2007-09

M1 - Renal, Fall 2007

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<http://hdl.handle.net/2027.42/64946>
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Nitrogen Metabolism (and Related Topics)

- Amino Acid Metabolism (Nitrogen metabolism)
- Folate Metabolism (“One-Carbon pathways”)
- Nucleotide Metabolism

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There are also PDF’s of class handouts with supplemental information available in the table of contents for this course.

Supplementary study material on the Web:
http://seqcore.brcf.med.umich.edu/mcb500
Protein Degradation:

- Endogenous proteins degrade continuously
  - Damaged
  - Mis-folded
  - Un-needed
- Dietary protein intake - mostly degraded

Nitrogen Balance - expresses the patient’s current status - are they gaining or losing net Nitrogen?
Transaminases Collect Amines

General reaction overview:

\[ R_1-C-COO^- + R_2-C-COO^- \rightarrow R_1-C-COO^- + R_2-C-COO^- \]

\( R_1 \) and \( R_2 \) are amino acids.
\( \alpha \)-keto acid (typically alpha-ketoglutarate).

Details of reaction mechanism:

1. Amino acid reacts with pyridoxal phosphate.
2. Pyridoxal phosphate is regenerated.
3. New amino acid is produced.

\[ R-C-COO^- + \text{pyridoxal phosphate} \rightarrow R-C-COO^- + \text{pyridoxal phosphate} \]
Transfer the amine back to an acceptor $\alpha$-keto acid
In peripheral tissues, transaminases tend to form Glutamate when they catabolize amino acids.

In other words, alpha-ketoglutarate is the preferred acceptor, and Glutamate is the resulting amino acid:

Some amino acid + α-ketoglutarate \rightarrow some alpha keto acid + Glutamate
Glutamate can donate its amines to form other amino acids as needed

A specific example - production of Aspartate in liver (described a few slides from now):

Glutamate + oxaloacetate $\rightarrow$ $\alpha$-ketoglutarate + aspartate
Getting Amines Into the Liver

**Glutamate Dehydrogenase:**

\[
\text{glutamate} \rightarrow \text{NAD(P)} \rightarrow \text{NAD(P)H} \rightarrow \alpha\text{-keto glutarate} + \text{ammonia}
\]

**Glutamine Synthetase:**

\[
\text{glutamate} \rightarrow \text{ATP + NH}_3 \rightarrow \text{ADP + P}_i \rightarrow \text{glutamine}
\]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamate donates its amino group to form aspartate:

Glutamate–aspartate aminotransferase:
Carbamoyl phosphate synthetase I
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

Citrulline

Argininosuccinate

ATP → AMP + PPi
Argininosuccinate lyase

Argininosuccinate $\xrightarrow{\text{Argininosuccinate lyase}}$ Arginine + Fumarate
Arginase

\[ \text{Arginine} \rightarrow \text{Urea} \rightarrow \text{Ornithine} \]

\[ \text{H}_2\text{O} \]

\[ \text{(-)OOCC}-\text{C}-\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2\text{-C-NH}_2 \]

\[ \text{(+)^3NH} \]

\[ \text{(-)OOCC}-\text{C}-\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}(+) \]

\[ \text{(+)^3NH} \]
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine → Citrulline
- Citrulline → Argininosuccinate
- Argininosuccinate → Arginine
- Arginine → Ornithine

TCA Cycle:
- Oxaloacetate → Malate
- Malate → Fumarate
- Fumarate → α-Ketoglutarate
- α-Ketoglutarate → Citrate
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\text{NAD}(\mathbf{P}) \quad \text{glutamate} \quad \xrightarrow{\text{mito}} \quad \text{NAD}(\mathbf{P})\mathbf{H} \quad \alpha\text{-ketoglutarate} \quad \text{ammonia}
\]

Glutamine Synthetase:

\[
\text{ATP} + \text{NH}_3 \quad \xrightarrow{\text{glutamate}} \quad \text{ADP} + \text{P}_i \quad \text{glutamine}
\]
CPS I is Stimulated by NAG

\[
\begin{align*}
\text{glutamate} & \quad + \quad \text{acetyl CoA} \\
\text{N-acetyl glutamate} & \quad \text{(NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \rightarrow \quad \text{carbonyl phosphate} \\
\text{carbamat} & \quad \rightarrow \quad \text{carbamoyl phosphate}
\end{align*}
\]
Complicating the picture: Other tissues may be involved.
Why is Ammonia Toxic?
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA)
  (due to shifting equilibria of reactions involving these compounds)
Why is Ammonia Toxic?

• Possible neurotoxic effects on glutamate levels (and also GABA) (due to shifting equilibria of reactions involving these compounds)

• Possible metabolic/energetics effects:
  - alpha-ketoglutarate levels
  - glutamate levels
  - glutamine
Inherited Defects of Urea Cycle Enzymes: Diagnosis

Defects are diagnosed based on the metabolites seen in the blood and/or urine.

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>CPSD</strong></td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td><strong>OTCD</strong></td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td><strong>ASD</strong></td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td><strong>ALD</strong></td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td><strong>AD</strong></td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
The diagram illustrates the urea cycle and the synthesis of proteins and amino acids. Here is a step-by-step breakdown of the process:

1. **2ATP + HCO₃⁻ + NH₃ → Carbamoyl phosphate**
   - Carbamoyl phosphate is formed from ATP, bicarbonate, and ammonia.

2. **2ADP + P₃ → ATP**
   - ATP is regenerated from ADP and inorganic phosphate (P₃).

3. **Ornithine → Arginine**
   - Ornithine is converted to arginine in the liver.
     - **Ornithine → Citrulline**
     - **Citrulline → Aspartate**
     - **Aspartate → Argininosuccinate**
     - **Argininosuccinate → Fumarate**

4. **Urea → NH₃**
   - Urea is synthesized from ammonia and carbon dioxide (CO₂).

5. **H₂O → Urea**
   - Urea is hydrated to form water and ammonia.

The cycle involves the interconversion of various amino acids and their derivatives, highlighting the metabolic pathways involved in protein synthesis and nitrogen metabolism.
CPS I is Stimulated by NAG

\[
\text{(-) ooc} \quad \text{(-) ooc} \\
\text{NH}_3 \\
\text{(-)} \quad \text{CH}_2 \text{CH}_2 \text{C} \\
\text{O} \\
\text{OH} \\
\begin{align*}
\text{glutamate} & \quad + \quad \text{CoA-c=}=o \\
\end{align*}
\]

\[
\text{N-acetyl glutamate} \\
\text{(NAG)}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{HO-C-O^(-)} & \quad \text{ATP} \\
\text{bicarbonate} & \quad \text{ATP} \\
\text{HO-C-PO} & \quad \text{NH}_3 \\
\text{carbonyl phosphate} & \quad \text{carbamate} \\
\text{ADP} & \quad \text{ADP} \\
\text{P}_i & \quad \text{carbamoyl phosphate}
\end{align*}
\]
Clinical Management of Urea Cycle Defects

- Dialysis to remove ammonia
- Provide the patient with alternative ways to excrete nitrogenous compounds:
  - Intravenous sodium benzoate or phenylacetate
  - Supplemental arginine

- Levulose - acidifies the gut
- Low protein diet
Degrading the Amino Acid Carbon Backbone
Easily-degraded products after transamination:

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \xrightarrow{\text{glutaminase}} \text{glutamate} + \text{ammonia}
\]

...and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate} + \text{ammonia}
\]
Many amino acids are purely glucogenic:
    Glutamate, aspartate, alanine, glutamine, asparagine,…

Some amino acids are *both* gluco- and ketogenic:
    Threonine, isoleucine, phenylalanine, tyrosine, tryptophan

The only PURELY ketogenic Amino Acids
: leucine, lysine
Amino acids with 5-carbon backbones tend to form α-ketoglutarate

Arginine

Urea (via the urea cycle)

Ornithine

α-ketoglutarate

glutamate

glutamate - 5-semialdehyde

NAD(P)⁺⁺

NAD(P)⁺

NAD(P)H

glutamate

NH₃

H₂O

glutamine

α-ketoglutarate
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\begin{align*}
\text{Glycine} & \quad \text{(+) + NADH} \quad \text{THF} \\
\text{CO}_2 & \quad \text{NH}_4^{(+)} \\
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad \text{(+) + NADH} \quad \text{THF} \\
\text{THF} & \quad \text{N}^5-N^0-\text{methylene THF} \\
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \text{(+) + NADH} \quad \text{THF} \\
\text{THF} & \quad \text{N}^5-N^0-\text{methylene THF} \\
\end{align*}
\]

\[
\begin{align*}
\text{Serine} & \quad \text{(+) + NADH} \quad \text{THF} \\
\text{THF} & \quad \text{N}^5-N^0-\text{methylene THF} \\
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

(Normal path shown in black, pathological reaction shown in red)

\[
\begin{align*}
\text{Phenylalanine} & \quad \xrightarrow{\text{Enzyme: Phenylalanine hydroxylase}} \quad \text{Tyrosine} \\
\text{Phenylpyruvate} & \quad \xrightarrow{\text{Deficiency: Alkaptonuria, “Ochronosis”}} \quad \text{Homogentisate}
\end{align*}
\]
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

------------- Transamination -------------

--- Branched-chain \boldsymbol{\alpha}-keto acid dehydrogenase ---

(continues on to degradation path similar to \(\beta\)-oxidation of fatty acids)
Synthesis of Bioactive Amines

Tyrosine $\xrightarrow{\text{Tyrosine hydroxylase}}$ Dihydroxyphenylalanine (L-DOPA)

Dopamine $\xrightarrow{}$ Norepinephrine $\xrightarrow{}$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase
PLP-dependent decarboxylation
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

Histamine
NON-Essential Amino Acids:

Glutamate, aspartate, alanine, glutamine, asparagine, (proline), glycine, serine (cysteine, tyrosine)

Essential Amino Acids:

Arginine (!), phenylalanine, methionine, histidine, Isoleucine, leucine, valine, threonine, tryptophan, lysine