounds diet (including essential amino acids
Link http://bcs.whfreeman.com/lehninger6e/#824263__839453__

Marks’ Basic Medical Biochemistry A Clinical Approach. Fourth Edition
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