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:

\[ R_1\text{-}C\text{-}\text{coo}^{(-)} + R_2\text{-}C\text{-}\text{coo}^{(-)} \rightarrow R_1\text{-}C\text{-}\text{coo}^{(-)} + R_2\text{-}C\text{-}\text{coo}^{(-)} \]

alpha-keto acid (typically alpha-ketoglutarate)

Details of reaction mechanism:

\[ \text{pyridoxal phosphate} \]

\[ \text{pyridoxamine phosphate} \]
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):

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

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

Glutamine is hydrolyzed to glutamate and ammonia:

\[
\begin{align*}
\text{glutamine} & \rightarrow \text{glutamate} + \text{NH}_3
\end{align*}
\]

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

Glutamate donates its amino group to form aspartate:

\[
\begin{align*}
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} + \text{oxaloacetate} & \rightarrow \alpha\text{-keto glutarate} + \text{aspartate}
\end{align*}
\]
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

Ornithine

Citrulline
Argininosuccinate synthetase

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

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

\[
\text{aspartate}
\]
Argininosuccinate lyase

Argininosuccinate $\rightarrow$ Arginine $\rightarrow$ Fumarate
Arginase

\[
\text{Arginine} \rightarrow \text{Urea} \rightarrow \text{Ornithine}
\]
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

\[
\text{glutamate} + \text{acetyl CoA} \xrightarrow{\text{N-acetyl glutamate synthetase}} \text{N-acetyl glutamate (NAG)}
\]

(repeating the figure from page 3 of your handout)

bicarbonate \xrightarrow{\text{ATP}} \text{carbonyl phosphate} \xrightarrow{\text{ADP}} \text{carbamate} \xrightarrow{\text{ATP}} \text{carbamoyl phosphate}

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>Metabolite Abnormality</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

glutamate + acetyl CoA \rightarrow N\text{-}acetyl glutamate (NAG)

(repeating the figure from page 3 of your handout)

bicarbonate \rightarrow \text{carbonyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}
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:

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

\[
\text{Asparagine} \xrightarrow{\text{transamination}} \text{aspartate} + \text{ammonia}
\]

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 \(\alpha\)-ketoglutarate.
Degradation and Biosynthesis of Serine and Glycine

Glycine Synthase:

\[
\text{Glycine} \quad \xrightarrow{\text{THF} \quad \text{N}^5-\text{N}^6- \text{methylene THF}} \quad \text{NAD}^{(+)} \quad \xrightarrow{\text{NADH}} \quad \text{CO}_2 + \text{NH}_4^{(+)}
\]

Serine Hydroxymethyltransferase:

\[
\text{Serine} \quad \xrightarrow{\text{THF} \quad \text{N}^5-\text{N}^6- \text{methylene THF}} \quad \text{Glycine}
\]

Serine Dehydratase:

\[
\text{Serine} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{Serine} \quad \xrightarrow{\text{NH}_4^{(+)}} \quad \text{Glycine}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine $\rightarrow$ Tetrahydrobiopterin $+\ O_2$ $\rightarrow$ Dihydrobiopterin $+\ H_2O$ $\rightarrow$ Tyrosine

Enzyme: Phenylalanine hydroxylase

Homogentisate

Deficiency: Alkaptonuria “Ochronosis” $\rightarrow$ Enzyme: homogentisate dioxygenase

Phenylpyruvate

Phenylketonuria (no phenylalanine hydroxylase)
Branched Chain Amino Acids

Isoleucine

\[
\text{CH}_3\text{CH}_2\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3^{(+)} \\
\alpha\text{-KG}
\]

\[\text{Glu} \]

\[\text{CH}_3\text{CH}_2\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{O}
\]

\[\text{NAD}^+, \text{CoASH} \]

\[\text{NADH} + \text{CO}_2\]

Leucine

\[
\text{CH}_3\text{CHCH}_2 - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3^{(+)} \\
\alpha\text{-KG}
\]

\[\text{Glu} \]

\[\text{CH}_3\text{CHCH}_2 - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{O}
\]

\[\text{NAD}^+, \text{CoASH} \]

\[\text{NADH} + \text{CO}_2\]

Valine

\[
\text{CH}_3\text{CH} - \text{CH} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{NH}_3^{(+)} \\
\alpha\text{-KG}
\]

\[\text{Glu} \]

\[\text{CH}_3\text{CH} - \text{C} - \text{COO}^{(-)} \\
\text{CH}_3 \quad \text{O}
\]

\[\text{NAD}^+, \text{CoASH} \]

\[\text{NADH} + \text{CO}_2\]

(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 \[ \rightarrow \text{Tryptophan hydroxylase} \rightarrow 5\text{-hydroxytryptophan} \rightarrow \text{PLP-dependent decarboxylation} \rightarrow \text{Serotonin} \]

\[ \text{NH}_3 \quad (+) \]

\[ \text{NAD}^+ \]

\[ \text{CO}_2 \]
Synthesis of Bioactive Amines

Glutamate

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

Histidine

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