Amino Acid Metabolism:
The Urea Cycle and Urea Cycle Disorders

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INTRODUCTION

The metabolism of amino acids (AAs) not used for the synthesis of proteins or nitrogen-containing compounds takes place in the liver and can occur through two complex mechanisms: deamination or transamination. Transamination reactions result in the transfer of one AA to an AA carbon skeleton or alpha-keto group while deamination removes the amino group and does not transfer the amino group to another compound thus results in the product of ammonium. Ammonia/ammonium is a compound of nitrogen and hydrogen. Excessive amounts of ammonia can be formed in the body for one of three reasons: ammonia generated in the gastrointestinal tract from bacteria lysis and AA thus absorbed through the enterocyte into the body, ammonia ingested and absorbed from the foods in our diet, and/or ammonia formed due to chemical reactions such as deamination.

The concluding pathway for removal of surplus nitrogen from the body and the major route for detoxification of ammonium is known as the urea cycle. The urea cycle occurs only in the liver and involves an initial four key enzymes: carbamoyl phosphate synthetase I (CPSI), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL). N-acetylglutamate synthase (NAGS) and arginase-1 are also important enzymes of the urea cycle. A deficiency or defect in any of the key enzymes or transport carriers involved in the urea cycle may result in the development of a urea cycle disorder (UCD).

This paper will review the oxidation deamination of glutamate as well as the steps and key components of the urea cycle. In addition, UCDs will be discussed along with the specific UCD: ornithine transcarbamylase deficiency (OTCD).
REVIEW OF LITERATURE

Oxidation Deamination

Oxidative deamination reactions are part of the metabolic pathway for endogenous amino compounds. (1) Deamination reactions result in the product of ammonium (NH$_4^+$) due to the fact that the amino group removed from the AA is not transferred to another compound. Some AAs that are more commonly deaminated include glutamate, histidine, serine, glycine, and threonine. The enzymes facilitating the deamination reactions are typically lyases, dehydratases, or dehydrogenases.

Glutaminase is a phosphate-activated mitochondrial matrix enzyme which catalyzes the hydrolysis of the amide group of glutamine to glutamate and NH$_4^+$. (2) Glutaminase isoenzymes are encoded on sequence similar human genes, human glutaminase-1 (GLS1) and human glutaminase-2 (GLS2). GLS1 and GLS2 are located on different chromosomes and expressed differently in different organs. Therefore, GLS1 and GLS2 have different purposes and functions.

GLS1 is located on chromosome 2q32 and is believed to encode an initial mRNA transcript that undergoes tissue-specific alternative splicing thus generating three glutaminase isoenzymes: kidney-type glutaminase (KGA), glutaminase C (GAC), and glutaminase M (GAM). (2) KGA, GAC, and GAM are expressed in different organs and/or areas of the body, not including the liver. It is believed that GLS1 does not act as a catalyst in the hydrolysis of the amide group of glutamine to glutamate and NH$_4^+$. Furthermore, GLS2 is located on chromosome 12q13 and is vastly expressed in postnatal liver and brain tissues. GLS2 encodes a mitochondrial glutaminase which catalyzes the hydrolysis of glutamine to glutamate. (3)
Hu et al. investigated how GLS2 regulates energy metabolism by overexpressing GLS2 through transfection of a GLS2 expression vector with a C-terminal Flag tag or knocked down by siRNA oligo in a cell. (3) Immunofluorescence staining was employed in order to assure that the exogenous GLS2 protein expressed by the vector was localized in the mitochondria. Results indicated that exogenous GLS2 expression greatly increased the intracellular levels of glutamate in comparison with control cells transfected with empty vectors. Additionally, it was clear that the GLS2 knockdown by siRNA oligo reduced levels of glutamate in comparison with control cells transfected with a scrambled siRNA. Therefore, GLS2’s function in the investigation corresponded with the known biological function and demonstrated that GLS2 catalyzes the hydrolysis of glutamine to glutamate. The hydrolysis of glutamine to glutamate increases the end-product of NH$_4^+$ which is then utilized in the urea cycle in order to keep safe levels of NH$_4^+$ in the body.

**The Urea Cycle**

The urea cycle is the major mechanism of ammonia removal in humans. (2) Human hepatocytes utilize two end-products of metabolism, ammonia and carbon dioxide in the form of bicarbonate. Utilizing such end-products allows for the generation of AAs through a series of reactions known as the urea cycle. The urea cycle result in the generation of AA and produces urea and fumarate. Moreover, aspartate is consumed as a result of the cycle continuously functioning. The four major enzymes in which are necessary for the urea cycle to function properly are: carbamoylphosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase 1, and argininosuccinate lyase. N-acetylglutamate synthase is also vital for the urea cycle to properly function. In addition to the initial enzymes, other important
aspects are transport carriers and include arginase-1, mitochondria ornithine carrier, and the aspartate-glutamate carrier (citrin).

CPS1 is required for the activation of the urea cycle. The human gene encoding CPS1 has been previously mapped to chromosome 2. (4) CPS1 contains one active site in which the binding and deamination of glutamine takes place. CSP1 catalyzes the combination of ammonia and carbon dioxide/bicarbonate to form carbamoyl phosphate using 2 mol of ATP and Mg\(^{2+}\) in the mitochondrial matrix. Carbamoyl phosphate is then placed into the urea cycle. (2) Moreover, N-acetyl-glutamate (NAG) is necessary for such activity to take place considering NAG acts as an allosteric activator thus allowing ATP binding.

Once carbamoyl phosphate has entered the urea cycle, OTC is activated. OTC is considered to be a mitochondrial enzyme expressed specifically in the liver and small intestine. The human gene encoding OTC is located on chromosome Xp21.1. (5) OTC catalyzes the synthesis of citrulline from carbamoyl phosphate and ornithine which previously entered the mitochondria from the cytosol. Citrulline and aspartate leave the mitochondrial matrix and enter the cytoplasm through the OTC transporter and the aspartate-glutamate carrier (citrin), respectively.

Furthermore, ASS1 is needed for the cycle to continue. ASS1 is encoded by the human gene expressed on chromosome 9q34.1. (6) ASS1 catalyzes the reaction of citrulline and aspartate in the cytosol of hepatocytes to generate argininosuccinate. This reaction is known as the rate-limiting step of the urea cycle since two high-energy bonds (ATP) and Mg\(^{2+}\) are required.

After argininosuccinate has been generated, ASL is released. ASL is encoded by the human gene located on chromosome 7. (7) ASL is a cystolic enzyme in which catalyzes the
breakdown of argininosuccinate to arginine and fumarate. Both arginine and fumarate inhibit argininosuccinase activity. An increase in the concentration of arginine results in an increase in the synthesis of N-acetylglutamate. Moreover, NAGS, located on the 17q21.31 chromosome, catalyzes the formation of N-acetylglutamate (NAG) from glutamate and acetyl-coA inside the mitochondrial matrix. (8) The formation of NAG is necessary considering NAG is an allosteric activator for CSP1 thus NAG is indirectly needed for the initiation of the urea cycle.

Moreover, arginase is an enzyme which catalyzes the hydrolysis of arginine to ornithine and urea. Humans have two isozymes of arginase: arginase-1 and arginase-2. (9) Arginase-1 is a cytosolic protein thus found in the liver, erythrocytes, granulocytes, kidney, brain, and GI tract. Arginase-2 is located in the mitochondrial matrix thus is only found in the kidney, brain, GI tract, and fibroblasts. Therefore, arginase-1 located on the 6q23 chromosome, is the only form of arginase which can hydrolyze the formation ornithine and urea.

Once ornithine is produced, it is necessary for ornithine to be transported from the cytosol to the mitochondrial matrix. (10) It is necessary for ornithine to be transported using the mitochondrial ornithine carrier (ORNT) due to the fact that ornithine has to cross the mitochondrial membrane. Ornithine is then recycled back into the urea cycle thus is known to be a central component to the continuation of the cycle.

**Urea Cycle Disorders**

The common feature of urea cycle disorders (UCDs) is a defect in NH$_4^+$ elimination in the liver thus leading to hyperammonia. (11) Typically, NH$_4^+$ is maintained at low concentrations considering the conversion of NH$_4^+$ to urea through the urea cycle. When the urea cycle is disrupted, a surplus of NH$_4^+$ is developed considering NH$_4^+$ is not converted to urea. The surplus of circulating NH$_4^+$ eventually reaches the central nervous system (CNS). Several
UCDs are characterized as being enzyme deficient. Therefore, individuals deficient in any of the key urea cycle enzymes could potentially develop a UCD. Likewise, individuals that have deficient or defective transport carrier’s will also be susceptible to the development of a UCD.

OTCD is the most common UCD. (12) OTCD is caused by a defect of the mitochondrial enzyme OTC. The human gene encoding the OTC is located on chromosome Xp21.1 thus inherited as an X-linked trait. Being an X-linked trait is crucial considering it identifies the fact that the OTC gene is mapped on the short arm of the X chromosome. More specifically, the OTC gene spans a region of 73kb and containing 10 exons as well as 9 introns of variable size. OTC mRNA contains 1062 nt and it encodes 40kDa protein precursor of 345 amino acids including the amino terminus leader peptide of 32 amino acids. Past studies have detected up to approximately 230 mutations in the OTC gene.

Bisanzi et al, investigated the study of genetic analysis by direct sequencing of OTC cDNA, genomic DNA, and enzymatic restriction analysis on the patients’ genomic DNA and total RNA isolated from peripheral blood lymphocytes. (12) OTCD male patient (1.1, with mild form and onset of three years) and eight OTCD manifesting carriers (2.1, 3.1, 4.1, 5.1, 6.1, 7.1, 8.1, 9.1) segregated from nine different families were studied. The male patient with onset of three years showed clinical symptoms of OTCD including but not limited to vomiting, confusion, and lethargy as well as usual laboratory findings. The manifesting carriers presented clinical heterogeneity varying from mild to severe symptoms. Age onset varied from 8 months to 30 months, while the age at diagnosis varied from the first symptom to 48 years. The results of Bisanzi et al’s study identify three new mutations and six previously reported mutations of the OTC gene. The first new mutation identified is a new amino acid substitution S132P due to the nucleotide change c.394T>C revealed by OTC gene exons sequencing in the male patient 1.1.
The second new mutation identified was in manifesting carrier 2.1 considering the OTC gene exons sequencing identified a new amino acid substitution E98K due to the c.292G>A nucleotide change. The third and last new mutation identified in manifesting carrier 3.1 was a the transversion IVS1-3C>G in the OTC gene by direct sequencing at the heterozygous state in the genomic DNA. The previously described mutations identified in six manifesting carriers (4.1, 5.1, 6.1, 7.1, 8.1, 9.1) are: N161S, R141Q, T178M, R92X, A208T, M268T, respectively. Therefore, it is evident that the OTC gene is subject to heterogeneous and frequent genetic lesions which may lead to partial or complete OTC enzymatic deficiency. Although, it is important to note that the clinical heterogeneity and the variability of the age of onset in OTCD manifesting carriers make diagnosis very difficult.

**Alternative Pathways**

The management of hyperammonemic episodes in UCD is achieved by dietary protein restriction, supporitive management of catabolic stress, and the use of sodium phenylacetate/benzoate (Ucephan) or sodium phenylbutyrate (Buphenyl). (13) Ucephan and/or Buphenyl stimulate the excretion of nitrogen as phyenylacetylglutamine and hippuric acid. Subsequently, buphenyl does not accumulate in plasma and is activated to is CoA ester within minutes thus it can then be converted to phenylacetyl CoA via beta-oxidation. Ultimately, this process relaces urea as a means for eliminating excess nitrogen compounds.

**CONCLUSIONS**

AA deamination results in the product of ammonium which is removed from the body via the urea cycle. For instance, human glutaminase-2 catalyzes the hydrolysis of glutamine to glutamate resulting in the one of the end products being ammonium. Subsequently, the
ammonium produced is utilized in the urea cycle and eventually excreted from the body as urea in order to keep NH₄⁺ levels safe.

The urea cycle has enzymes and transport carriers which are necessary for the urea cycle to work properly. A defect and/or deficiency of any key components of the cycle could lead to the development of a UCD. The most common UCD is known as the OTCD. OCTD is believed to be the most common due to its location on the Xp21.1 (short arm chromosome). Therefore, the OCT gene is subject to heterogeneous and frequent genetic lesions thus potentially leading to partial or complete OTC enzymatic deficiency. In OTCD cases, both Ucephan and Buphenyl are used to stimulate the excretion of nitrogen.
REFERENCES


