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M1 - Renal, Fall 2007

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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:

\[
R_1\overset{\text{H}}{\text{C}}\overset{\text{NH}_2}{\text{amino acid}} + R_2\overset{\text{coo}(-)}{\text{C}}\overset{\text{alpha-keto acid (typically alpha-ketoglutarate)}}{\text{coo}(-)} \rightarrow R_1\overset{\text{H}}{\text{C}}\overset{\text{NH}_2}{\text{amino acid}} + R_2\overset{\text{coo}(-)}{\text{C}}\overset{\text{coo}(-)}{\text{amino acid (typically glutamate)}}
\]

Details of reaction mechanism:

1. Amino acid + pyridoxal phosphate
2. Formation of pyridoxamine phosphate
3. Release of pyridoxal phosphate
4. Formation of α-keto acid
5. Reaction with α-keto acid
6. Formation of product

\[
R_1\overset{\text{H}}{\text{C}}\overset{\text{NH}_2}{\text{amino acid}} + \overset{\text{H}^+}{\text{R-C}}\overset{\text{coo}(-)}{\text{C}}\overset{\text{NH}_2, \text{OC}}{\text{pyridoxamine phosphate}} \rightarrow R_1\overset{\text{H}}{\text{C}}\overset{\text{NH}_2}{\text{amino acid}} + \overset{\text{R-C}}{\text{C}}\overset{\text{COO}(-)}{\text{amino acid (typically glutamate)}}
\]
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):

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

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \xrightarrow{\text{ATP+NH}_3} \quad \text{glutamine} \\
& \quad \xrightarrow{\text{ADP+P}_i} \quad \text{glutaminase}
\end{align*}
\]
In the Liver: Precursors for Urea Cycle

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:
Carbamoyl phosphate synthetase I

bicarbonate $\rightarrow$ carbonyl phosphate $\rightarrow$ carbamate $\rightarrow$ carbamoyl phosphate
Ornithine Transcarbamoylase

Carbamoyl phosphate

\[
\text{NH}_2\text{C} - \text{OPo}_3^{-} \quad \text{Pi}
\]

Ornithine

\[
(-)\text{OOC} \quad \text{C} \quad \text{C}_2\text{H}_2\text{NH}_3^+ \quad \text{NH}_3^+
\]

Citrulline

\[
(-)\text{OOC} \quad \text{C} \quad \text{C}_2\text{H}_2\text{NH}_3^+ \quad \text{C} \quad \text{NH}_2
\]
Argininosuccinate synthetase

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

\[
\text{ATP} \rightarrow \text{AMP} + \text{PP}_i
\]

\[
\text{aspartate}
\]
Argininosuccinate lyase

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

$\text{(-)}\text{O}_2\text{CCH}_2\text{C}^-\text{NH}_2\text{C}*=\text{NH}^{(+)}$ $\xrightarrow{\text{Argininosuccinate lyase}}$ $\text{(-)}\text{O}_2\text{CCH}_2\text{C}^-\text{NH}_2\text{C}^*=\text{NH}^{(+)}$

$\text{(-)}\text{O}_2\text{C}^-\text{C}*=\text{C}^-\text{CO}_2^{(-)}$ $\xrightarrow{\text{Argininosuccinate lyase}}$ $\text{(-)}\text{O}_2\text{C}^-\text{C}*=\text{C}^-\text{CO}_2^{(-)}$
Arginase

\[
\begin{align*}
&\text{Arginine} \\
&\quad \xrightarrow{\text{H}_{2}O} \text{Urea} \\
&\quad \xrightarrow{\text{H}^+} \text{Ornithine}
\end{align*}
\]
Urea Cycle Connects to TCA Cycle

Urea Cycle:
- Ornithine
- Citrulline
- Argininosuccinate
- Arginine

Urea formation from Arginine:

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

Glutamate Dehydrogenase:

\[
\begin{align*}
\text{glutamate} & \quad \text{NAD(P)} \\
& \quad \text{(mito)} \\
& \quad \text{NAD(P)H} \\
& \quad \alpha\text{-ketoglutarate} \\
& \quad \text{ammonia}
\end{align*}
\]

Glutamine Synthetase:

\[
\begin{align*}
\text{glutamate} & \quad \text{ATP} + \text{NH}_3 \\
& \quad \text{ADP} + \text{P}_i \\
& \quad \text{glutamine}
\end{align*}
\]
CPS I is Stimulated by NAG

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
& \quad \xrightarrow{\text{ADP}} \quad \text{carbamate} \\
& \quad \xrightarrow{\text{P}_i} \quad \text{carbamoyl phosphate}
\end{align*}
\]
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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Description</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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \text{ATP} \\
\text{HO} - \text{C} - \text{O}^{(-)} & \quad \rightarrow \quad \text{HO} - \text{C} - \text{O}^{(P)} \\
\rightarrow \quad \text{ADP} & \quad \text{carbonyl phosphate} \\
\rightarrow \quad \text{NH}_3 & \quad \rightarrow \quad \text{HO} - \text{C} - \text{NH}_2 \\
\rightarrow \quad \text{P}_i & \quad \rightarrow \quad \text{carbamate} \\
\rightarrow \quad \text{ADP} & \quad \rightarrow \quad \text{carbamoyl phosphate}
\end{align*}
\]
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:

\[
\begin{align*}
\text{Glutamine} & \rightarrow \text{glutamate} + \text{ammonia} \\
\text{Asparagine} & \rightarrow \text{aspartate} + \text{ammonia}
\end{align*}
\]

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
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\begin{align*}
\text{Glycine Synthase:} & \quad (-)\text{OOC} - \text{C} - \text{NH}_3 \\
& \quad \text{coverts to} \quad \text{CO}_2 + \text{NH}_4^+ \\
& \quad \text{via THF and N}^5 - N^0 - \text{methylene THF}
\end{align*}
\]

Serine Hydroxymethyltransferase:

\[
\begin{align*}
\text{Serine} & \quad (-)\text{OOC} - \text{CH} - \text{NH}_3 \\
& \quad \text{coverts to} \quad (-)\text{OOC} - \text{C} - \text{NH}_3 \\
& \quad \text{via THF and N}^5 - N^0 - \text{methylene THF}
\end{align*}
\]

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad (-)\text{OOC} - \text{CH} - \text{NH}_3 \\
& \quad \text{dehydrates to} \quad (-)\text{OOC} - \text{C} - \text{NH}_2 \\
& \quad \text{then to} \quad (-)\text{OOC} - \text{C} - \text{O}
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine
(Normal path shown in black, pathological reaction shown in red)

Phenylalanine

\[ \text{(+) Tetrahydrobiopterin + O}_2 \rightarrow \text{Dihydrobiopterin + H}_2\text{O} \]

Enzyme: Phenylalanine hydroxylase

Tyrosine

Homogentisate

Phenylpyruvate

Deficiency: Alkaptonuria
(“Ochronosis”)
Branched Chain Amino Acids

Isoleucine  Leucine  Valine

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

------------ Branched-chain \(\alpha\)-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 $\rightarrow$ Norepinephrine $\rightarrow$ Epinephrine
Synthesis of Bioactive Amines

Tryptophan → 5-hydroxytryptophan → PLP-dependent decarboxylation → Serotonin
Synthesis of Bioactive Amines

\[ \text{Glutamate} \xrightarrow{\text{Glutamate decarboxylase (PLP-dependent)}} \text{\(\gamma\)-aminobutyric acid (GABA)}} \]

\[ \text{Histidine} \xrightarrow{\text{Histidine decarboxylase (PLP-dependent)}} \text{Histamine} \]
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