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:

\[
\begin{align*}
R_1\text{C}\text{H} &\text{COO}^(-) + R_2\text{C}\text{H} &\text{COO}^(-) \\
\text{NH}_2 &\longrightarrow & \alpha\text{-keto acid (typically alpha-ketoglutarate)} \\
\text{amino acid} & & \alpha\text{-keto acid} \\
\text{amino acid} & & \text{amino acid (typically glutamate)} \\
\end{align*}
\]

Details of reaction mechanism:

amino acid

\[
\begin{align*}
R\text{C}\text{H} &\text{COO}^(-) + \text{NH}_2 + \text{O} &\longrightarrow & \text{H}_2\text{O} \\
\text{amino acid} & & \text{pyridoxal phosphate} \\
\end{align*}
\]
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 → 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:

\[
\text{H} \quad \xrightarrow{\text{NAD}(P)} \quad \text{NAD}(P)H \quad \xrightarrow{\text{NH}_3} \quad \text{NH}_2
\]

\[
\text{O}_2 \text{C} \text{CH}_2 \text{CH}_2 \text{C} - \text{CO}_2^- \quad \xrightarrow{\text{mito}} \quad \text{O}_2 \text{C} \text{CH}_2 \text{CH}_2 \text{C} - \text{CO}_2^- + \text{NH}_3
\]

Glutamine Synthetase:

\[
\text{H} \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{H} \quad \xrightarrow{\text{ADP} + P_i} \quad \text{H}
\]

\[
\text{OOC} - \text{C} - \text{CH}_2 \text{CH}_2 \text{COO}^- \quad \xrightarrow{\text{ATP} + \text{NH}_3} \quad \text{OOC} - \text{C} - \text{CH}_2 \text{NH}_2 \text{NH}_2
\]

\[
\text{glutamate} \quad \rightarrow \quad \text{glutamine}
\]
In the Liver: Precursors for Urea Cycle

Glutamine is hydrolyzed to glutamate and ammonia:

![Chemical structure of glutamine hydrolysis 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:

![Chemical reaction of glutamate-aspartate aminotransferase]

\[
\text{Glutamate-aspartate aminotransferase:} \\
\text{Glutamate} \quad \text{oxaloacetate} \quad \alpha\text{-keto glutarate} \quad \text{aspartate}
\]
2ATP + HCO₃⁻ + NH₃ → Carboxamoyl phosphate

2ADP + P⁺ → Ornithine

Liver mitochondrion

Citrulline

Liver cytoplasm

Ornithine

Urea

H₂O

Arginine

Argininosuccinate

Fumarate

ATP

Aspartate

AMP + P⁺
Carbamoyl phosphate synthetase I

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

Carbamoyl phosphate

\[ \text{NH}_2\text{C} - \text{OPO}_3^{(-)} \]

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

Ornithine

\[ \text{NH}_2\text{C} - \text{H}_2\text{C} - \text{H}_2\text{C} - \text{NH}_2 \]

Citrulline

\[ \text{NH}_2\text{C} - \text{H}_2\text{C} - \text{H}_2\text{C} - \text{NH}_2 \]
**Argininosuccinate synthetase**

\[
\begin{align*}
\text{Citrulline} & \rightarrow \text{Aspartate} & \text{Argininosuccinate} \\
\text{ATP} & \rightarrow \text{AMP} + \text{PP}_i
\end{align*}
\]
Argininosuccinate lyase

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

Arginine + H₂O → Ornithine + Urea
Getting Amines Into the Liver

Glutamate Dehydrogenase:

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

Glutamine Synthetase:

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

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

(repeating the figure from page 3 of your handout)

\[
\begin{align*}
\text{bicarbonate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbonyl phosphate} \\
\text{carbamate} & \quad \xrightarrow{\text{ATP}} \quad \text{carbamoyl phosphate}
\end{align*}
\]
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>Condition</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

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

(repeating the figure from page 3 of your handout)

bicarbonate + ATP $\rightarrow$ carbamoyl phosphate

\[\text{carbamoyl phosphate} \rightarrow \text{carbamate} \rightarrow \text{carbamoyl phosphate}\]
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:

We also already know how to degrade Glutamine:

\[
\text{Glutamine} \rightarrow \text{glutamate} + \text{ammonia}
\]

…and by analogy, how to degrade Asparagine:

\[
\text{Asparagine} \rightarrow \text{aspartate} + \text{ammonia}
\]
Amino Acids are categorized as ‘Glucogenic’ or ‘ketogenic’ or both.

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:

\[
\begin{align*}
\text{Glycine} & \quad \text{THF} \quad N^5 - N^0 - \text{methylene THF} \\
& \quad \text{NAD}^{+} \quad \text{NADH} \quad CO_2 \quad NH_4^{+}
\end{align*}
\]

Serine Hydroxymethyl-transferase:

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

Serine Dehydratase:

\[
\begin{align*}
\text{Serine} & \quad H_2O \\
& \quad \text{H}_2O \quad \text{NH}_4^{+} \quad \text{H}_2O
\end{align*}
\]
Methionine Cycle
And Biological Methyl Groups
Phenylalanine and Tyrosine

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

Phenylalanine

(+)
NH₃

(Tetrahydrobiopterin + O₂)

Dihydrobiopterin + H₂O

Enzyme:
Phenylalanine hydroxylase

HO

(-)

Tyrosine

(+)
NH₃

Homogentisate

Deficiency:
Alkaptonuria
“Ochronosis”

Enzyme:
Homogentisate dioxygenase

(you don’t need to know the rest)

Phenylalanine

(-)

Phenylpyruvate

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

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

Tryptophan $\xrightarrow{\text{NAD}^+}$ 5-hydroxytryptophan $\xrightarrow{\text{Tryptophan hydroxylase}}$ 5-hydroxytryptamine $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin

Tryptophan $\xrightarrow{\text{NH}_3}$ 5-hydroxytryptophan $\xrightarrow{\text{NAD}^+}$ 5-hydroxytryptamine $\xrightarrow{\text{PLP-dependent decarboxylation}}$ Serotonin

$\xrightarrow{\text{CO}_2}$
Synthesis of Bioactive Amines

Glutamate decarboxylase (PLP-dependent)

γ-aminobutyric acid (GABA)

Histidine decarboxylase (PLP-dependent)

Histidine
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