Chapter 3

Ammonia toxicity to the brain

Olivier Braissant

Various acquired or inherited disorders, such as urea cycle defects, can cause hyperammonemia. In contrast to the mature CNS, the developing brain is much more susceptible to the deleterious effects of hyperammonemia, which can provoke irreversible damage such as cortical atrophy, ventricular enlargement or demyelination, in turn leading to cognitive impairment, seizures and cerebral palsy. While the mechanisms involved are still poorly understood, recent studies show that CNS exposure to ammonium alters several amino acid pathways and neurotransmitter systems, cerebral energy metabolism, nitric oxide synthesis, oxidative stress and signal transduction pathways. These pathomechanisms are associated with alterations in neuronal differentiation and patterns of cell death in the CNS. These recent findings on the mechanisms of ammonia toxicity to the brain have further suggested some new neuroprotective strategies, including the use of *N*-methyl-D-aspartate receptor antagonists, nitric oxide inhibitors, creatine, acetyl-L-carnitine, ciliary neurotrophic factor or inhibitors of MAPK and glutamine synthetase (GS).

Ammonia is produced by amino acid metabolism and intestinal flora, and is mostly present as ammonium (NH_4^+) in physiological conditions. The urea cycle, fully expressed in the liver exclusively, maintains low serum NH_4^+ concentrations by converting NH_4^+ to urea prior to renal excretion. Although the CNS cannot convert NH_4^+ to urea, NH_4^+ is nevertheless maintained at low levels in the brain by the astrocytic enzyme GS, synthesizing glutamine (Gln) from glutamate (Glu) and NH_4^+ [1].

Excessive NH_4^+ is toxic for the CNS. In adults, liver failure results in hyperammonemia, which in turn leads to the potentially severe neuropsychiatric disorder hepatic encephalopathy (HE), characterized by altered mental status and coma. In the absence of irreversible cerebral edema, HE symptoms in adults are largely reversible when NH_4^+ returns to normal levels. In children, hyperammonemia is caused by numerous inherited or acquired disorders, among which the best known are inherited urea cycle disorders (UCD) [2,3]. The susceptibility of the developing brain to hyperammonemia leads to severe cognitive impairment, seizures and cerebral palsy; neonates and infants with severe hyperammonemia develop cortical atrophy, ventricular enlargement, demyelination or gray and white matter hypodensities [2,4,5]. The extent of irreversible damage depends on CNS maturational stage and on the magnitude and duration of NH_4^+ exposure [4–6].

Hyperammonemia in humans

While hyperammonemia can develop secondary to various inherited or acquired causes, much of our understanding of ammonia neurotoxicity in humans stems from patients with UCD.

Nonspecific symptoms common to the immature & mature CNS

Nonspecific symptoms are common in most UCD patients presenting in the neonatal period (poor feeding, vomiting, somnolence, irritability, tachypnea) [3]. As NH_4^+ rises in blood, hypothermia, lethargy and coma progress rapidly [2]. In cases of partial UCD, clinical presentation can occur as late as months or years postnatally and are often triggered by illness or catabolic stress. In this case, hyperammonemia is generally less severe and the symptoms are usually milder than in newborns. Patients with late-onset hyperammonemia can present with loss of appetite, cyclic vomiting, lethargy or behavioral abnormalities [7]. Mental retardation and learning difficulties are frequent.

Cerebral edema: a common feature of the NH_4^+-exposed CNS

In response to elevated serum NH_4^+, developing and mature CNS respond similarly: Gln content in astrocytes rises through increased GS activity, and astrocytes swell. Under high NH_4^+ levels, osmoregulation is insufficient and cerebral edema develops, affecting all areas of the brain. In its most severe form, increased intracranial pressure eventually leads to brain herniation. In advanced cerebral edema, seizures, abnormal posture and neuromuscular irritability are frequent. CNS edema first causes hyperventilation and respiratory alkalosis, later progressing to hypoventilation and apnea. Without any treatment, most infants die, while mental retardation is the norm in survivors [5,6]. Edema associated with HE can be followed by magnetic resonance spectroscopy (MRS) and MRI [8].

Irreversible effects of ammonia on the developing brain

Irreversible damage to the developing brain results in mental retardation in most surviving children with UCD [2,5]. Neonatal onset leads to the most severe brain damage and the lowest IQ score, with significant volume loss of different parts of the developing brain as assessed by later MRI. Diffuse cortical atrophy, lesions in the basal ganglia and thalamus, myelination

delay and injury of the oligodendro-axonal unit are frequent. Cerebral MRI in UCD neonates suggests that some of these lesions might already be acquired *in utero*.

If hyperammonemia is diagnosed before irreversible cerebral insults, patients may have a normal neurodevelopment. Many, however, remain mentally retarded or have learning difficulties [7]. Brain MRIs of late-onset UCD patients show cortical injury including acute ischemia, ventricular dilatation and myelination defects [8,9].

Only a few UCD cases have been analyzed by autopsy. Findings included microcephaly, shrinkage of hemispheres coupled with multiple cysts, ventricular dilatation, atrophy or necrosis of various brain nuclei or myelination defects. Microscopically, spongious brain tissue with extensive neuronal loss (in cortex and hippocampus particularly) was observed, together with gliosis and astrocytes with water-clear, oval nuclei characteristic of Alzheimer's type II astrocytes [10].

Experimental models to study NH_4^+ toxicity to the brain

During the last 15 years, various experimental models, both *in vivo* and *in vitro*, have been analyzed and developed, which have provided new knowledge regarding NH_4^+ toxicity to the brain.

In vivo: *Spf* mice, KO mice and rat models of hyperammonemia

Sparse-fur (*spf*) mice have a single point substitution in the ornithine transcarbamylase (*OTC*) gene, with X-linked transmission, mimicking the human disease. Two *spf* strains have been described: sparse-fur (*spf*) and sparse-fur with abnormal skin and hair (*spf*^{ash}). Hepatic OTC activity is 13% in *spf* and 5–10% in *spf*^{ash}, as compared with normal mice. Adult *Spf/Y* mice show NH_4^+ blood and brain levels increased by 1.5- and 5-fold, respectively [11]; neuropathologic studies in *spf* mice show similar brain alterations as those observed in OTC patients [12]. Several knock-out (KO) mice have been engineered to model UCDs: carbamoylphosphate synthetase I (CPS-I), argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL), and arginases I and II KO mice, as well as double-KO mice for arginases I+II. CPS-I mice die 36 h postnatally, with highly elevated plasma ammonia. ASS$^{-/-}$ and ASL$^{-/-}$ mice die a few days after birth, with plasma NH_4^+ increased fourfold. Arginase I and arginases I+II KO mice die from hyperammonemia 14 days postnatally, with a tenfold increase in plasma NH_4^+ [13].

Different rat models have been developed to analyze the effects of hyperammonemia on the CNS. Pregnant rats can be fed a diet containing NH_4-acetate from day 1 of pregnancy until weaning, followed by feeding the pups after weaning with a high NH_4^+-containing diet; alternatively, hyperammonemia can be induced in adult rats by intraperitoneal injections of NH_4^+-acetate, continuous intravenous infusion of NH_4Cl, intravenous urease infusion, administration of a NH_4^+-acetate-containing diet or surgical portocaval shunt coupled with NH_4^+ treatment [14]. These *in vivo* rat models are of particular interest to follow the hyperammonemic CNS by high-resolution MRS and MRI [15].

In vitro: monotypic brain cell cultures & organotypic mixed-cell cultures

NH_4^+ toxicity has been studied in monotypic primary cultures of neurons or astrocytes as well as in organotypic cultures of hippocampal rat brain slices. These models provide several clues regarding the mechanisms of cellular NH_4^+ toxicity, but they do not allow for the analysis of the effects of hyperammonemia on the developing CNS, especially with respect to the relationships between developing neurons and glia (see [1] for a review).

3D primary reaggregated brain cell cultures are an interesting alternative as a valid experimental model to study the effects of NH_4^+ on the developing CNS. These cultures, classified as organotypic and prepared from the brain of rat embryos, contain all types of brain cells (neurons, astrocytes, oligodendrocytes, microglia) and grow in a manner resembling *in vivo* CNS. In this model, hyperammonemia is mimicked by treating cultures with NH_4Cl. Compared with classical monotypic cultures, 3D brain cell cultures allow for the analysis of irreversible NH_4^+ toxicity in a model that mimics brain complexity at different maturational stages. These cultures are also a useful tool to examine the effects of hyperammonemia in isolation, devoid of the confounding variables found in animal models owing to the secondary effects of hyperammonemia (see [3] for a review).

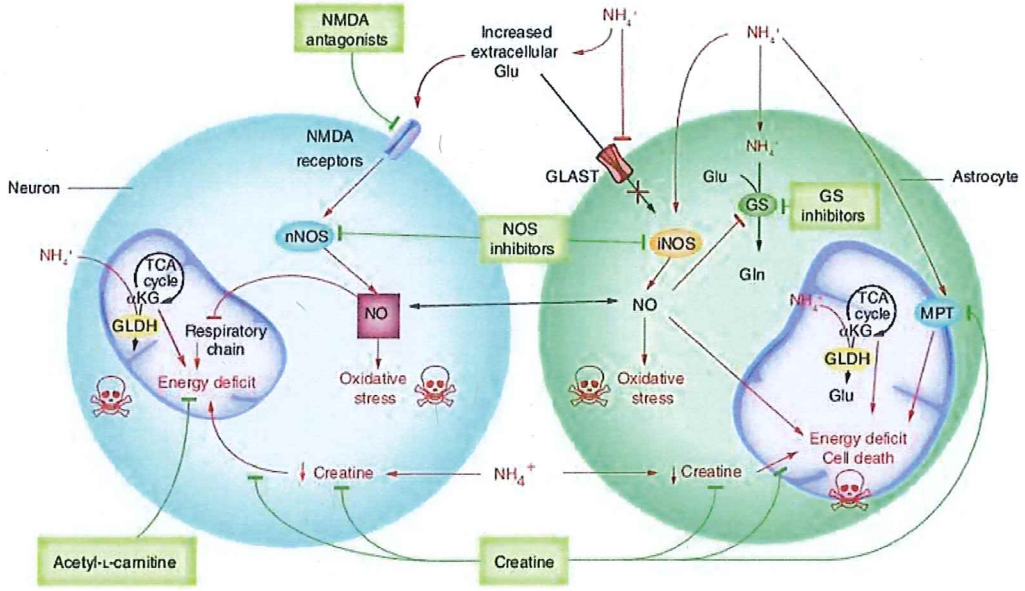
Mechanisms of CNS ammonium toxicity

Amino acids disturbances

By synthesizing Gln from NH_4^+ and Glu, the astrocytic enzyme GS is the major CNS pathway of NH_4^+ removal. Accordingly, hyperammonemia with high NH_4^+ levels increases Gln in brain cells, as seen in OTC patients, *Spf* mice, NH_4^+-infused rats and organotypic brain cell cultures (Figure 3.1) [15]. Gln is osmotically active and its NH_4^+-induced increase leads to cytotoxic edema by astrocyte swelling. Recent *in vivo* studies in rats under single injection or continuous infusion of NH_4^+, after longitudinal analysis by high-resolution MRS, have indeed shown that Gln in the CNS increases immediately after the initiation of NH_4^+ exposure, and continues to increase linearly during the NH_4^+ challenge, suggesting that no delay in Gln accumulation occurs; this also suggests an increase of anaplerosis coupled with the NH_4^+ detoxification pathway by GS [14,16]. Attenuating NH_4^+-induced edema in the brain by using GS inhibitors such as methionine sulfoximine has been proposed (Figure 3.1). Astrocyte swelling under NH_4^+ exposure may also be subsequent to Gln transport into mitochondria, where Gln may be

cleaved back to ammonia, producing reactive oxygen species and inducing mitochondrial permeability transition (MPT; 'Trojan Horse' hypothesis) [17].

Figure 3.1 NH_4^+ toxicity for the brain.



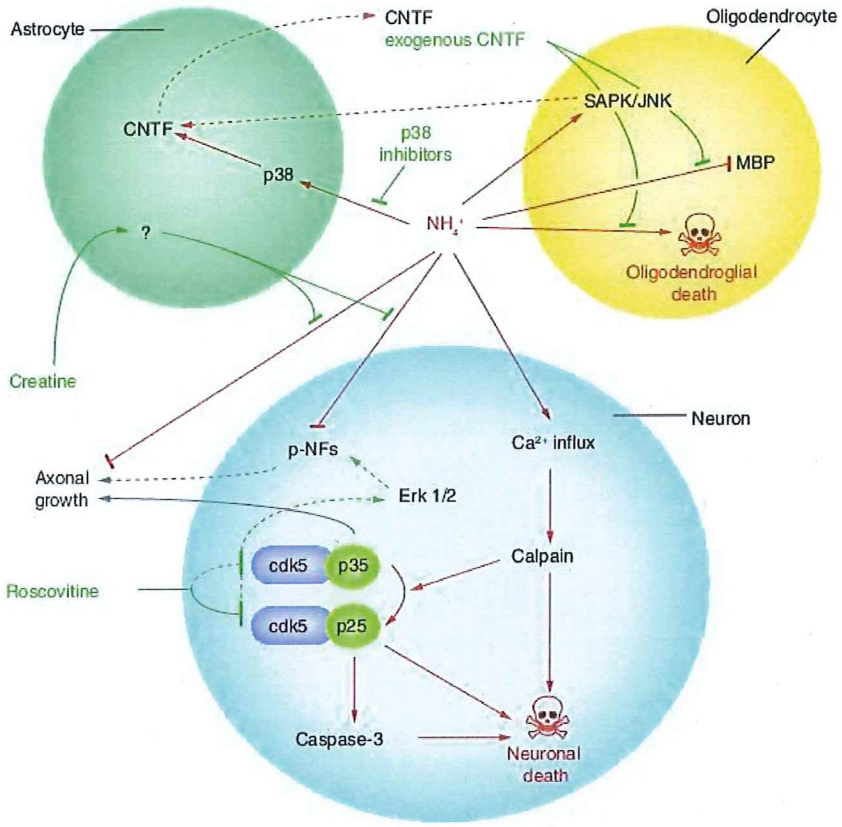
Toxic effects of NH_4^+ to neurons and astrocytes are shown in red. In particular, NH_4^+ exposure generates oxidative stress, energy deficit and cell death in the CNS through disturbances of the NO pathway, inhibition of the TCA cycle, opening of the MPT and secondary creatine deficiency. Possible protective effects of creatine, acetyl-L-carnitine, NMDA antagonists as well as GS and NOS inhibitors against NH_4^+ toxicity are shown in green.

αKG: α-ketoglutarate; GLAST: NA^+-dependent Glu/Asp transporter; GLDH: Glutamate dehydrogenase; Gln: Glutamine; Glu: Glutamate; GS: Glutamine synthetase; iNOS: Inductible nitric oxide synthase or NOS2; MPT: Mitochondrial permeability transition; NMDA: *N*-methyl-D-aspartate; nNOS: Neuronal nitric oxide synthase or NOS1; NO: Nitric oxide; NOS: Nitric oxide synthase; TCA: Tricarboxylic acid.

Astrocyte swelling can also cause a secondary release of Glu in the intercellular space, which, coupled with the conversion of Glu and NH_4^+ to Gln, can decrease intracellular pools of Glu and induce death of glutamatergic neurons [18]. Indeed, Glu is significantly decreased in the cerebral cortex of *Spf* mice and NH_4^+-exposed brain cell 3D cultures. To a lesser extent, NH_4^+ excess can also be detoxified by glutamate dehydrogenase converting α-ketoglutarate to Glu, leading to α-ketoglutarate depletion from the tricarboxylic acid cycle (Figure 3.1).

Patients with UCD (except arginase I deficiency) present with decreased plasma Arg, hence the indication of Arg supplementation [2,9]. As Arg is the precursor for NO and creatine (Cr) synthesis, decompensated UCD is associated with disturbances in NO and Cr metabolism, both in the CNS and periphery (Figures 3.1 &3.2). *Spf* mice display deficient Arg synthesis, as observed in the CNS of OTC patients. By contrast, intracellular Arg increases when brain cells with a normal Arg supply are exposed to NH_4^+. This has been shown in brain cell organotypic cultures, in rat cerebellar synaptosomes and in rat primary astrocytes. The NH_4^+-induced expression of ASS and ASL in astrocytes may also contribute to this process (reviewed in [3]).

Figure 3.2 Proposed mechanisms leading to brain cell death under NH_4^+ exposure, and effects on intracellular and extracellular signaling pathways.



Toxic effects of NH_4^+ are shown in red, while possible protective effects of creatine, roscovitine and exogenous CNTF are shown in green. NH_4^+ activates calpain, which can induce neuronal death directly. Activated calpain also cleaves p35 to p25 and activates caspase-3, causing neuronal death. Roscovitine decreases neuronal death by inhibiting cdk5/p25 and the subsequent caspase-3 activation. Creatine protects axonal growth under NH_4^+ exposure in a glial cell-dependent way. NH_4^+ activates MAPKs in brain cells, and particularly p38 in astrocytes, which increases their release of CNTF. Exogenous CNTF exerts a protective effect on oligodendrocytes, through SAPK/JNK. Full arrows show clearly demonstrated pathways, while dotted lines suggest possible pathways still to be fully characterized.

CNTF: Ciliary neurotrophic factor; Erk1/2: Extracellular signal-regulated kinases 1/2; MBP: Myelin basic protein; p-NF: Phosphorylated neurofilament; SAPK/JNK: Stress-activated protein kinase or c-Jun NH_2-terminal kinase.

Alterations in neurotransmission systems

NH_4^+ exposure leads to astrocyte swelling, pH- and Ca^{2+}-dependent Glu release from astrocytes, inhibition of Glu re-uptake by astrocytes (GLAST transporter) and excess depolarization of glutamatergic neurons [1,19]. This in turn induces excess extracellular Glu accumulation, which is excitotoxic essentially through *N*-methyl-D-aspartate (NMDA) receptor activation. NMDA receptor activation in turn leads to an array of metabolic alterations affecting NO metabolism and Na^+/K^+-ATPase. ATP shortage, mitochondrial dysfunction, free radical accumulation and oxidative stress ultimately ensue and lead to cell death (Figure 3.1) [3,20]. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and metabotropic glutamate receptors are also affected by NH_4^+ exposure. NH_4^+-induced glutamatergic excitotoxicity can in turn alter other neurotransmission systems (activation of γ-aminobutyric acid or benzodiazepine receptors) [21].

Hyperammonemia also impairs cholinergic and serotoninergic neurotransmissions, which can be imbalanced in UCD, in humans and rats under HE, in rats fed with NH_4^+, in *Spf* mice, as well as in NH_4^+-exposed 3D brain cell cultures [3,22].

Cerebral energy deficit

Spf mice show decreased ATP in the CNS coupled with lower cytochrome C oxidase expression and activity, suggesting that ATP reduction might be due to a slowing of the electron transport chain enzymes; the deficit in brain energy metabolites under hyperammonemia might also be due to tricarboxylic acid cycle inhibition via α-ketoglutarate dehydrogenase (Figure 3.1). Hyperammonemia also disturbs the Cr/phosphocreatine/creatine kinase system, which is essential for cellular energy through buffering and regeneration of ATP, both in the periphery and in the brain (Figure 3.1). *Spf* mice show decreased brain Cr, and NH_4^+ exposure generates a secondary Cr deficiency in brain cells [1,23]. Cr has been proposed to protect from NH_4^+-induced cerebral energy deficits and Cr is also neuroprotective by inhibiting MPT opening (Figure 3.1). Using Cr as a neuroprotective agent may be facilitated by the NH_4^+-induced activity of the Cr transporter SLC6A8 in the astrocytes surrounding the blood–brain barrier [23].

Primary astrocyte cultures, but not neuronal cultures, exposed to NH_4^+ show opening of MPT [24], leading to altered oxidative phosphorylation, cessation of ATP synthesis, production of reactive oxygen species and cell death (Figure 3.1). Increased

superoxide production and decreased antioxidant enzyme activity were also observed in the brain of NH_4 -infused rats. This was prevented both by nitroarginine-mediated NO synthase (NOS) inhibition and by NMDA receptor antagonists, suggesting that NH_4^+-induced oxidative stress is at least in part due to increased NO formation through excessive NMDA receptor activation. Acetyl-L-carnitine was also proposed as neuroprotective under NH_4^+ exposure, as it may restore cytochrome C oxidase activity and protect brain cells against oxidative stress by free-radical scavenger action (Figure 3.1) [14,25,26].

Alteration of NO synthesis

NO is produced from Arg by NOS, which constitutes the citrulline–NO cycle in concert with ASS and ASL; the citrulline–NO cycle is well expressed in the CNS. NO metabolism in the brain is affected in a number of ways by NH_4^+ exposure. Effects vary depending on whether exposure is acute or chronic, on brain cell type, and whether Arg supply is normal or decreased (Figure 3.1).

In neurons under normal Arg supply, activation of NMDA receptors by NH_4^+ exposure activates neuronal NOS (nNOS or NOS1) and stimulates NO synthesis. This was demonstrated both *in vivo* and *in vitro*. Likewise, in astrocytes, NH_4^+ exposure induces inducible NOS (iNOS or NOS2) expression and enhances NO synthesis. This is also coherent with the observation that brain cell cultures exposed to NH_4^+ increase their Arg content and induce expression of ASS and ASL in astrocytes, thereby stimulating the citrulline–NO cycle [1]. The excessive formation of NO under NH_4^+ exposure can impair mitochondrial respiration by interacting with superoxide anions, leading to formation of highly toxic peroxynitrites. Neuronal and glial death under NH_4^+ exposure can thus occur from secondary ATP depletion, increased free radicals and oxidative stress. Moreover, NH_4^+-induced production of NO can inhibit GS, thus potentiating the consequences of hyperammonemia on the CNS [27]. Recent data also suggest that ammonia may alter blood–brain barrier permeability through a mechanism involving increased NO and oxidative stress in the brain microcapillary endothelium, thus contributing to vasogenic edema induced in acute liver failure conditions [28].

NH_4^+ effects on NO are different in UCD. Except for arginase I deficiency, other UCDs are associated with Arg shortage, thus impairing the citrulline–NO cycle. Accordingly, NOS activity and NO synthesis are decreased in the CNS of *Spf* mice. Arginase I deficiency presents with elevated plasma Arg levels, thereby inducing an upregulation of NO metabolism [9].

Impairment of axonal & dendritic growth

Cortical atrophy, ventricular enlargement, and gray and white matter hypodensities are characteristic neuroimaging findings in children having suffered from hyperammonemia, suggesting neuronal fiber loss or defects in neurite outgrowth. Alteration of neurite outgrowth by NH_4^+ exposure might be triggered by dysregulation of cytoskeletal elements [3]. Rats fed with NH_4^+-acetate develop decreased phosphorylation of the dendritic microtubule-associated protein 2 (MAP-2), together with an increase of MAP-2 binding to microtubules. NH_4^+ exposure of 3D developing brain mixed-cell primary cultures decreases medium-weight neurofilament (NFM) expression and phosphorylation, and inhibits axonal growth. This occurs only in developing brain cells but not after neuronal differentiation, in line with the clinical differences between pediatric and adult patients. Cr cotreatment under NH_4^+ exposure appears to protect axonal growth in NH_4^+-exposed 3D organotypic cultures of rat brain cells, where it restores NFM expression and phosphorylation in a glial cell-dependent manner (Figure 3.2) [15]. In the same model, Cr also prevent the loss of cholinergic neurons.

Cell death & signaling transduction pathways

Irreversible damage caused by NH_4^+ exposure on the developing CNS is consistent with brain cell death, which was shown in neurons and oligodendrocytes of NH_4^+-exposed organotypic brain cell cultures [29]. In particular, NH_4^+ induces neuronal apoptosis through activation of caspases and calpain. NH_4^+-induced calpain activation cleaves the cdk5 activator p35 to p25, which induces neurodegeneration (Figure 3.2). Inhibitors of the cdk5 pathway, such as roscovitine, may be used to protect neurons from NH_4^+-induced death.

NH_4^+ exposure may also trigger endogenous protective mechanisms to prevent or limit brain damage. Ciliary neurotrophic factor (CNTF), an injury-associated survival factor expressed by astrocytes, is upregulated by NH_4^+ through p38 MAPK activation [30], with secondary roles of the two other MAPKs, SAPK/JNK and Erk1/2 in oligodendrocytes and neurons, respectively (Figure 3.2). Erk1/2, SAPK/JNK and p38 are activated in primary astrocytes by NH_4^+, and phosphorylation of Erk1/2 and p38 appears responsible for NH_4^+-induced astrocyte swelling, while phosphorylation of SAPK/JNK and p38 is involved in NH_4^+-induced inhibition of Glu uptake by astrocytes [31]. Inhibitors of the p38 pathway and exogenous CNTF have been proposed as neuroprotective agents to avoid some of the deleterious effects of NH_4^+ on astrocytes and oligodendrocytes, respectively (Figure 3.2).

Channels & transporters

Brain edema due to hyperammonemia is thought to occur essentially through astrocyte swelling. In primary astrocyte cultures exposed to NH_4^+, the Na^+–K^+–Cl^- cotransporter-1 (NKCC1) is activated in response to NH_4^+ exposure, thus increasing water entry in astrocytes. It has also been shown that connexin 43 (Cx43), aquaporin 4 (Aqp4) and the astrocytic inward-rectifying K^+

channels Kir4.1 and Kir5.1 are decreased in astrocytes of *Spf* mice [32]. Kir4.1 is also downregulated in the cortex of rats with liver failure. NH_4^+ is known to cross some aquaporins, which might link cerebral metabolism to volume control. Astrocytes may respond to elevated blood NH_4^+ by inducing a protective downregulation of Cx43, Aqp4 and Kir4.1/Kir5.1. Thus, slowing NH_4^+ influx but decreasing water and K^+ efflux from CNS may come with a price: increased brain extracellular K^+ and water lead to brain vasogenic edema (see [15] for a review, and references therein).

Impairment of cognitive performance

As observed in UCD patients, several animal models with hyperammonemia show impaired cognitive performance. *Spf* mice show cognition deficits during hyperammonemic episodes. Prenatal and neonatal exposure to NH_4^+ in rats appears to impair memory or conditioned learning, while no such effect is observed in adults. Long-term potentiation, considered as the molecular basis of some forms of memory and learning, is significantly decreased in hippocampal slices from rats prenatally and neonatally exposed to ammonia. Long-term potentiation impairment in hyperammonemia might be responsible for some of the cognitive alterations found in hyperammonemic rats and *Spf* mice, and could be involved in some aspects of mental retardation in pediatric patients exposed to NH_4^+ (see [1] for a review, and references therein).

Conclusion

Hyperammonemia during brain development is associated with neuronal cell loss and cerebral atrophy, leading to mental retardation and cerebral palsy in pediatric patients with, for example, UCD. In survivors, the toxic effects of NH_4^+ on the CNS can be observed with brain tissue atrophy, ventricular enlargement or gray and white matter hypodensities, due to cytotoxic brain edema, cell death, impairment of neurite outgrowth, defects in nerve cell migration or hypomyelination. The NH_4^+-induced pathogenic mechanisms involve disturbances of amino acid pathways, neurotransmission, brain energy, NO synthesis, axonal and dendritic growth, or signal transduction systems (Figures 3.1 &3.2). These toxic effects of NH_4^+ are specific to the developing brain, as neuronal damage is not observed in the CNS of adult patients with hyperammonemia due to liver failure. In the mature brain, the main effect of NH_4^+ toxicity is the rise of Gln in astrocytes, while osmoregulation is insufficient and cerebral edema develops, affecting all CNS areas. Why the developing brain is so vulnerable to fluctuations in serum NH_4^+ levels remains to be elucidated. Apart from using NH_4^+ scavengers, new neuroprotective strategies have been proposed, making use of NMDA receptor antagonists, NOS inhibitors, Cr, acetyl-L-carnitine, inhibition of CDK5/p25, CNTF or inhibitors of MAPKs and GS (Figures 3.1 &3.2).

Understanding ammonia toxicity for the CNS, or unraveling new therapeutic targets to protect the brain from hyperammonemia, requires experimental approaches focusing on the CNS in its multicellular complexity (*in vivo* rodent models; *ex vivo* or *in vitro* 3D organotypic brain cell cultures). The extraordinary development of high-resolution MRS should contribute significantly to directing future investigations, in particular by focusing on intra- and extra-cellular metabolic and signaling pathways disturbed in the brain during NH_4^+ exposure.

Summary

- The developing brain is much more susceptible to the deleterious effects of ammonium than the adult brain. Hyperammonemia provokes irreversible damage to the developing CNS.
- Recent studies show that ammonium exposure alters several amino acid pathways and neurotransmitter systems, cerebral energy metabolism, nitric oxide synthesis, oxidative stress and signal transduction in developing brain cells.
- Alterations in neuronal differentiation and patterns of cell death are among the consequences of ammonia toxicity to the developing brain.
- New neuroprotective strategies have been proposed, including the use of *N*-methyl-D-aspartate receptor antagonists, nitric oxide inhibitors, creatine, acetyl-L-carnitine, ciliary neurotrophic factor or inhibitors of MAPKs and glutamine synthetase.
- Understanding ammonia toxicity for the CNS, or unraveling new therapeutic targets to protect the brain from hyperammonemia, requires experimental approaches focusing on the CNS in its multicellular complexity.

Key Point Box 3.1

The developing brain is much more susceptible to ammonia toxicity than the mature one, and irreversible damage can occur more easily.

Key Point Box 3.2

Cerebral edema due to ammonium-induced glutamine increase in astrocytes is a common feature of hyperammonemic patients.

Key Point Box 3.3

Ammonium induces excitotoxicity through increased extracellular glutamate and activation of *N*-methyl-D-aspartate receptors.

Key Point Box 3.4

Ammonium induces cerebral energy deficit through ATP decrease, tricarboxyclic acid cycle inhibition and secondary creatine deficiency.

Key Point Box 3.5

Ammonium-induced defect of brain cell migration and differentiation can lead to neuronal death during development.

Financial & competing interests disclosure

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About the Author

Olivier Braissant

Olivier Braissant obtained his PhD at the University of Lausanne, Switzerland, in 1994. After a postdoc on nuclear receptors in the CNS, he moved in 1997 to the University Hospital of Lausanne in the Service of Biomedicine. He is Head of a research section focused on brain development, and how various inborn errors of metabolism, including urea cycle diseases, can affect it.