2007-09

M1 - Renal, Fall 2007

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http://hdl.handle.net/2027.42/64946
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:

\[
R_1\text{C}\text{--}\text{coo}^{(-)} + R_2\text{C}\text{--}\text{coo}^{(-)} \rightarrow R_1\text{C}\text{--}\text{coo}^{(-)} + \alpha\text{-keto acid (typically alpha-ketoglutarate)} + R_2\text{C}\text{--}\text{coo}^{(-)} + \text{amino acid (typically glutamate)}
\]

Details of reaction mechanism:

1. Amino acid
2. H^+
3. Pyridoxal phosphate
4. H_2O
5. Pyridoxamine phosphate
Transfer the amine back to an acceptor α-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 → 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):

\[
\text{Glutamate} + \text{oxaloacetate} \rightarrow \alpha\text{-ketoglutarate} + \text{aspartate}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{Glutamate} & \xrightarrow{\text{NAD}(P)} \text{α-ketoglutarate} + \text{NH}_3 \\
\text{NH}_2 & \xrightarrow{\text{mito}} \text{NAD}(P)H
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{Glutamine} & \xrightarrow{\text{ATP} + \text{NH}_3} \text{Glutamate} \\
\text{NH}_3 & \xrightarrow{\text{ADP} + P_i} \text{Glutamine}
\end{align*}
\]
Ammonia can also be formed by the glutamate dehydrogenase reaction and several other reactions as well.

Glutamine is hydrolyzed to glutamate and ammonia:

Glutamate donates its amino group to form aspartate:

Glutamate-aspartate aminotransferase:
Carbamoyl phosphate synthetase I

bicarbonate $\xrightarrow{\text{ATP}}$ carbonyl phosphate $\xrightarrow{\text{NH}_3}$ carbamate $\xrightarrow{\text{ATP}}$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

\[
\text{Citrulline} \rightarrow \text{aspartate} \rightarrow \text{Argininosuccinate}
\]
Argininosuccinate lyase

Argininosuccinate → Arginine

Fumarate
Arginase

\[
\text{Arginase} \quad \xrightarrow{H_2O} \quad \text{Ornithine}
\]

Arginine \rightarrow Urea \rightarrow Ornithine
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\text{Glutamate} \quad \text{ATP} \quad \text{NH}_3 \quad \text{ADP} \quad \text{Pi} \quad \text{Glutamine}
\]
CPS I is Stimulated by NAG

\[
\begin{align*}
\begin{array}{c}
\text{glutamate} \\
\text{acetyl CoA}
\end{array}
& \xrightarrow{\text{N-acetyl glutamate synthetase}} \\
& \text{N-acetyl glutamate (NAG)}
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \xrightarrow{\text{ATP}} \text{carbonyl phosphate} \\
& \xrightarrow{\text{ADP}} \text{carbamate} \\
& \xrightarrow{\text{ADP}} \text{carbamoyl phosphate}
\end{align*}
\]
Complicating the picture: Other tissues may be involved

Muscle:
- Amino acids: Transamination, Deamination
- Alanine → Glutamate → Glutamine
- purine deamination: NH₄⁺

Intestine:
- Glutamine
- Alanine → NH₄⁺ → Citrulline

Kidney:
- Glutamine
- NH₃
- NH₄⁺
- Citrulline → Arginine

Liver:
- Glutamine
- Alanine → Glu → Aspartate
- NH₄⁺ → Urea

Arginine
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.

<table>
<thead>
<tr>
<th>Defect</th>
<th>Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD</td>
<td>No elevation except ammonia; diagnosed by elimination.</td>
</tr>
<tr>
<td>OTCD</td>
<td>Elevated CP causes synthesis of Orotate</td>
</tr>
<tr>
<td>ASD</td>
<td>Elevated citrulline</td>
</tr>
<tr>
<td>ALD</td>
<td>Elevated argininosuccinate</td>
</tr>
<tr>
<td>AD</td>
<td>Elevated arginine</td>
</tr>
</tbody>
</table>
CPS I is Stimulated by NAG

\[
\begin{align*}
\text{(-)} \quad \text{OOC} & \quad \text{C} & \quad \text{CH}_2\text{CH}_2\text{C} & \quad \text{CH}_3 \quad \text{OH} \\
\text{NH}_2 & \quad \text{(+) -} \\
\text{glutamate} & \quad \text{+} & \quad \text{CoA} & \quad \text{C} & \quad \text{O} \\
\text{N-acetyl glutamate} & \quad \text{(NAG)} & \quad \text{synthetase} & \quad \text{N-acetyl glutamate} & \quad \text{(NAG)} \\
\end{align*}
\]

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{HO} & \quad \text{C} \quad \text{O} \quad \text{((-)} \quad \text{ATP} \\
\text{bicarbonate} & \quad \text{ADP} & \quad \text{carbonyl phosphate} & \quad \text{ADP} & \quad \text{carbamoyl phosphate} \\
\text{NH}_3 & \quad \text{ADP} & \quad \text{carbamate} & \quad \text{ADP} & \quad \text{carbamoyl phosphate} \\
\text{O} & \quad \text{P} & \quad \text{P_i} & \quad \text{P} & \quad \text{P}
\end{align*}
\]
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
- Leulose - 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:

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

…and by analogy, how to degrade Asparaginase:

\[
\text{Asparagine} \xrightarrow{\text{asparaginase}} \text{aspartate + ammonia}
\]
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.
Degradation and Biosynthesis of Serine and Glycine

**Glycine Synthase:**

\[
\begin{align*}
\text{Glycine} & \quad \rightarrow \quad \text{CO}_2 + \text{NH}_4^{(+)} \\
\text{THF} & \quad \rightarrow \quad \text{N}^5\text{-N}^{10}\text{-methylene THF}
\end{align*}
\]

**Serine Hydroxymethyltransferase:**

\[
\begin{align*}
\text{Serine} & \quad \rightarrow \quad \text{Glycine} \\
\text{THF} & \quad \rightarrow \quad \text{N}^5\text{-N}^{10}\text{-methylene THF}
\end{align*}
\]

**Serine Dehydratase:**

\[
\begin{align*}
\text{Serine} & \quad \rightarrow \quad \text{H}_2\text{O} \\
\text{Serine} & \quad \rightarrow \quad \text{NH}_4^{(+)}
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups

Methionine

S-Adenosyl Methionine

Homocysteine

S-Adenosyl Homocysteine

Serine

Cysteine

(remainder of homocysteine degraded for energy)
Phenylalanine and Tyrosine

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

Phenylalanine → Tetrahydrobiopterin + O₂ → Dihydrobiopterin + H₂O

Enzyme: Phenylalanine hydroxylase

Phenylalanine → Tyrosine

Tyrosine → Homogentisate

Enzyme: homogentisate dioxygenase

Deficiency: Alkaptonuria “Ochronosis”

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine

Leucine

Valine

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

----------- Branched-chain α-keto acid dehydrogenase ------

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

Tyrosine → Dihydroxyphenylalanine (L-DOPA) → Dopamine → Norepinephrine → Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → Serotonin

Tryptophan hydroxylase

PLP-dependent decarboxylation

NAD+ → CO₂
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent) catalyzes the conversion of glutamate to γ-aminobutyric acid (GABA).

Histidine decarboxylase (PLP-dependent) catalyzes the conversion of histidine to 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