#### **EDUCATIONAL REVIEW**

# Hyperammonemia in review: pathophysiology, diagnosis, and treatment

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**Abstract** Ammonia is an important source of nitrogen and is required for amino acid synthesis. It is also necessary for normal acid base balance. When present in high concentra tions, ammonia is toxic. Endogenous ammonia intoxication can occur when there is impaired capacity of the body to excrete nitrogenous waste, as seen with congenital enzymatic deficiencies. A variety of environmental causes and medica tions may also lead to ammonia toxicity. Hyperammonemia refers to a clinical condition associated with elevated ammonia levels manifested by a variety of symptoms and signs, including significant central nervous system (CNS) abnormalities. Appropriate and timely management requires a solid understanding of the fundamental pathophysiology, differential diagnosis, and treatment approaches available. The following review discusses the etiology, pathogenesis, differential diagnosis, and treatment of hyperammonemia.

**Keywords** Hyperammonemia · Dialysis · Urea cycle defects · Treatment · Pathophysiology

#### Introduction

Ammonia is an important source of nitrogen and is required for amino acid synthesis. Nitrogenous waste results from

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the breakdown and catabolism of dietary and bodily proteins, respectively. In healthy individuals, amino acids that are not needed for protein synthesis are metabolized in various chemical pathways, with the rest of the nitrogen waste being converted to urea. Ammonia is important for normal animal acid base balance. During exercise, ammonia is produced in skeletal muscle from deamination of adenosine monophos phate and amino acid catabolism. In the brain, the latter processes plus the activity of glutamate dehydrogenase mediate ammonia production. After formation of ammonium from glutamine, a ketoglutarate, a byproduct, may be degraded to produce two molecules of bicarbonate, which are then available to buffer acids produced by dietary sources. Ammonium is excreted in the urine, resulting in net acid loss. The ammonia level generally remains low (<40 mmol/L) due to the fact that most ammonia produced in tissue is converted to glutamine. Glutamine is also excreted by the kidneys and utilized for energy production by gut cells, which convert the nitrogen byproduct into alanine, citrulline, and ammonia, which are transported to the liver via the bloodstream. Ammonia enters the urea cycle in hepatocytes or is ultimately converted to glutamine. [1 5]

Ammonia is toxic when present in high concentrations. Endogenous ammonia intoxication can occur when there is impaired capacity of the body to excrete nitrogenous waste, as seen with congenital enzymatic deficiencies. Patients with urea cycle defects (UCD), organic acidemias, fatty acid oxidation defects, bypass of the major site of detoxification (liver) (such as that seen in cirrhosis), Reye syndrome, postchemotherapy, or exposure to various toxins and drugs can all present with elevations in ammonia. Delayed diagnosis or treatment of hyperammonemia, irrespective of the etiology, leads to neurologic damage and potentially a fatal outcome, and thus it becomes a medical emergency when present. [6]

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#### Ammonia toxicity (pathogenesis)

Hyperammonemia refers to a clinical condition characterized by elevated serum ammonia levels and manifests with hypotonia, seizures, emesis, and abnormal neurologic changes (including stupor). It may also exert a diabetogenic effect mediated by ammonia inhibition of the insulin secretion induced by a glucose load [7]. Hyperammonemia can cause irreparable damage to the developing brain, with presenting symptoms such as posturing, cognitive impairment (mental retardation), seizures, and cerebral palsy. The total duration of hyperammonemic coma and maximum ammonia level (but not the rapidity of ammonia removal), is negatively correlated with the patient's neurological outcome. This is concerning when ammonia levels at presentation exceed 300 mmol/L. If left untreated, the outcome can be fatal. [7, 8]

Pathologic brain changes induced by hyperammonemia

Deaths secondary to hyperammonemia reveal upon *autop sia cadaverum* brain edema, brainstem herniation, astrocytic swelling, and white-matter damage [8]. Neuropathologic findings seen in children with hyperammonemia include ventriculomegaly, cerebral cortical atrophy, basal ganglia lesions, neuronal loss, gliosis, intracranial bleed, and areas of focal cortical necrosis and myelination deficiencies. Electroencephalogram (EEG) denotes slow delta-wave activity, consistent with a metabolic disturbance [9–19]. The brains of neonates and infants with hyperammonemia demonstrate areas of cortical atrophy or gray/white matter hypodensities, which are remnants of lost neuronal fibers. There is also an increased number of oligodendroglia and microglia in striatum. [17]

Amino acid disturbances in the brain

#### Glutamine

Glutamine synthesis mediated by the astrocytic enzyme glutamine synthetase is the major pathway for ammonia detoxification in the brain and cerebrospinal fluid. In conditions in which excess ammonia exists, brain osmotically active glutamine concentrations increase, leading to astrocytic damage and swelling. Consequently, the astrocyte promotes intercellular glutamate release, which decreases the glutamate intracellular pool and ultimately leads to cell death of the glutamatergic neurons [17, 20–22]

#### Arginine

Although arginine is an essential amino acid for the fetus and the neonate, they still synthesize part of it through intestinal expression of carbamyl phosphate synthetase -1 (CPS), ornithine transcarbamylase (OTC), argininosuccinate synthetase (AS), and argininosuccinase (AL). In the adult, arginine is synthesized through two pathways: in the intestine, CPS-1 and OTC synthesize citrulline; and AS and AL in the renal proximal tubules synthesize arginine from citrulline. Thus, patients with UCD (except Arginase deficiency I) have low serum arginine levels and need this amino acid to be replaced, although citrulline is a better replacement choice for patients with CPS-1 and OTC [17, 23]

Alterations in neurotransmission systems

Glutamatergic system

An excess of extracellular glutamate accumulates in brain when the latter is exposed to ammonia. The mechanism involves astrocytic swelling, pH and calcium (Ca<sup>2+</sup>)-dependent release of glutamate by astrocytes, inhibition of glutamate uptake by astrocytes through inhibition of the glutamine aspartate transporter (GLAST), and excess depolarization of glutamatergic neurons. Excess extracellular glutamate secondary to ammonia exposure promotes toxic cellular hyperexcitability through activation of N-methyl-D-aspartate (NMDA) receptors and leads to alteration in nitric oxide (NO) metabolism, disturbances in sodium/potassium adenosine triphosphatase (Na<sup>+</sup>/K<sup>+</sup>-ATPase), (causing neuronal sodium/potassium influx), ATP shortage, mitochondrial dysfunctions, free-radical accumulation, and oxidative stress, leading ultimately to cell death. Toxic stimulation of NMDA receptors can also be caused by quinolinic acid, which is an oxidation product of tryptophan acting on NMDA receptors and plays a role in the neurotoxicity seen in UCD patients (associated with death of spiny neurons in the striatum). Tryptophan and quinolinic acid concentration increases in brain and cerebrospinal fluid, respectively, in the presence of hyperammonemia. [17, 24-28]. In experimental animal models, as a protective cellular mechanism against excitotoxicity, a reduction in NMDA receptors is noted secondary to the increased release of glutamate. Nevertheless, in patients with UCD, despite the downregulation of NMDA receptors, there is still a certain degree of cellular toxicity that leads to cell death. Interestingly, prolonged cortical neuron survival has been reported to occur in hyperammonemic animals given the NMDA receptor antagonists MK-801 and 2-amino-5-phosphonovaleric acid (APV) [29]. The glutamatergic stimuli secondary to prolonged brain exposure to ammonia can also affect activation of other neurotransmission systems, such as gamma-aminobutyric acidd (GABA) or benzodiazepine receptors [30]



#### Cholinergic system

This system plays a key role in cognitive development. Cholinergic neurons loss, with decreased choline acetyltransferase (ChAT) activity level, has been observed in animal models with hyperammonemia. [17, 31]

#### Serotonergic system

Tryptophan (precursor for serotonin) and 5-hydroxyindoleacetic acid (5-HT) (metabolite of serotonin) are elevated in cerebrospinal fluid of children with UCD. Experimental hyperammonemic animal models demonstrate alterations in serotonergic activity through loss of 5-HT2 receptors and an increase in 5-HT1A receptors. These physiologic responses may play a role in anorexia and sleep disturbances noted in children with UCD [32–34]

#### Cerebral energy deficit

Hyperammonemic experimental animal models develop increased superoxide production and decreased activities of antioxidant enzymes in brain cells. Blockage of nitric oxide synthase by nitroarginine or NMDA receptor antagonists prevent the latter changes, suggesting therefore that ammonia-induced oxidative stress is due to the increased nitric oxide formation as a consequence of NMDA receptor activation [34]. Indeed, excessive formation of nitric oxide seen with ammonia exposure impairs mitochondrial respiration, depletes ATP reserves, and increases free radicals and oxidative stress, which ultimately leads to neuron death [17, 26]. Creatine, too, plays a key role in cellular energy formation and is decreased in the brain cells of hyperammonemic animals due to altered creatine transport and synthesis. Acetyl-L-carnitine enhances restoration of ATP and phosphocreatine levels in ischemia and has been reported to enhance the recovery of cerebral energy deficits caused by hyperammonemia, thus having a neuroprotective effect (through restoration of cytochrome C oxidase activity and free-radical scavenging) [35-37]. Cotreatment with creatine (while there is exposure to ammonia) protects axonal growth, thereby suggesting a possible neuroprotective role for this substance. [17, 38]

#### Cerebral exposure

Once the brain has been exposed to ammonia, intracerebral endogenous protective mechanisms that prevent or limit brain damage are triggered. Astrocytes express an injury-associated survival protein—ciliary neurotrophic factor (CNTF)—which is upregulated by ammonia through p38 mitogen-activated protein kinase (MAPK) activation. Ammonia activates a variety of kinase pathways including extracellular signal

regulated kinase (ERK)1/2, stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and p38 in astrocytes. Phosphorylation of ERK1/2 and p38 are responsible for ammonia-induced astrocyte swelling, and phosphorylation of SAPK/JNK and p38 are responsible for ammonia-induced inhibition of astrocytic uptake of glutamate. Adjunct treatment with CNTF has shown to induce protective effects on oligodendrocytes by reversing the demyelination induced by hyperammonemia [17, 39, 40]

#### K<sup>+</sup> and water channels

Although the brain edema that develops in hyperammonemic patients with UCD has been thought to occur secondary to astrocytic swelling, recently, this has been challenged. In astrocytes of hyperammonemic animals, it has been noted that a reduction of proteins that regulate the K<sup>+</sup> and water transport at the blood-brain barrier, including connexin 43, aquaporin 4 (aquaporins are membrane proteins that mediate water movement across the membrane), and the astrocytic inward-rectifying K<sup>+</sup> channels Kir4.1 and Kir5.1, allows ammonia to easily permeate through the aquaporin channels. In the setting of hyperammonemia, downregulation of aquaporins occurs (reflecting a protective mechanism of astrocytes to prevent ammonia entering the cell), but simultaneously, the excretion of K<sup>+</sup> and water is altered, and an increase in brain extracellular K<sup>+</sup> and water develops, contributing to brain edema formation [41, 42].

#### Differential diagnosis of hyperammonemia

Diagnostically, the presence of acidosis, ketosis, hypoglycemia, and low bicarbonate levels indicate that the root cause of hyperammonemia is probably due to an organic acidemia, systemic carnitine deficiency, Reye syndrome, toxins, drug effect, or liver disease. On the other hand, if there is normal lactate and no metabolic acidosis associated with the hyperammonemia, then UCD, dibasic aminoaciduria, or transient hyperammonemia of the newborn are more likely. The association of elevated liver enzymes with hyperammonemia is most consistent after insult from hepatotoxins, Reye syndrome, or carnitine deficiency [6] (Table 1).

#### Urea cycle disorders

The urea cycle produces arginine by de novo synthesis. Six enzymatic reactions comprise the cycle, which occurs in the liver. The first three reactions are located intramitochondrially and the rest are cytosolic. The enzymatic substrates are ammonia, bicarbonate, and aspartate. After each turn of the cycle, urea is formed from two atoms of nitrogen (see Fig. 1) [43]. Key enzymes in the urea cycle include CPS-1,



Table 1 Differential diagnosis of hyperammonemia

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Causes	Disease
Urea cycle disorders	Argininosuccinic aciduria
	Citrullinemia
	Carbamyl Phosphate Synthetase (CPS) deficiency 1
	Ornithine transcarbamylase deficiency
	Argininemia
Organic acidemias	Propionic acidemia
	Methylmalonic acidemia
	Isovaleric acidemia
	Maple syrup urine disease
	Dibasic aminoacidurias
	Type 1
	Type 2 (Lysinuric protein intolerance)
	Hyperammonemia hyperornithinemia homocitrullinuria
	Transient hyperammonemia of the newborn
Drug related causes	Salicylates
	Carbamazepine
	Valproic acid
	Topiramate
	Tranexamic acid
	Chemotherapeutic agents:
	Aspariginase 5 fluorouracil
	Rituximab
Miscellaneous causes	Hypoglycin intoxication
	Pregnancy
	Distal Renal Tubular Acidosis (RTA)
	Carnitine transport defects
	Urinary tract dilatation
	Reye's syndrome

OTC, AS, AL, and arginase. There are cofactors necessary for optimal enzyme activity, the most clinically important of which is N-acetyl glutamate (NAG). In UCD, there is an increase in glutamine, which transports nitrogen groups to the liver for ammonia formation within hepatic mitochondria, and ammonium combines with bicarbonate in the presence of NAG to form carbamyl phosphate. If NAG is absent, hyperammonemia develops [1, 6]

The presence of an enzymatic defect in the urea cycle results in arginine becoming an essential amino acid (except in arginase deficiency), and waste nitrogen accumulates mainly as ammonia and glutamine [6] Measurement of plasma amino acids aids in differentiating between the different urea cycle enzyme deficiencies. Citrulline is the product of CPS and OTC and the substrate for AS and AL, thus its value is of critical importance. In CPS and OTC deficiencies, plasma citrulline levels are low. These two defects are differentiated

by the fact that orotic acid is elevated in OTC deficiency and absent in CPS deficiency. In AS (citrullinemia) and AL (argininosuccinic aciduria) deficiencies, plasma citrulline levels are increased (see Fig. 2). If the serum citrulline level is normal, then disorders such as argininemia, lysinuric protein intolerance, and the hyperammonemia-hyperornithinemia-homocitrullinuria syndrome should be considered [6]

The prevalence of UCD is estimated at 1:8,200 in the USA. The overall incidence of defects presenting clinically is estimated at approximately 1 in 45,000 live births [1, 44]. Each specific disorder is considered below.

Argininosuccinic aciduria: argininosuccinase deficiency 1

The genetic deficiency responsible for this disorder is located on chromosome 7-q11.2. The diagnosis is suspected by observing hyperammonemia without acidosis in the presence of increased AL levels in plasma and urine. Plasma citrulline is also elevated. Measuring AL activity in erythrocytes or fibroblasts confirms the diagnosis. Distinguishing clinical features include mental retardation, trichorrhexis nodosa (fragile hair with a nodular appearance), and an erythematous maculopapular skin rash. The latter two are associated with arginine deficiency and disappear with arginine supplementation. Liver involvement is characterized by hepatomegaly and elevated transaminases, and the associated histopathology displays enlarged hepatocytes and fibrosis [6, 45]

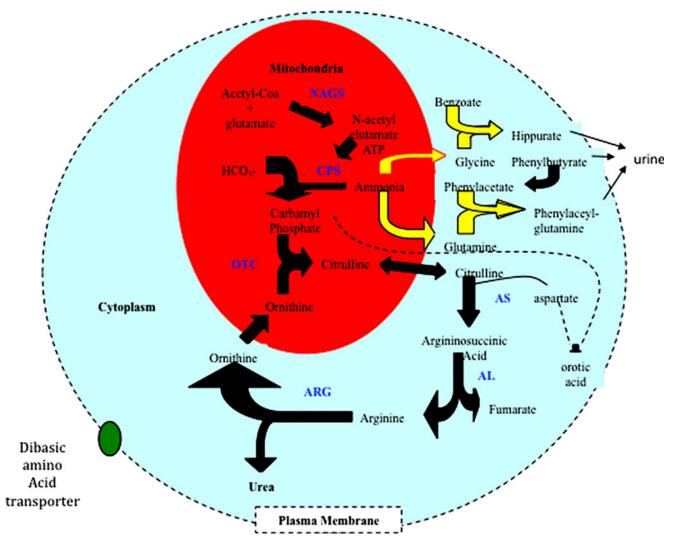
Citrullinemia: argininosuccinate synthetase deficiency

The genetic deficiency responsible for this disorder is located on chromosome 9q34. Common findings include hyperammonemia and low serum urea nitrogen and arginine levels, but the pathognomonic finding is a markedly elevated plasma citrulline level in the absence of AS. Affected individuals are able to partially incorporate the waste nitrogen into urea-cycle intermediates, which makes treatment easier [6, 45]

Carbamyl phosphate synthetase (CPS) deficiency 1

This represents the most severe form of UCD, with early development of hyperammonemia in the neonatal period, although it can also become apparent in adolescence. The genetic deficiency responsible for this disorder is located on chromosome 2q35. Biochemical findings in this entity include recurrent hyperammonemia, absent citrulline, and low levels of arginine, urea nitrogen, and urinary orotic acid. Diagnosis is confirmed by demonstrating <10% normal CPS activity in hepatic, rectal, or duodenal tissue [6, 45, 46].





**Fig. 1** Urea cycle pathway. *Black arrows* indicate primary pathway. *Yellow arrows* show alternative pathways used to eliminate nitrogen in patients with urea cycle defects. Enzymes are in *blue*. *CPS* carbamoylphosphate synthetase, *OTC* ornithine transcarbamoylase,

AS argininosuccinate synthetase, AL argininosuccinate lyase, ARG arginase, NAGS N acetylglutamate synthase. Reproduced with permission from Walters and Brophy [43]

#### Ornithine transcarbamylase deficiency

This represents the most common urea cycle enzyme defect, with an incidence of 1 in 14,000, and is the only one inherited as a sex-linked trait. OTC catalyzes the reaction of ornithine and carbamoyl phosphate to produce the amino acid citrulline. The *OTC* gene is located on the short arm of the X chromosome at Xp21, which is expressed specifically in the liver and gut. Around 350 pathological mutations have been reported, and in approximately 80% of patients, a mutation is found. In girls in whom there is partial expression of the X-linked OTC deficiency disorder, mild symptoms can be seen. Only 15% of female carriers are symptomatic, and most asymptomatic carriers have a normal IQ score. Biochemical diagnostic findings include hyperammonemia, absent serum citrulline,

and elevated urinary orotic acid. Liver biopsy helps confirm the diagnosis [1, 45, 47, 48]

Argininemia: arginase deficiency 1

Afflicted patients do not develop symptoms in infancy. Children develop toe walking as a characteristic feature, which progresses to spastic diplegia. Finding elevated serum arginine, urine orotic acid, and ammonia levels makes the diagnosis.

#### Organic acidemias

This group of autosomally recessive inherited disorders results from enzyme deficiencies in amino acid degradation pathways. Most of the disorders are due to



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