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Review article

Brain mitochondria as potential therapeutic targets for managing hepatic encephalopathy

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ABSTRACT

Hepatic encephalopathy (HE) is a critical clinical complication. There is a consensus that ammonia plays a pivotal role in the pathogenesis of HE. Ammonia is a neurotoxin which induces a wide range of functional disturbances in the central nervous system (CNS). On the other hand, HE is associated with the increased free radical formation, tissue inflammation, disturbed neurotransmission, astrocytes swelling, brain edema, and brain herniation. In view of the severe CNS complications ensued HE, potential therapeutic points of intervention need to be vigorously investigated. A role for CNS mitochondrial damage and energy crisis has been considered in HE. It has been found that ammonia induces mitochondrial impairment as a result of a multifaceted interaction of different signaling molecules. Hence, ammonia-induced mitochondrial injury and compromised brain energy metabolism might play a vital role in the pathogenesis of ammonia neurotoxicity. This review focuses on the concept that mitochondrial dysfunction and cellular energy crisis indeed plays a critical role in the pathogenesis of hyperammonemia-induced brain injury. Further, it will highlight the potential therapeutic value of mitochondrial protecting agents and energy providers in the management of HE. The data collected in this review might provide clues to new therapeutic interventions aimed at minimizing HE-associated complications.

1. Ammonia as a neurotoxic molecule

Hepatic encephalopathy (HE) is the disturbance of CNS due to liver failure [1]. HE is characterized by several symptoms including cognitive, psychiatric, and motor deficits [2]. Although the clear mechanism (s) involved in the pathogenesis of HE remain to be determined, there is agreement that ammonia is the primary molecule responsible for HE-induced CNS complications [3]. The key detoxification process of ammonia is its incorporation in the urea, which mainly takes place in the liver. When liver function is compromised, its capacity of ammonia fixation will be impaired [1]. Consequently, serum and brain ammonia reaches a toxic level. HE is a complex syndrome which might lead to permanent brain injury, coma, and even patient death if not appropriately managed [1].

As mentioned, the urea cycle is the most efficient path for ammonia detoxification. In tissues which lack an efficient and functional urea cycle (*e.g.*, Brain), blood-born ammonia is metabolized to glutamine (Gln) by the glutamine synthase (GS) enzyme [4]. Gln production from ammonia mainly takes place in the brain astrocytes [4]. Although Gln formation seems to be a detoxification process, several investigations indicate that Gln accumulation in the brain tissue might also lead to several problems during HE [5–8].

Ammonia induces a wide range of complications in the CNS. Alteration in the intracellular pH, CNS electrophysiological derangements, increased glutamatergic neurotransmission (excitotoxicity), oxidative/nitrosative stress, neuroinflammation, astrocytes swelling, brain edema, and brain herniation all are attributed to ammonia as a neurotoxic molecule [9–11]. Hence, ammonia affects brain function by multiple mechanisms. On the other hand, ammonia-induced mitochondrial dysfunction and energy crisis seems to play a relevant role in the pathogenesis of HE-induced CNS injury.

CNS is highly and continuously dependent on the mitochondrial energy (ATP) production. Therefore, any agent which interfere with oxidative phosphorylation might lead to the energy crisis and CNS damage. Ammonia-induced mitochondrial dysfunction could play a pivotal role in altered cerebral function during HE. Hence, targeting this vital organelle might provide a promising therapeutic point of intervention in the management of ammonia-induced CNS complications. This review is devoted to a discussion of the role of brain mitochondrial dysfunction and energy crisis in HE.

In the forthcoming sections first, an intriguing hypothesis about the mechanism of ammonia accumulation in mitochondria is discussed.

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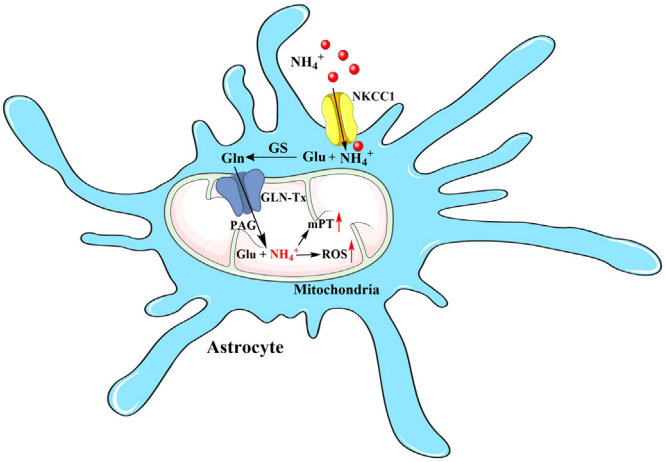

Fig. 1. A diagrammatic outline illustrating the "Trojan Horse" hypothesis of ammonia accumulation in mitochondria and its consequent mitochondrial impairment. Glu: Glutamate; Gln: Glutamine; GS: Glutamine synthase; GLN-Tx: Glutamine transporter; PAG: Phosphate-activated glutaminase; mPT: Mitochondrial permeability transition; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; NH_4^+: Ammonium ion; NKCC1: Na-K-Cl co-transporter 1. The same mechanism might occur in neuronal mitochondria.

Then, the interplay between high brain ammonia level and mitochondrial dysfunction in CNS is reviewed. Finally, the potential therapeutic value of mitochondrial protecting agents and energy providers against HE-induced CNS injury are highlighted.

2. The "Trojan Horse" hypothesis of ammonia accumulation in the mitochondrial matrix

Glutamine (Gln) synthesis is the primary mechanism for ammonia removal in the brain [4]. This process mainly takes place in astrocytes by the enzyme glutamine synthase (GS) [4] (Fig. 1). Although Gln synthesis seems to be a detoxification process for ammonia, increased Gln level in astrocytes is likely to be associated with mitochondrial dysfunction [12–14] (Fig. 1). Hence, some investigations indicated a pathogenic role for Gln in HE [5–8]. Furthermore, it seems that Gln acts as an osmolyte which leads to an influx of water into the cells [12,15]. Although the role of Gln in brain edema is controversial, this amino acid might be attributed to astrocytes swelling and brain edema during HE [12,15].

The "Trojan Horse" hypothesis of ammonia-induced neurotoxicity is one of the most exciting theories for the mechanism of ammonia-induced mitochondrial dysfunction [6,16] (Fig. 1). In this theory, it is proposed that Gln-derived ammonia within the mitochondrial matrix interfere with mitochondrial function, a mechanism which might finally lead to astrocytes swelling and brain edema [6,16] (Fig. 1).

It has been documented that the activation of a Na-K-Cl co-transporter (NKCC1) is a key step for ammonia accumulation in astrocytes (Fig. 1) [17–19]. NKCCs are a class of transporters which play an important role in regulating cell volume in many conditions [17,18]. GS metabolizes ammonia and glutamate (Glu) to Gln upon ammonia accumulation in brain astrocytes (Fig. 1). It has been found that Gln is actively transported to mitochondria by transporters [6,16] (Fig. 1). Secondarily to its excessive accumulation in mitochondria, Gln might impair the mitochondrial function [6,16] (Fig. 1). Most of Gln in the mitochondrial matrix is metabolized by mitochondrial glutaminase (Fig. 1). The products of glutaminase activity are Glu and ammonia (Fig. 1). Hence, ammonia reaches a high level in the mitochondrial matrix. Consequently, the normal mitochondrial function will compromise. Gln itself is also reported to induce oxidative stress in astrocytes. Astrocytes Gln accumulation is also associated with mitochondrial dysfunction and cell swelling [5,12,16,20] (Fig. 1). Hence, Gln could play a significant role in the mechanism of ammonia-induced mitochondrial impairment. In support of the role of Gln in the mitochondrial dysfunction are studies showing that glutamine synthesis inhibition alleviated ammonia-induced astrocytes damage and mitochondrial dysfunction [21–24]. Some investigations suggested the inhibition of glutamine synthesis as a therapeutic option in hyperammonemia [21,22]. Although inhibition of glutamine synthesis might mitigate the detrimental effects of Gln accumulation in the brain, but preventing ammonia detoxification *via* glutamine synthesis could also be dangerous and attributed to deleterious side effects. On the other hand, there is no investigation which measures Gln-born ammonia in the mitochondrial matrix. Hence, we might not be able to estimate the actual role of Gln in ammonia-induced mitochondrial dysfunction. Moreover, there is no study on the potential pathogenic role of Glu accumulation in the mitochondrial matrix (Fig. 1). The importance of Gln in the mechanism of ammonia-induced brain injury, mitochondrial dysfunction, and energy crisis is also discussed in more details in the forthcoming sections.

3. Ammonia interferes with cerebral energy metabolism

Disruption in brain bioenergetics has long been considered as a pathogenic factor in several neurodegenerative disorders [25–28]. Impaired mitochondrial function leads to the cessation of ATP synthesis and cellular ATP depletion. Ammonia-induced mitochondrial injury and brain bioenergetics disturbances seem to play a relevant role in the mechanism of ammonia neurotoxicity [29,30]. Ammonia-induced disruption of brain energy metabolism could also be a significant factor contributing to the inhibition of CNS development and mental retardation in newborns with congenital hyperammonemia [31,32]. High and constant dependence of the brain tissue to ATP makes cellular mitochondria as a critical and potential therapeutic point of intervention in the management of HE.

It has repeatedly been shown that acute ammonia intoxication produces marked alterations in brain energy metabolism [33–35]. It has been reported that ammonia inhibits tricarboxylic acid cycle enzymes, induces mitochondrial permeability transition (mPT) and swelling, inhibited mitochondrial electron transport chain complexes, enhanced glutamatergic neurotransmission, provoked oxidative/nitrosative stress, and lead to neuroinflammation. At the following sections, an overview of the effect of ammonia on the enzymes responsible for energy metabolism, mitochondrial respiratory chain complexes, neuroinflammation, and mitochondrial permeability transition pore (mPT) is given to provide an idea on the relevance of disturbed energy metabolism to HE-induce brain energy crisis and finally highlight these mechanisms as potential therapeutic targets.

3.1. Inhibition of tricarboxylic acid cycle and suppression of oxidative phosphorylation by ammonia

The effect of ammonia on the rate-limiting enzymes involved in cerebral energy metabolism is one of the proposed mechanisms for CNS injury during HE [34,36–38]. Inhibiting the tricarboxylic acid (TCA; Krebs) cycle enzymes is suggested to be one of the primary mechanisms underlying ammonia-induced mitochondrial dysfunction, energy crisis, and neurotoxicity [36–38] (Fig. 2). It has been reported that ammonia inhibited α-ketoglutarate dehydrogenase (α-KGDH), a rate-limiting enzyme in the TCA cycle (Fig. 2) [36–38]. The inhibition of other enzymes such as isocitrate dehydrogenase and malate dehydrogenase is also reported in the presence of ammonia (Fig. 2) [35,39]. Some investigations indicated that pyruvate dehydrogenase might also be inhibited by ammonia [37] (Fig. 2). The inhibition of pyruvate dehydrogenase will further limit the availability of substrates for the TCA cycle (Fig. 2). A significant decrease in TCA cycle intermediates such as malate, citrate, and α-KG has been documented in hyperammonemia conditions [40]. Hence, inhibiting the rate-limiting enzymes in the TCA

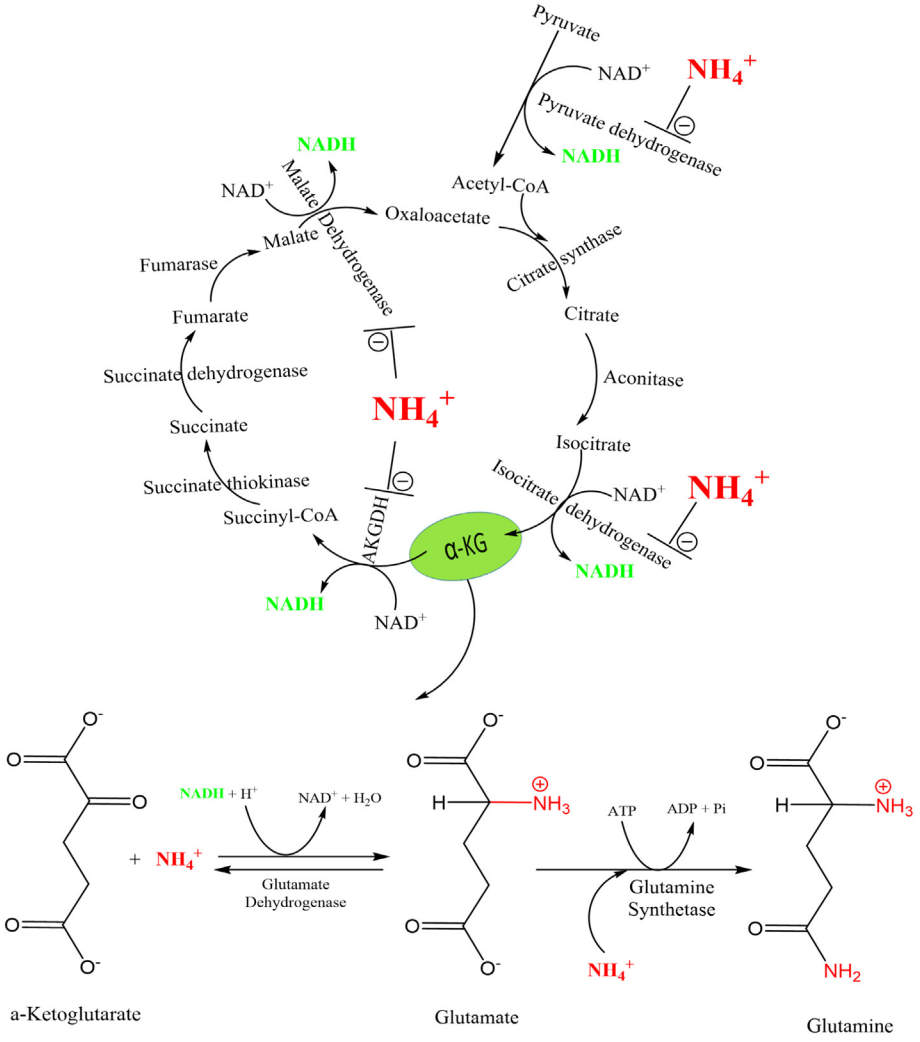

Fig. 2. Ammonia-induced inhibition of the tricarboxylic acid (TCA) cycle. TCA cycle enzymes are responsible for providing reducing equivalents (NADH) which are further used for cellular energy (ATP) production. The ammonia-induced arrest of the TCA cycle leads to depletion of mitochondrial NADH and consequently energy production failure and ATP depletion. On the other hand, alpha-ketoglutarate combines with ammonia to produce glutamate and finally glutamine molecule. This might serve as a detoxification process for ammonia. NH_4^+: Ammonium ion; α-KG: alpha-ketoglutarate; AKGDH: alpha-ketoglutarate dehydrogenase.

cycle might be one of the primary mechanisms underlying ammonia neurotoxicity and mitochondrial dysfunction (Fig. 2). TCA cycle enzymes inhibition also limits the mitochondrial content of the reducing equivalents (NADH) (Fig. 2), which are necessary for mitochondrial respiratory chain activity. Therefore, the inhibition of TCA cycle enzymes disturbs cerebral energy metabolism. On the other hand, it has been found that α-KG is essential for ammonia detoxification (Fig. 2). α-KG is combined with two ammonia molecule to form Gln (Fig. 2). Therefore, any changes in α-KG resources (*e.g.*, inhibition of Krebs cycle enzymes) might disturb ammonia detoxification [41].

The effect of ammonia on TCA cycle enzymes seems to be significantly inconsistent in different experimental models. Although it has been found that ammonia could robustly inhibit TCA enzymes *in vitro* [34,35], some studies mentioned that ammonia might not significantly affect TCA enzymes *in vivo* [42,43]. It has been suggested that the discrepancy between different models might be associated with non-mitochondrial reactions associated with ammonia [35]. The discrepancy in the result of ammonia-induced inhibition of TCA cycle enzymes might also be associated with dissimilar models of HE and the length of the investigations.

In addition of the effect of ammonia on the TCA cycle, changes in cerebral glucose utilization, as well as changes in the glycolysis process, also have been suggested to be involved in the effect of ammonia on cerebral energy metabolism [34,35,41,44]. It has been reported that brain glucose consumption is diminished in experimental models of HE [45]. It has also been proposed that lactate accumulation during hyperammonemic episodes could also play a relevant role in the brain metabolic derangements [39,46]. Moreover, impairment in the malate-aspartate shuttle has been reported during hyperammonemia [34]. The malate-aspartate shuttle is liable for the transfer of reducing equivalents (NADH) across the inner mitochondrial membrane [34]. Hence, impairment in the malate-aspartate shuttle could result in impaired mitochondrial energy metabolism. All these data mention the fundamental role of brain metabolic derangements in HE.

It has been documented that ammonia inhibited mitochondrial respiratory chain complexes in HE episodes [47,48] (Fig. 3). Some investigations mentioned the suppressed respiration in ammonia-exposed mitochondria [34,49,50]. Data obtained from different experimental models also indicated a progressive decrease in the activity and expression of ETC complexes in the brain of hyperammonemic animals [34,49–52]. Inhibition of ETC components by ammonia (*e.g.*, complex II) is clinically important since new roles for these proteins have recently emerged in cell signaling and neurodegeneration [53]. Other ETC components such as cytochrome *c* oxidase (COX; Complex IV) are also essential to produce sufficient ATP for neuronal survival. Previous studies also suggested that a reduced cerebral COX activity may be an

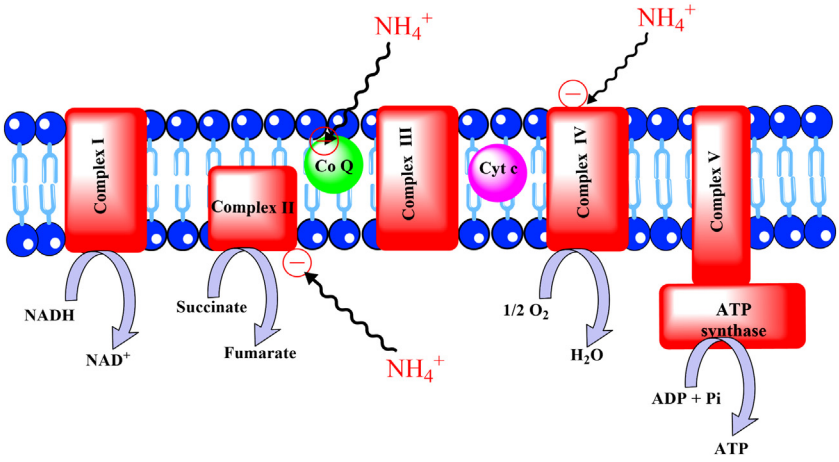

Fig. 3. The inhibitory effect of ammonia on mitochondrial electron transport complexes impairs ATP synthesis. NH_4^+: Ammonium ion; Cyt c: Cytochrome c; Co Q: Coenzyme Q.

index of neurodegenerative events in hyperammonemia [51]. Ammonia-induced inhibition of ETC complexes might also play a role in the enhancement of mitochondria-facilitated ROS formation and oxidative stress [54]. Disturbances in the ETC by ammonia finally lead to impaired ATP production and cellular energy crisis. The blockade of ETC and impaired ATP production could trigger the cascade of apoptosis and cell death. As previously mentioned, the TCA cycle enzymes inhibition also leads to deprivation in NADH (Fig. 2). NADH serves as the respiratory chain substrate (Fig. 3). Therefore, NADH deprivation further impairs mitochondrial respiration (Fig. 3). Hence, ammonia-induced mitochondrial ETC inhibition could play a significant role in the pathogenesis of HE-associated mitochondrial dysfunction and brain energy crisis (Fig. 3).

In conclusion, the ammonia-induced arrest of TCA cycle, the defect in mitochondrial ETC, impaired malate-aspartate shuttle, and changes in cerebral glucose utilization are associated with suppression of oxidative metabolism and deteriorated brain energy status during HE. All these events could finally lead to altered cerebral function in hyperammonemia. On the basis of these findings, a pivotal role for brain energy crisis could be suggested in the pathophysiology of ammonia neurotoxicity. Therefore, targeting ammonia-induced bioenergetic failure could represent a viable therapeutic approach against HE.

At the next following sections, the interrelation between mitochondrial dysfunction and ammonia-induced complications including oxidative/nitrosative stress in the CNS, excitotoxic response, neuroinflammation, and brain edema are discussed.

3.2. The relationship between oxidative/nitrosative stress and mitochondrial impairment in HE and hyperammonemia

Reactive oxygen/nitrogen species (ROS/RNS) are generated as the inevitable byproducts of normal cellular metabolic activities. On the other hand, the disproportion generation of ROS/RNS poses a serious problem for cellular homeostasis and causes oxidative/nitrosative injury. Oxidative/nitrosative stress induces a cascade of pathogenic events which finally damage the tissue. Elevated brain ammonia level is known to be attributed to the oxidative/nitrosative stress in this organ [29,55,56]. It has been found that ammonia enhanced the generation of ROS and NOS in astrocytes [29,56,57]. Hence, oxidative/nitrosative stress could play a vital role in the pathophysiology of ammonia-induced CNS injury. Readers could find outstanding investigations about the role of oxidative/nitrosative stress in hyperammonemia-induced CNS injury elsewhere [11,58]. Here, we will focus on the relationships between ammonia-induced oxidative/nitrosative stress, mitochondrial dysfunction, and energy crisis in the CNS. In this regard, the capability of antioxidant therapy is then highlighted at the end of this review.

It has been found that primary antioxidant defense mechanisms are defected in the brain tissue or astrocytes exposed to pathological ammonia concentrations [54,59,60]. The activity of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Mn-SOD and Cu-SOD) has been found to be suppressed in hyperammonemia and HE [59,60]. On the other hand, the uptake of glutathione precursors and the synthesis of glutathione in astrocytes have also been reported to be interrupted by ammonia [61]. It has been demonstrated that the transport of cystine to astrocytes is impaired by ammonia [61]. Once taken up by cells, cystine is reduced to cysteine [61]. Cysteine is the rate-limiting precursor of glutathione synthesis. Hence, ammonia could interrupt astrocytes glutathione synthesis [61]. All these events could finally lead to severe oxidative stress and a defect in proper cellular mitochondrial function.

Several mechanisms are proposed to be involved in the ammonia-induced oxidative/nitrosative stress and its connection to mitochondrial impairment in the brain tissue. Mitochondria could act as a source of ROS which finally might entail oxidative stress and cellular dysfunction. It had long been understood that cellular mitochondria are the primary source of cellular oxygen radicals [62]. Therefore, oxidative/nitrosative stress could act as a cause or a consequence of mitochondrial dysfunction [63]. Thus, ammonia-induced oxidative stress and mitochondrial dysfunction are mechanistically interrelated. Interestingly, it has been documented that oxidative/nitrosative stress could play a critical role in the activation of transporters such as NKCC1 [17,19]. As mentioned, activation of NKCC1 could result in disturbances of cellular ion homeostasis and translocation of ions and water to the astrocytes [17,19]. These events play an important role in astrocytes swelling and brain edema (Refer to next sections).

Increase in cytoplasmic Ca^{2+} is also a critical factor which affects normal mitochondrial function [63]. Oxidative stress might lead to elevated intracellular Ca^{2+} [63] (Fig. 4). Increased cytoplasmic Ca^{2+} is attributed to the induction of mitochondrial mPT, collapse of mitochondrial membrane potential ($\Delta\Psi$), osmotic swelling of mitochondrial matrix, uncoupling of oxidative phosphorylation, and interruption of ATP synthesis [64,65]. Hence, ammonia-induced oxidative stress might also be connected to mitochondrial dysfunction through cellular Ca^{2+} dysregulation (Fig. 4). It has also been found that cellular endoplasmic reticulum is a critical target affected is hyperammonemia states [22,66]. Ammonia-induced activation of glutamate receptors is also associated with an increased cytoplasmic Ca^{2+} level, which could contribute to mitochondrial dysfunction (Figs. 4 and 5).

Another mechanism for ammonia-induced oxidative stress and mitochondrial ROS overload could be mediated through peripheral benzodiazepine receptors (PBRs) [58,67]. PBRs are transmembrane proteins located in the outer mitochondrial membrane [58,67]. PBRs seem

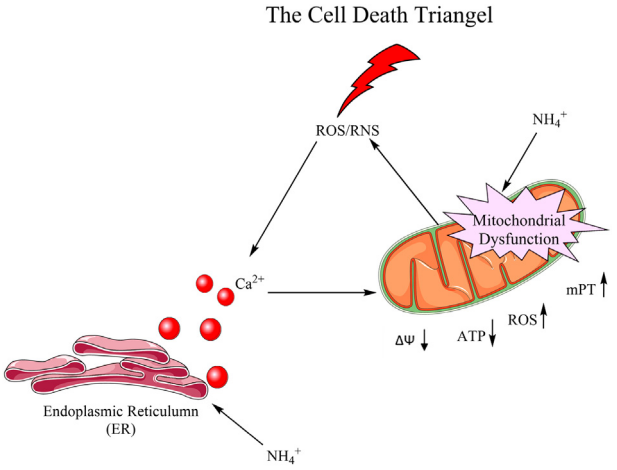

Fig. 4. The interrelationship between ammonia (NH_4^+)-induced mitochondrial impairment, oxidative/nitrosative stress, cytoplasmic calcium disturbances, and cell death. mPT: Mitochondrial permeability transition; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; ΔΨ: Mitochondrial membrane potential; ATP: Adenosine triphosphate.

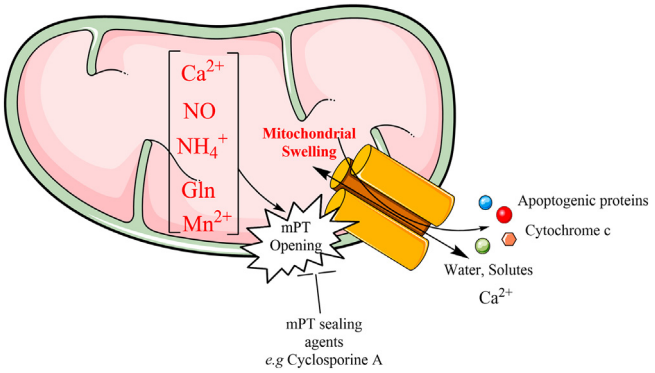

Fig. 5. Factors responsible for the assembly of the mitochondria permeability transition (mPT) pore components and mitochondrial permeabilization. Several chemicals are identified which are capable of mPT induction during hepatic encephalopathy (HE). The mPT opening during hepatic encephalopathy is relevant to mitochondrial swelling and cell death. Mn^{2+}: Manganese; NO: Nitric Oxide; Ca^{2+}: Calcium; NH_4^+: Ammonium ion; Gln: Glutamine.

to be one of the mPT components [9,68]. It has been found that PBRs are up-regulated in hyperammonemic conditions [9,68]. On the other hand, ligands of PBRs have been shown to induce oxidative stress in cells [9,68]. Hence, the ammonium ion might act as a PBR ligand to induce oxidative stress and mitochondrial permeabilization (Fig. 5).

Increased nitric oxide synthetase (NOS) activity is another major factor which plays a critical role in the pathogenesis of oxidative/nitrosative stress-induced mitochondrial impairment during hyperammonemia (Fig. 4) [9]. It has been found that NOS activity is increased in experimental models of HE [9,69]. Excessive formation of NO could trigger the formation of highly toxic peroxynitrite radical in astrocytes and neurons [9,69]. These events might finally lead to cell death.

Nitric oxide (NO) is characterized as a mPT inducer [70–72] (Fig. 5). It is well-known that mPT opening leads to mitochondrial membrane potential dissipation and release of apoptosis signaling molecules (Fig. 5). In addition, mitochondrial respiratory chain complexes are also reported to be inhibited by NO or peroxynitrite ($ONOO^-$) as its highly reactive radical metabolite [71]. It has also been found that NO and peroxynitrite radical inactivated electron transport complex I and III [73,74]. On the other hand, it has been reported that NOS inhibitors attenuated the induction of mPT by ammonia [69,75]. Hence, NO is also a key player in HE-induced mitochondrial dysfunction and brain energy crisis (Fig. 5).

All these findings implicate that oxidative stress plays a pivotal role in the mechanisms of ammonia neurotoxicity. Ammonia induces oxidative stress in the brain could ensue by deleterious events such as mitochondrial injury and energy failure (Fig. 4). Hence, antioxidants might play a role in the management of ammonia-induced mitochondrial dysfunction and brain injury. The significance of antioxidant therapy in HE is discussed in the forthcoming sections.

Another interesting mechanism of mitochondrial ROS production and oxidative stress could be mediated through ammonia-induced excitotoxicity response. The effect of ammonia on glutamate receptors (excitotoxicity response) and its relevance to ammonia-induced oxidative stress, mitochondrial dysfunction, and brain energy crisis are discussed in more details in the forthcoming sections.

3.3. Ammonia-induced excitotoxic response in the CNS and its relevance to the brain energy crisis

One of the critical findings of the mechanism of ammonia neurotoxicity is the effect of this chemical on the CNS glutamatergic system [76,77]. Glutamate (Glu) is the major excitatory neurotransmitter in the CNS. Several investigations described the interrelation between glutamate excitotoxicity and ammonia neurotoxicity [76–78]. Herein, we mention this connection to discuss the role of mitochondrial injury in the pathogenesis of ammonia-induced CNS damage.

A leading contributor to the toxic effects of ammonia in the brain is the over-activation of *N*-methyl-D-aspartate (NMDA) type of glutamate receptors [77,79] (Fig. 6). This over-activation is also known as the "excitotoxic response" of NMDA receptors [77,79]. It has been found that the excitotoxic response plays a crucial role in the pathogenesis of ammonia neurotoxicity (Fig. 6) [77,79]. As previously mentioned, conversion of ammonia to glutamine (Gln) is the primary metabolic path in the brain tissue which takes place in astrocytes by glutamine synthetase enzyme (GS) (Fig. 1). Gln is further metabolized to Glu in neurons (Fig. 1). It has been well-documented that in hyperammonemia the cycling of Gln-Glu between neurons and astrocytes is interrupted [80] (Fig. 6). Consequently, the extracellular concentration of Glu is increased (Fig. 6). As mentioned, Glu is the major excitatory neurotransmitter in the brain which activates the NMDA receptor signaling. Deleterious events such as excessive nitric oxide and peroxynitrite radical formation might ensue NMDA receptor overactivation [69,81]. Hence, the ammonia-induced excitotoxic response is tightly linked to oxidative/nitrosative stress (Fig. 6). As mentioned, oxidative/nitrosative stress and mitochondrial impairment are interconnected events. Therefore, the oxidative/nitrosative response associated with glutamate-induced excitotoxicity response could play a significant role in the brain mitochondrial dysfunction and consequently energy failure (Figs. 4 and 5). On the other hand, it has also been documented that ammonia impairs Glu uptake by astrocytes [82,83]. Glu uptake by astrocytes is also a crucial pathway since ammonia detoxification in these cells relies on the amidation of Glu to Gln (Fig. 6) [82,83]. It has been found Glu uptake by astrocytes is in close relation to Na^+/K^+ ATPase pump activity [82,83]. In this regard, mitochondrial dysfunction and cellular ATP crisis impairs the clearance of Glu from the synaptic cleft and potentiates excitotoxicity (Fig. 6).

Glu-mediated NMDA receptors over activation also lead to increased intracellular Ca^{2+} [69,81]. As mentioned, the increased cytoplasmic Ca^{2+} level is associated with severe mitochondrial dysfunction and membrane potential dissipation [63] (Figs. 4 and 5). Mitochondrial Ca^{2+} accumulation also impairs mitochondrial respiration, decreases ATP synthesis and increases the formation of free radicals which lead to more oxidative stress [84] (Figs. 4 and 5). Hence, disturbances of intracellular Ca^{2+} homeostasis could complicate mitochondrial function and acts as a significant factor in disturbing the brain energy metabolism during the ammonia-induced excitotoxic response.

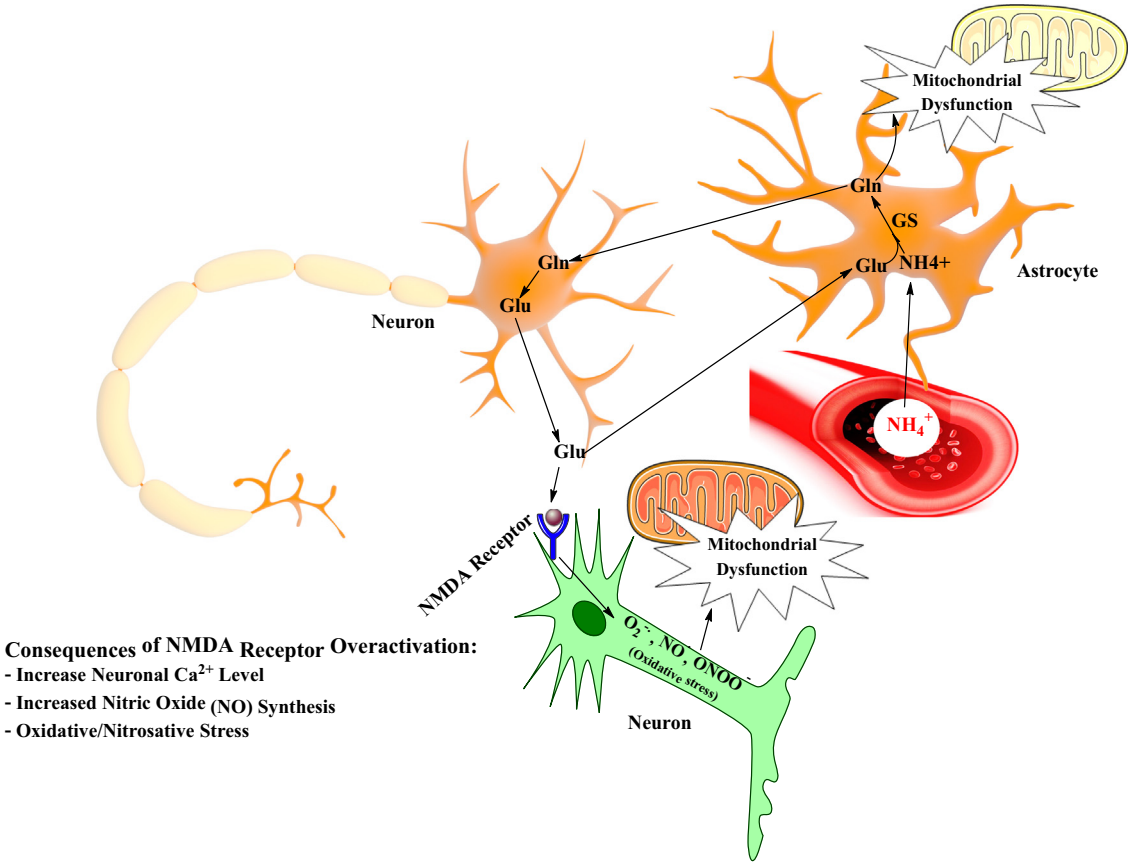

Fig. 6. Glutamine-glutamate cycle between astrocytes and neurons. It has been assumed that excessive glutamate production during hyperammonemia leads to overactivation of the brain glutamatergic system (Excitotoxicity). On the other hand, the excitotoxic response is firmly connected to other complications such as oxidative stress and mitochondrial impairment during hyperammonemia. Glu: glutamate; Gln: glutamine; GS: Glutamine synthase; NMDA: *N*-methyl-D-aspartate receptor.

All these data indicate that excessive activation of NMDA receptors could lead to cellular ATP depletion (Fig. 5). Hence, NMDA-receptor over activation-mediated depletion of ATP during hyperammonemia might also play a role in the energy crisis induced by this noxious neurotoxic chemical (Fig. 5). It has also been mentioned that activation of NMDA receptors leads to increased nitric oxide (NO) synthesis (Fig. 5) [75,85]. Overproduction of NO leads to excessive NOS and nitrosative stress (Fig. 5). As previously mentioned, NO is another critical factor which affects mitochondrial function (Fig. 4).

During the ammonia-induced excitotoxic response, increased consumption of ATP (*e.g.,* by NMDA receptors overactivation and transporters activity), decreased ATP synthesis (*e.g.,* by mitochondrial dysfunction) or both are contributed in the brain energy deficit during hyperammonemia and HE. It has been shown that the cytotoxic effects of ammonia on astrocytes are significantly attenuated by NMDA receptors antagonists and NOS inhibitors [85]. Hence, managing ammonia-induced excitotoxicity and its consequences might serve as a potential point of intervention in attenuating HE-induced CNS complications (Fig. 5).

3.4. Mitochondria and astrocytes swelling: relevance to HE-associated brain edema

Brain edema is a critical complication during HE and hyperammonemia which could even lead to brain herniation [86,87]. There are no precise molecular mechanisms for brain edema in hyperammonemic patients. On the other hand, some studies proposed a connection between mitochondrial dysfunction and brain edema [6,88,89]. As mentioned in the previous sections mitochondrial mPT could be induced during hyperammonemia through different mechanisms (Fig. 4). The mPT opening makes a point of “no return” during apoptosis and cell death [84]. The mPT opening will lead to dissipation of the mitochondrial membrane potential, movement of metabolites to the cytoplasm, decreased ATP synthesis, the release of cell death mediators, and ROS generation [84] (Fig. 5). In this section, the interrelation between mitochondrial mPT opening, and astrocytes swelling, and its relevance to HE-associated brain edema is highlighted.

Cerebral edema and intracranial pressure is a common feature of HE [90]. There is a consensus that HE-induced brain edema is primarily due to astrocytes swelling [23,91]. It has been found that astrocytes show nuclear and cytosolic alterations in HE [92,93]. Astrocytes swelling appear to be a key component in the ammonia-associated brain edema, intracranial pressure and brain herniation [92,93]. Hence, astrocytes swelling play a role in ammonia neurotoxicity and patients' death.

Several mechanisms have been proposed to be involved in the astrocytes swelling during HE [6,88,89]. Besides other factors which are proposed to be implicated in the HE-associated astrocytes swelling and brain edema [94–96], one of the most exciting mechanisms for ammonia-induced astrocytes swelling is connected to cellular mitochondria [89]. There is substantial evidence that induction of mPT contributes to the pathogenesis of ammonia-induced astrocytes swelling [6,88,89]. In line with this speculation, the blockade of mPT has been shown to reduce the extent of astrocytes swelling and brain edema as a deleterious consequence of hyperammonemia [88,97]. On the other hand, some studies mentioned the importance of the cotransporter NKCC1 in ammonia-induced cell swelling and brain edema during HE [17,19]. As previously mentioned, this transporter could be activated by conditions such as oxidative stress [17,19]. It has been found that NKCC1 inhibitors and antioxidant molecules could diminish cell

swelling in ammonia-exposed astrocytes [17,19]. These events could confirm the connection between ammonia-induced oxidative stress and cell swelling/brain edema.

The concept that cellular mitochondria undergo swelling in the presence of various substances is known for a long time. Structurally, mPT consists of several proteins. Under conditions favorable for the mPT opening, the component proteins assemble to form a pore [84,98]. Several chemicals and conditions might lead to the induction of mPT during HE (Fig. 4) [84,98]. Different studies have suggested that mPT induction could be the cause of energetic failure presented in HE and hyperammonemia [98]. On the other hand, the mPT opening is closely associated with the signaling pathways of cell death (Fig. 4).

Elevated cytoplasmic Ca^{2+} is a well-known factor in mPT opening [99] (Fig. 5). On the other hand, several factors associated with HE and hyperammonemia could lead to mPT induction (Fig. 5). Elevated astrocytes Gln level is thought to be one of the major causes of ammonia-induced mPT opening, cell swelling, and cerebral edema [12,13] (Fig. 5). As mentioned, Gln is one of the most suspicious molecules responsible for ammonia-induced mitochondrial dysfunction [12]. Gln is converted to ammonia in the mitochondrial matrix and causes mPT opening (Figs. 1 and 5). This event finally leads to mitochondrial dysfunction and release of cell death mediators to the cytoplasm (Fig. 5). Other molecules such as manganese, nitric oxide (which is abundantly formed during ammonia-induced excitotoxicity), and the ammonia molecule itself are also mPT inducing molecules [6,88,89,100] (Fig. 5). Oxidative stress and mPT opening are also interrelated events [101]. As ammonia-induced oxidative stress is a common event during HE, reactive oxygen species might also play a role in mPT induction and mitochondrial dysfunction [101,102]. Norenberg et al. suggested that a cascade of events initiated by ammonia-induced oxidative stress, which are connected to mPT and mitochondrial swelling, results in astrocytes volume dysregulation and finally brain edema [89]. Another mechanistic view proposed that excessive Gln accumulation in astrocytes during the detoxification process of ammonia could lead to a hyperosmotic state and the shift of water to astrocytes cytoplasm [103]. On the other hand, astrocytes exposure to high Gln increased free radical production and oxidative stress [20,21,104,105]. As mentioned, oxidative stress and mitochondrial swelling are two interconnected events [106] (Fig. 4). Moreover, some other investigations questioned the “glutamine osmotic hypothesis” of astrocytes swelling. Finally, it should be mentioned that cell swelling and transport of electrolytes through the cell membrane is principally an energy-dependent process. Hence, any defect in normal mitochondrial function and cellular ATP level might impair solutes transport and lead to cell swelling. As a result, protecting mitochondria and preserving appropriate cellular ATP levels could serve as a potential point of intervention against cell swelling and brain edema as a severe complication associated with HE.

All these data might mention the importance of mPT sealing agents in preventing mitochondria, and consequently, astrocytes swelling and brain edema during hyperammonemia and HE (Fig. 5).

3.5. Neuroinflammation during HE: relevance to mitochondrial injury and CNS bioenergetic disturbances

Inflammatory response and neuroinflammation could play an essential role in the pathogenesis of HE [10,107–110]. The significant increase in the cerebral level of proinflammatory cytokines such as TNF-α and IL- 1β has been repeatedly documented in the pathogenesis of HE [111,112] (Fig. 7). On the other hand, some investigations confirmed a connection between brain edema and neuroinflammation [107,111,113] (Fig. 7). There is also enough evidence suggesting a connection between neuroinflammation and excitotoxicity response during HE [114,115] (Fig. 7). The interplay between increased circulatory and CNS proinflammatory cytokines, neuroinflammation, and oxidative stress has also been mentioned in previous studies [57,116,117] (Fig. 7). As mentioned in the previous sections, excitotoxicity and oxidative stress are two major events firmly interconnected to brain mitochondrial dysfunction and energy crisis in HE episodes. All these data suggest a possible connection between neuroinflammation and mitochondrial injury (Fig. 7). The current section aimed to review the possible interconnection between neuroinflammation, mitochondrial impairment, and cellular energy crisis during HE.

The relevance of neuroinflammation and excitotoxicity also has been mention in several studies. Neuroinflammation might lead to excitotoxicity through a range of mechanisms (Fig. 7). Among them, microglia activation is a significant mechanism which connects neuroinflammation to excitotoxicity and CNS injury (Fig. 7). It has been found that brain microglia become activated in the brain of animal models of HE [118,119]. Activation of brain microglia also could play a critical role in the pathogenesis of other neuroinflammatory disorders such as Alzheimer disease, stroke, and multiple sclerosis (MS) [120–122]. Microglia are the primary sources of inflammatory cytokines in the CNS [123]. Astrocytes also could act as a source of cytokines during ammonia-induced neuroinflammation [119,124,125] (Fig. 7). Hence, ammonia-induced neuroinflammation could play a significant role in the brain tissue mitochondrial dysfunction (Fig. 7).

Recent evidence indicates that inflammation and oxidative stress play a significant role in ammonia neurotoxicity [10,95,111]. Inflammation and oxidative stress seem to be interconnected. It has been well-documented that elevated serum and tissue level of cytokines aggravate ROS formation which is involved in the pathogenesis of several neurological disorders [126,127]. On the other hand, inflammatory cells are a major source of ROS [128]. Therefore, brain tissue aggregation of inflammatory cells such as neutrophils during HE could primarily contribute to elevated tissue ROS and oxidative stress. As previously mentioned, elevated ROS and mitochondrial dysfunction are two associated events [63] (Fig. 4). Some studies reported that that excessive production of nitric oxide (NO) induced by cytokines (*e.g.*, IL-1β) could disrupt cellular energy metabolism through the inhibition of mitochondrial respiration in different cell types [129]. Hence, the interplay between neuroinflammation and oxidative stress could deteriorate the mitochondrial function (Fig. 7). Neuroinflammation would contribute to increased oxidative stress and consequently mitochondrial impairment and energy crisis (Fig. 7). Therefore, new therapeutic agents aimed to treat neuroinflammation might also decrease oxidative stress and mitochondrial dysfunction in the brain (Fig. 7).

Increased gastrointestinal (GI) permeabilization to toxic substances such as bacterial lipopolysaccharides (LPS) is a crucial event associated with cirrhosis-associated HE of subclinical HE [130]. GI-derived LPS is a robust inflammation-inducing agent especially in chronic HE and cirrhosis [131,132] (Fig. 7). LPS-induced upregulation of microglia activation and cytokine release also has been documented [133]. Hence, the LPS-induced release of cytokines also could play a significant role in neuroinflammation during HE (Fig. 7). A systemic inflammatory syndrome resulting from GI permeability, LPS infiltration to the systemic circulation, and sepsis are common events in patients with HE [134,135] (Fig. 7). There is also a connection between systemic inflammation and the severity of CNS injury during HE [134]. LPS translocation from GI could also affect circulatory cytokines which finally might deteriorate CNS neuroinflammation (Fig. 7). Interestingly, it has been found that pro-inflammatory cytokines and LPS also inhibit Glu reuptake to the astrocytes [119,136,137] (Fig. 7). LPS is also able to enhance Glu release from astrocytes [138]. These events might enhance the excitotoxicity response during HE (Fig. 7). Therefore, a connection between NMDA receptors over-activation, excitotoxicity, and neuroinflammation is possible [139]. Consequently, the mitochondrial injury might occur (Fig. 7).

It has been found that increased brain lactate level which occurs during HE could also play a role in the aggravation of neuroinflammation [119]. Increased brain lactate during HE and hyperammonemia could be the cause or the consequence of HE [140]. On the

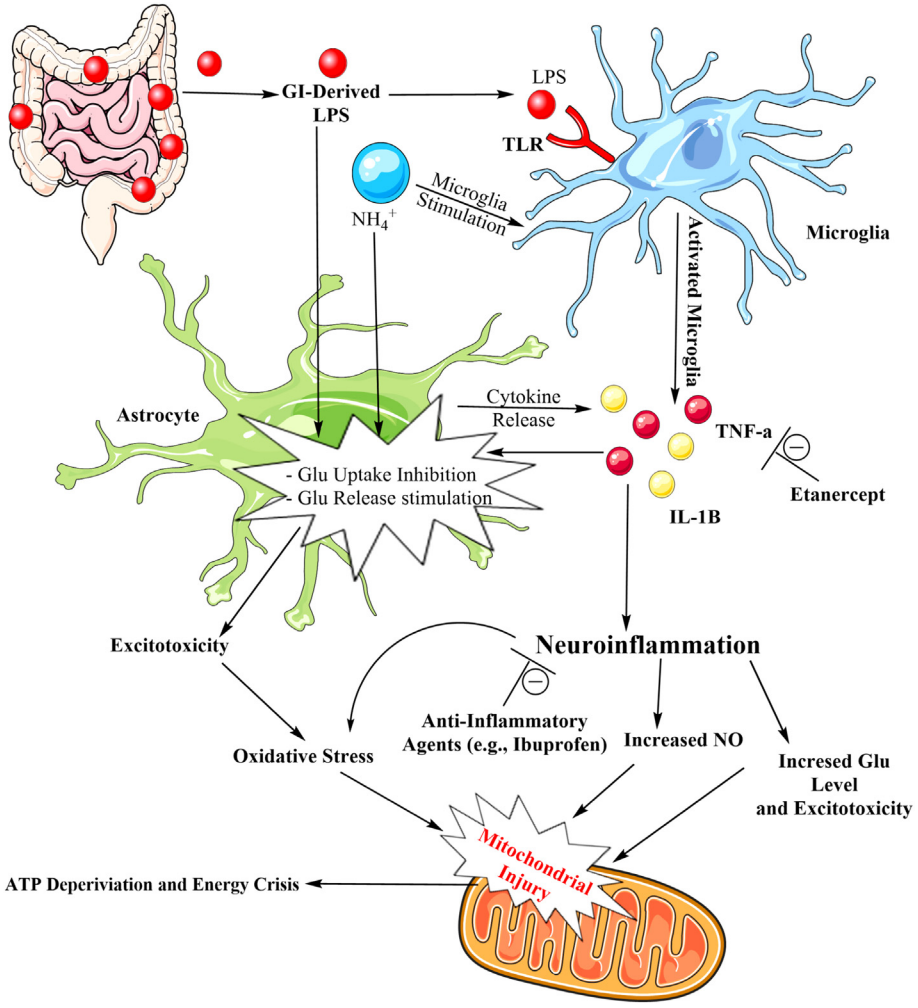

Fig. 7. Neuroinflammation and inflammatory cytokines could adversely affect mitochondrial function during hepatic encephalopathy (HE). Systemic inflammation and gastrointestinal (GI)-derived bacterial lipopolysaccharide (LPS) could also play a critical role in HE-associated CNS inflammation, especially in chronic HE and cirrhosis. On the other hand, ammonia-induced neuroinflammation is firmly connected to oxidative stress. NH_4^+: Ammonium ion; TLR: Toll-like receptor; NO: Nitric oxide; Glu: Glutamate.

other hand, lactate might serve as a "fuel" for mitochondria energy metabolism and ATP production [141]. Indeed, further investigations are needed to enhance our understanding of such controversies about the role of lactate in the aggregation of HE.

In an attempt to alleviate HE complications, several strategies have been tested to mitigate the neuroinflammation and its following events. It has been reported that administration of non-steroidal anti-inflammatory drugs improved locomotor activity during hyperammonemia and HE [108,109] (Fig. 7). On the other hand, it has also been documented that counteracting inflammatory cytokines (*e.g.*, Etanercept administration; Fig. 7) could robustly mitigate neuroinflammation and its consequences during HE [93,112].

All the data discussed in this section reveal a pivotal role for neuroinflammation and its connection to oxidative stress, excitotoxicity, and mitochondrial impairment. However, due to the complicated nature of inflammatory signal pathways in the CNS, more studies are needed to reveal the precise role of neuroinflammation and its relationship to oxidative stress and mitochondrial dysfunction, and considering these events as a therapeutic point of intervention during HE.

3.6. Role of manganese in HE, mitochondria dysfunction, and brain energy crisis

Brain manganese (Mn) accumulation is another factor which is not directly associated with ammonia-induced CNS injury, but play a significant role in the pathogenesis of mitochondrial dysfunction and brain energy crisis during HE. Hence, in this section, we reviewed the potential role of Mn in the brain bioenergetics failure during HE.

Mn is a trace element which plays a pivotal role in several physiological processes as well as in the structure of many vital enzymes [142]. On the other hand, overexposure to Mn seems to play a role in the pathogenesis of a range of neurodegenerative diseases [143]. Accumulation of Mn in the CNS is a common feature of chronic HE [144–147]. Mn is principally excreted in bile in healthy subjects [145,148] (Fig. 8). Hence, diminished removal of Mn in chronic liver diseases is the primary cause of elevated blood and brain Mn during HE [145,149,150] (Fig. 8). In liver cirrhosis, failure of biliary excretion of Mn leads to blood Mn overload and subsequent brain accumulation of this metal [145,146,149,150]. Albumin and transferrin are major blood carrier proteins for Mn [151]. As proteins such as albumin modify the quantity of Mn transported across the blood-brain barrier, impaired plasma albumin level during HE and cirrhosis might also account for CNS Mn accumulation and neurotoxicity [144,152]. Measurement of blood and brain Mn concentration in patients with chronic liver disease and HE revealed 2 to 7 folds increases in these subjects [145,153,154]. It has also been shown that Mn is significantly accumulated in the basal ganglia of patients with end-stage liver disease [149,155]. Larargues et al. also reported that brain Mn content was significantly elevated in

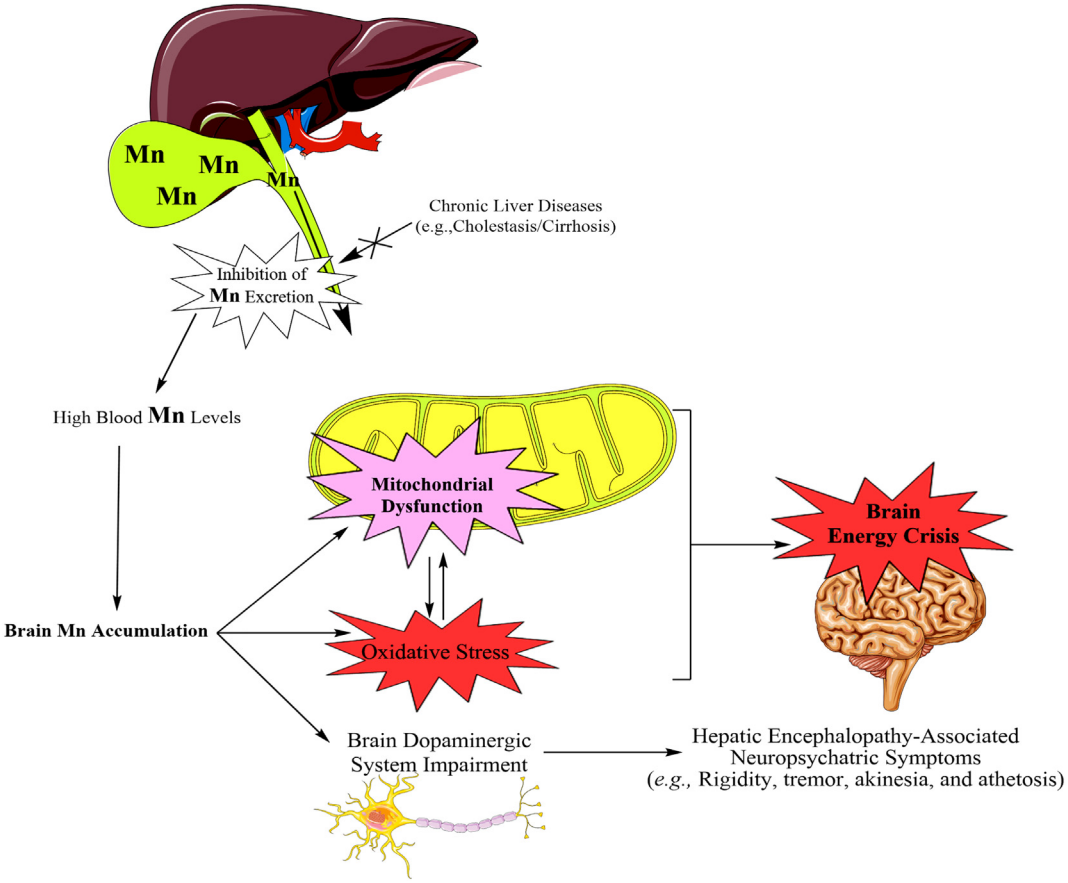

Fig. 8. Effect of manganese (Mn) on brain mitochondrial impairment, oxidative stress, and hepatic encephalopathy-associated neuropsychiatric symptoms. Mn deposition in the CNS is a crucial factor for the deterioration of the brain function during chronic hepatic encephalopathy (*e.g.*, in cirrhotic patients).

patients with liver diseases with different etiologies [153].

Elevated brain Mn level is postulated to be associated with HE-induced neuropsychiatric disturbances and behavioral deficits (Fig. 8) [146,156–158]. Dopaminergic neurons in the basal ganglia structures are particularly susceptible to the toxic effects of the Mn [159]. Hence, Mn effects on the CNS dopaminergic system play a critical role in the development of neurotoxicity during HE [146,149,155–158]. It has been reported that increased pallidal Mn concentrations coincided with reduced dopamine concentration as well as dopamine release from nerve endings, and dopamine displacement from its storage sites in the presynaptic neurons [147]. It has been proposed that Mn-induced dopaminergic neurons toxicity might be attributed to the extrapyramidal symptoms (rigidity, tremor, akinesia, and athetosis) of HE [160,161] (Fig. 8).

As mentioned, Mn is a neurotoxic metal [161]. Mn neurotoxicity might be mediated *via* several mechanisms including striatal dopamine depletion, glutamatergic over-activation (excitotoxicity), glutamatergic neurons cell death, or induction of oxidative stress in the brain tissue (Fig. 8) [161–164]. Mn-induced cytotoxicity toward astrocytes is a common feature in patients with HE [165]. It has been found that excessive accumulation of Mn in astrocytes impairs their capability of ammonia detoxification. Moreover, it has been reported that increased astrocytes Mn content might alter the release of glutamate and elicit the excitotoxicity (Fig. 8) [166,167]. Therefore, the brain Mn level is another factor which might be associated with mitochondria dysfunction during HE [144,146,149] (Fig. 8).

Although the precise mechanism(s) responsible for Mn-induced extrapyramidal symptoms and astrocytes cytotoxicity in HE is not fully cleared, some investigations mentioned the role of oxidative stress, defect in cellular antioxidant mechanisms, and mitochondrial dysfunction in this complication [150,168–170] (Fig. 8). Mn is capable of inducing oxidative stress in the brain tissue [170–172]. Moreover, Mn is known to induce mitochondrial injury and energy failure [168–170,173].

Interestingly, brain astrocytes serve as a primary storage site for Mn [174,175]. It has been found that astrocytes have a specific Mn transport system [165,174,175]. It has been found that Mn accumulates in the astrocytes. At the cellular level, Mn is preferentially accumulated in mitochondria [176,177]. It has been found that Mn is accumulated within the mitochondrial matrix *via* the Ca^{2+} ion uniporters [178,179]. Mitochondrial Mn deposition might lead to the collapse of mitochondrial membrane potential, impaired oxidative phosphorylation, and finally depletion of mitochondrial ATP [180–182] (Fig. 8). It has been reported that Mn-treated astrocytes and neural cells have lower ATP content [183,184]. The Mn-induced mitochondrial injury might also lead to the release of numerous apoptogenic factors into the cytosol which enhances cell death.

In conclusion, due to the critical role of Mn in the pathogenesis of HE-induced CNS injury, mitochondrial dysfunction, and energy crisis, decreasing brain Mn level or protecting brain mitochondria against this neurotoxic metal could be a critical site of interference in HE [185]. Hence, therapeutic approaches aimed at the removal of Mn (*e.g.,* chelation therapy) and protecting mitochondria and astrocytes might be of potential value in preventing HE-induced mitochondrial injury and CNS bioenergetic failure.

As discussed in the previous sections, brain mitochondria could serve as an essential therapeutic point of intervention in the management of HE. Hence, a critical discussion is how we could prevent mitochondrial dysfunction and brain energy crisis in HE. At the forthcoming parts, the importance of therapeutic strategies against

ammonia-induced mitochondrial dysfunction and CNS injury is discussed.

4. Brain mitochondria as potential therapeutic points of intervention during HE

HE is a multifaceted clinical disorder. Therefore, the therapeutic strategies against HE should simultaneously consider many parameters involved in the pathogenesis of this complication. Traditional treatments for HE in chronic and acute liver diseases aims to reduce blood and brain ammonia levels. For this purpose, non-absorbable antibiotics (*e.g.*, Neomycin, Rifaximine) or polysaccharides (*e.g.*, Lactulose) are administered to prevent ammonia production by intestinal flora [186]. Although traditional therapy of HE could significantly enhance patients' survival, it might also lead to several adverse effects. On the other hand, lowering blood and brain ammonia level may not be entirely effective against the vast complications associated with HE or preventing its neurological sequel.

As mentioned, ammonia is not the only factor responsible for CNS injury during HE. Hence, the conventional management of HE might not affect problems associated with glutamine, cellular Ca^{2+} overload, Mn-induced brain injury, excitotoxicity, and severe oxidative/nitrosative stress in the CNS. A wide range of protective agents has been evaluated for their effect against ammonia neurotoxicity. Carnitine, *N*-acetylcysteine, taurine, carnosine, ascorbate, vitamin E, sulforaphane, resveratrol, and many other chemicals have been investigated against HE [82,187]. The beneficial effects of these chemicals are generally explained by scavenging of reactive oxygen species and improvement in mitochondrial function.

As discussed in the current review, mitochondrial dysfunction and brain energy crisis could play a significant role in altered cerebral function during hyperammonemia and HE (Fig. 9). Therefore, targeting this vital organelle might provide a promising therapeutic point of intervention in the management of ammonia-induced CNS complications. On the other hand, selecting the appropriate experimental models is an important point for evaluating the adverse effects of ammonia on brain mitochondrial function and energy status. Several experimental tools have been developed to evaluate the effects of acute, chronic, or subclinical hyperammonemia on brain function. These models could be achieved by surgical intervention or hepatotoxins administration. Bile duct ligation (BDL) is one of the most reliable experimental tools for evaluating the mechanisms involved in the pathogenesis of HE-induced organ injury [32,188,189]. This model shares many clinical features of cirrhosis and subclinical hyperammonemia in human [189]. On the other hand, several studies have mentioned thioacetamide (TAA)-treated rats as a model to investigate the effect of ammonia on mitochondrial impairment, brain energy status, and tissue injury [92,188,190,191]. TAA-treated animals could be used as a model of acute hyperammonemia [92,188,190]. These models could be applied as appropriate tools to investigate ammonia-induced brain mitochondria impairment.

As ammonia-induced CNS complications and mitochondrial dysfunction are firmly interconnected events (Fig. 9), manipulation in each path might serve as an option to preserve mitochondrial functionality and brain ATP level. Therefore, antioxidants, anti-excitotoxicity agents, Mn-chelation, inhibitors of nitric oxide synthesize (NOS), or administration of mitochondrial function regulating chemicals and energy providers might preserve mitochondrial function and prevent the brain injury during HE.

Several studies mentioned the primary role of oxidative/nitrosative stress in the pathogenesis of ammonia neurotoxicity [55,57,81]. As mentioned in different parts of this review oxidative/nitrosative stress could be firmly connected to other CNS complications associated with HE and hyperammonemia (*e.g.*, excitotoxicity, inflammation, *etc.*). On the other hand, ammonia-induced mitochondrial impairment, as well as NMDA receptors over-activation (Excitotoxicity), have been identified

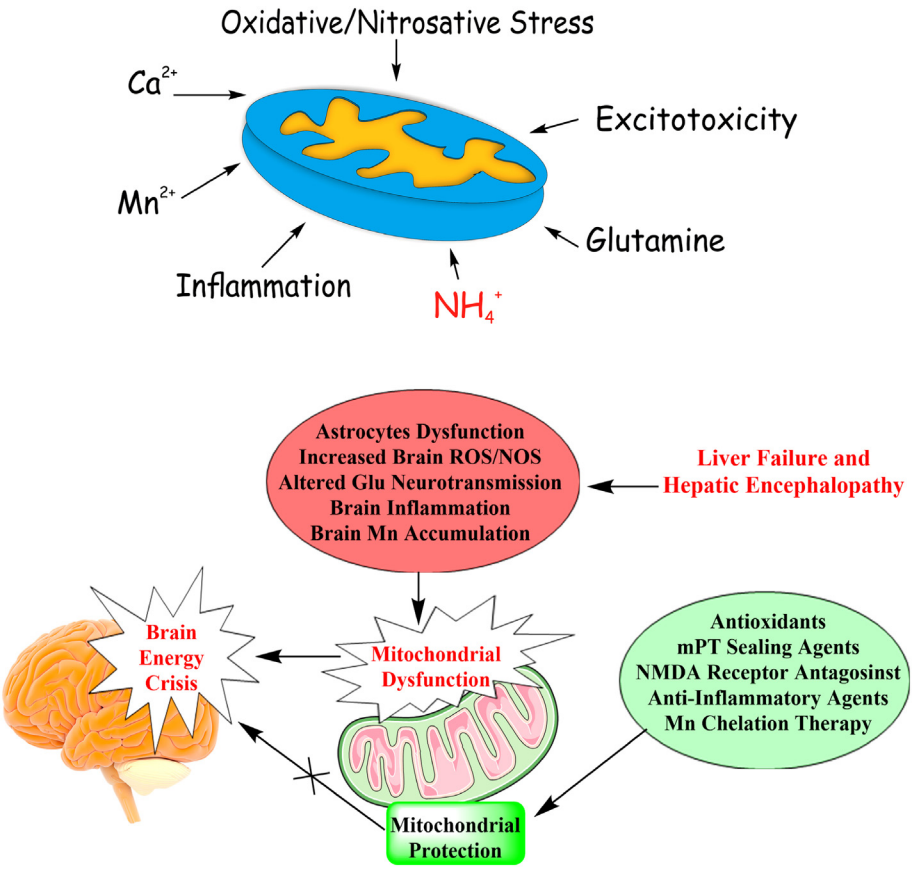

Fig. 9. A summary of the relationship between hyperammonemia, mitochondrial impairment, and brain energy crisis. Protecting brain mitochondria might serve as a viable therapeutic point of intervention in the management of hyperammonemia and hepatic encephalopathy. Glu: Glutamate; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; Ca^{2+}: Calcium; Mn^{2+}: Manganese; NMDA: *N*-Methyl-D-Aspartate; mPT: Mitochondrial permeability transition.

as the primary source of reactive oxygen species during HE [62,77,192]. Hence, administration of antioxidants might provide neuroprotection against HE. The neuroprotective effects of antioxidants against HE have been mentioned in several investigations [193–196]. Some antioxidants and thiol-reducing agents such as taurine, NAC, resveratrol, and sulforaphane have been proved to be beneficial in experimental models and human cases of HE and hyperammonemia [59,197–201]. These agents have been shown to be able to modulate critical astrocytes related parameters and function during HE. Oxidative stress, neuroinflammation, cellular glutathione (GSH) synthesis, and glutamine synthetase enzyme activity are key factors affected by these chemicals [82,124,187,201]. On the other hand, hypothermia as a major clinical intervention used in the management of HE also mentioned decreasing free radical production, oxidative stress, and its associated complications in the brain tissue [202]. On the other hand, antioxidant supplementation might also be effective in enhancing patients cognitive function and quality of life in subclinical HE. The mechanism of neuroprotection provided by antioxidants could be mediated through their direct interaction with reactive species. The antioxidative properties of these molecules might also be attributed to their effect on mitochondrial function and mitigating mitochondria-facilitated ROS formation [59,195,197–200,203–206]. Administration of antioxidants might also attenuate liver damage and enhance its ability for ammonia detoxification [59,195,197–200,203–206]. Consequently, the toxic effects of ammonia toward CNS are mitigated. All these data mention the value of antioxidants as a neuroprotective strategy against HE. However, the antioxidant therapy may not be completely effective without considering other factors involved in the pathogenesis of HE.

Hyperactivation of the NMDA receptors and excitotoxicity is one of the most deleterious events associated with HE and hyperammonemia. As mentioned in different parts of the current review, hyperactivation of NMDA receptors is associated with a battery of events including severe oxidative stress, cytoplasmic Ca^{2+} overload, as well as mitochondrial impairment and cellular energy crisis [9,32,85,192,207]. Therefore, it is reasonable that NMDA receptor antagonists might have a protective role in hyperammonemia-associated brain injury. Interestingly, it has been reported that ammonia inhibits kynurenic acid synthesis in the brain [208]. Kynurenic acid is an endogenous NMDA receptor antagonist. On the other hand, inhibitors of NMDA receptors alleviated the consequences of NMDA hyperactivation such as cytoplasmic Ca^{2+} overload and impairment in mitochondrial function [9,32,85,192,207]. NMDA antagonists also have beneficial effects in attenuating HE-induced cognitive impairment and locomotor dysfunction [209,210]. Several safe neuroprotective chemicals such as the amino acid taurine revealed significant antiexcitotoxic properties in previous studies [211,212]. All these data mention a critical role for NMDA receptors and excitotoxicity in HE. As excitotoxicity is firmly linked with Ca^{2+} overload, oxidative stress and mitochondrial impairment, NMDA inhibition might also mitigate oxidative stress and prevent brain energy crisis (Fig. 9). Hence, NMDA antagonism could be a therapeutic strategy against ammonia neurotoxicity in combination with other options such as antioxidants supplementation.

Neuroinflammation is a significant problem associated with HE. It has been well-documented that neuroinflammation is connected to other complications such as oxidative stress, excitotoxicity, and mitochondrial dysfunction [119,136]. As previously mentioned, an elevated level of cytokines enhances the excitotoxicity response, promotes oxidative stress and impairs mitochondrial function [123,129,137,138]. Based on these findings, we might be able to propose that the administration of anti-inflammatory agents might alleviate ammonia-induced neurotoxicity and mitochondrial impairment. Several investigations revealed that administration of pharmaceuticals such as non-steroidal anti-inflammatory drugs (NSAIDs) diminished HE-associated neuropsychiatric symptoms, cognitive impairment, and locomotor disturbances in experimental animal models and human cases of hyperammonemia [108,213–215]. Therapeutic strategies such as administration of etanercept, a TNF-α neutralizing molecule, has been found to ameliorate brain inflammatory response and improve cognitive function and locomotor activity in HE [112]. It is well documented that ammonia-induced neuroinflammation is mechanistically connected to the occurrence of oxidative stress in the brain tissue or enhancement of the ammonia-induced excitotoxic response [10,107,108,110,115]. Hence, the beneficial effects of anti-inflammatory agents in hyperammonemia might be connected to the alleviation of the oxidative stress and its consequences. These data might suggest anti-inflammatory agents as potential candidates for neuroprotection in ammonia toxicity (Fig. 9).

Induction of mitochondrial permeability transition (mPT) is a serious adverse effect of ammonia as a neurotoxin [13,14,97,100,101]. As mentioned, mPT induction and mitochondrial permeabilization are connected to astrocytes swelling and brain edema as deleterious complications associated with HE [97]. It has been found that administration of cyclosporine A as an mPT inhibitor mitigated ammonia-induced astrocytes injury [97]. Other neuroprotective agents such as taurine and carnosine are also good inhibitors of mitochondrial swelling and permeabilization [216,217]. Hence, administration of mPT sealing agents might be of benefit against ammonia neurotoxicity in combination with other mitochondria targeting strategies (Fig. 9).

As mentioned Mn deposition in the CNS is a critical factor affecting brain bioenergetics especially during chronic HE and cirrhosis [149,218]. In this context, some studies declared the importance of Mn chelation therapy as a choice against Mn neurotoxicity [219,220]. However, the effectiveness of such strategies has questioned. Chelation therapy might interrupt the homeostasis of other metals in the human body or lead to complications such as severe renal injury.

It is important to mention again that all the problems associated with ammonia neurotoxicity are firmly interrelated (Fig. 9). Application of each of the protective strategies might provide partial protection in HE. Hence, combination therapy might be more effective against HE. On the other hand, mitochondrial dysfunction seems to be a common endpoint associated with these adverse effects (Fig. 9). Therefore, protecting this organelle might provide viable therapeutic options against HE (Fig. 9).

5. Future perspectives

Understanding the molecular basis of neurological alterations in HE significantly improved the therapeutic strategies against this severe clinical complication. This manuscript has reviewed the evidence supporting a role for mitochondrial injury and brain energy crisis in the pathogenesis of HE.

HE treatment strategies are primarily directed to reducing ammonia generation and its detoxification. Although ammonia is the primary culprit toxicant implicated in the pathogenesis of CNS injury during HE, many other factors could play an essential role in the pathogenesis of these complications in concert with ammonia. Hence, any therapeutic interventions should consider all key players involved in the mechanism of HE-induced CNS injury. On the other hand, it has been well characterized that along with pathological and functional alterations in astrocytes; the mitochondrial injury might play a crucial role in the pathophysiology CNS complications during hyperammonemia. Therefore, targeting cellular mitochondria and brain energy metabolism might be a useful strategy for attenuating HE neurological consequences. This interference might justify further attempt to alleviate neurological sequel of HE with appropriate mitochondria protecting agents.

Most evidence indicating a pathogenic role for ammonia-induced mitochondrial impairment in HE is derived from *in vitro* cell culture studies. Hence, evaluating the effects of protective agents and mechanisms of HE-associated CNS injury in the *in vivo* conditions need further investigations. On the other hand, the majority of these

investigations have employed astrocytes which are critically involved in ammonia-induced neurotoxicity. Therefore, there is little information about the direct effects of ammonia on neurons. Interestingly, it has been found that ammonia could also affect neurons energy turnover [221,222]. Hence, the whole effect of ammonia on brain energy crisis may not just be due to its effects on astrocytes. The direct effects of ammonia and other toxic molecules involved in HE-induced CNS injury on neurons need further investigations to be cleared.

In summary, mitochondrial impairment seems to be a fundamental component and a point of convergence for the events involved in the pathogenesis of HE (Fig. 9). This concept might stimulate the development of new therapeutic strategies against HE. Indeed, future investigations are needed to prove if mitochondria protection and mitigation of brain energy crisis is a viable clinical option in the management of HE.

Conflicts of interest

There are no conflicts of interest.

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References

[1] V. Felipo, Hepatic encephalopathy: effects of liver failure on brain function, Nat. Rev. Neurosci. 14 (2013) 851–858, https://doi.org/10.1038/nrn3587.
[2] K. Weissenborn, K. Giewekemeyer, S. Heidenreich, M. Bokemeyer, G. Berding, B. Ahl, Attention, memory, and cognitive function in hepatic encephalopathy, Metab. Brain Dis. 20 (2005) 359–367, https://doi.org/10.1007/s11011-005-7919-z.
[3] R.F. Butterworth, Pathophysiology of hepatic encephalopathy: a new look at ammonia, Metab. Brain Dis. 17 (2002) 221–227, https://doi.org/10.1023/A:1021989230535.
[4] I. Suárez, G. Bodega, B. Fernández, Glutamine synthetase in brain: effect of ammonia, Neurochem. Int. 41 (2002) 123–142, https://doi.org/10.1016/S0197-0186(02)00033-5.
[5] J. Albrecht, M. Dolińska, Glutamine as a pathogenic factor in hepatic encephalopathy, J. Neurosci. Res. 65 (2001) 1–5, https://doi.org/10.1002/jnr.1121.
[6] J. Albrecht, M.D. Norenberg, Glutamine: a Trojan Horse in ammonia neurotoxicity, Hepatology 44 (2006) 788–794, https://doi.org/10.1002/hep.21357.
[7] S. Dadsetan, H.S. Waagepetersen, A. Schousboe, L.K. Bak, Glutamine and ammonia in hepatic encephalopathy, in: R. Rajendram, V.R. Preedy, V.B. Patel (Eds.), Glutamine in Clinical Nutrition, Springer, New York, 2015, pp. 219–227.
[8] J.M. Matés, C. Pérez-Gómez, I.N. de Castro, M. Asenjo, J. Márquez, Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death, In. J. Biochem. Cell Biol. 34 (2002) 439–458, https://doi.org/10.1016/S1357-2725(01)00143-1.
[9] V. Felipo, R.F. Butterworth, Neurobiology of ammonia, Prog. Neurobiol. 67 (2002) 259–279, https://doi.org/10.1016/S0301-0082(02)00019-9.
[10] C. Bémeur, R.F. Butterworth, Liver-brain proinflammatory signalling in acute liver failure: role in the pathogenesis of hepatic encephalopathy and brain edema, Metab. Brain Dis. 28 (2013) 145–150, https://doi.org/10.1007/s11011-012-9361-3.
[11] C. Bemeur, P. Desjardins, R.F. Butterworth, Evidence for oxidative/nitrosative stress in the pathogenesis of hepatic encephalopathy, Metab. Brain Dis. 25 (2010) 3–9, https://doi.org/10.1007/s11011-010-9177-y.
[12] A.R. Jayakumar, K.V.R. Rao, C.R.K. Murthy, M.D. Norenberg, Glutamine in the mechanism of ammonia-induced astrocyte swelling, Neurochem. Int. 48 (2006) 623–628, https://doi.org/10.1016/j.neuint.2005.11.017.
[13] K.V. Rama Rao, A.R. Jayakumar, M.D. Norenberg, Induction of the mitochondrial permeability transition in cultured astrocytes by glutamine, Neurochem. Int. 43 (2003) 517–523, https://doi.org/10.1016/S0197-0186(03)00042-1.
[14] M.D. Norenberg, K.V.R. Rao, A.R. Jayakumar, Ammonia neurotoxicity and the mitochondrial permeability transition, J. Bioenerg. Biomembr. 36 (2004) 303–307, https://doi.org/10.1023/B:JOBB.0000041758.20071.19.
[15] M.D. Norenberg, A.S. Bender, U.I. M.D, A.B. M.D, K.-A.H. M.D, T.K. M.D, A. Marmarou, H.-J.R. M.D, K.T. M.D, Astrocyte swelling in liver failure: Role of glutamine and benzodiazepines, Brain Edema IX, Springer, Vienna, 1994, pp. 24–27.
[16] K.V.R. Rao, M.D. Norenberg, Glutamine in the pathogenesis of hepatic encephalopathy: the Trojan Horse hypothesis revisited, Neurochem. Res. 39 (2014) 593–598, https://doi.org/10.1007/s11064-012-0955-2.
[17] A.R. Jayakumar, M.D. Norenberg, The Na-K-Cl co-transporter in astrocyte swelling, Metab. Brain Dis. 25 (2010) 31–38, https://doi.org/10.1007/s11011-010-9180-3.
[18] T.N. Nagaraja, N. Brookes, Intracellular acidification induced by passive and active transport of ammonium ions in astrocytes, Am. J. Phys. 274 (1998) C883–C891, https://doi.org/10.1152/ajpcell.1998.274.4.C883.
[19] A.R. Jayakumar, M. Liu, M. Moriyama, R. Ramakrishnan, B. Forbush, P.V.B. Reddy, M.D. Norenberg, Na-K-Cl cotransporter-1 in the mechanism of ammonia-induced astrocyte swelling, J. Biol. Chem. 283 (2008) 33874–33882, https://doi.org/10.1074/jbc.M804016200.
[20] J. Albrecht, M. Zielińska, M.D. Norenberg, Glutamine as a mediator of ammonia neurotoxicity: A critical appraisal, Biochem. Pharmacol. 80 (2010) 1303–1308, https://doi.org/10.1016/j.bcp.2010.07.024.
[21] N. Svoboda, H.H. Kerschbaum, L-glutamine-induced apoptosis in microglia is mediated by mitochondrial dysfunction, Eur. J. Neurosci. 30 (2009) 196–206, https://doi.org/10.1111/j.1460-9568.2009.06828.x.
[22] C.L. Willard-Mack, R.C. Koehler, T. Hirata, L.C. Cork, H. Takahashi, R.J. Traystman, S.W. Brusilow, Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat, Neuroscience 71 (1996) 589–599, https://doi.org/10.1016/0306-4522(95)00462-9.
[23] M.D. Norenberg, K.V.R. Rao, A.R. Jayakumar, Mechanisms of ammonia-induced astrocyte swelling, Metab. Brain Dis. 20 (2005) 303–318, https://doi.org/10.1007/s11011-005-7911-7.
[24] G. Seifert, K. Schilling, C. Steinhäuser, Astrocyte dysfunction in neurological disorders: a molecular perspective, Nat. Rev. Neurosci. 7 (2006) 194–206, https://doi.org/10.1038/nrn1870.
[25] M.T. Lin, M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, Nature 443 (2006) 787–795, https://doi.org/10.1038/nature05292.
[26] D.C. Wallace, A mitochondrial paradigm for degenerative diseases and ageing, in: G.B. Organizer, J.A. Goode (Eds.), Ageing Vulnerability: Causes and Interventions, John Wiley & Sons, Ltd, 2001, pp. 247–266.
[27] F. Aboul-Enein, H. Lassmann, Mitochondrial damage and histotoxic hypoxia: a pathway of tissue injury in inflammatory brain disease? Acta Neuropathol. 109 (2005) 49–55, https://doi.org/10.1007/s00401-004-0954-8.
[28] P. Mao, P.H. Reddy, Is multiple sclerosis a mitochondrial disease? Biochim. Biophys. Acta 1802 (2010) 66–79, https://doi.org/10.1016/j.bbadis.2009.07.002.
[29] M. Skowrońska, J. Albrecht, Oxidative and nitrosative stress in ammonia neurotoxicity, Neurochem. Int. 62 (2013) 731–737, https://doi.org/10.1016/j.neuint.2012.10.013.
[30] R.F. Butterworth, J.-F. Giguère, J. Michaud, J. Lavoie, G.P. Layrargues, Ammonia: key factor in the pathogenesis of hepatic encephalopathy, Neurochem. Pathol. 6 (1987) 1–12, https://doi.org/10.1007/BF02833598.
[31] M. Walterfang, O. Bonnot, R. Mocellin, D. Velakoulis, The neuropsychiatry of inborn errors of metabolism, J. Inherit. Metab. Dis. 36 (2013) 687–702, https://doi.org/10.1007/s10545-013-9618-y.
[32] O. Braissant, V.A. McLin, C. Cudalbu, Ammonia toxicity to the brain, J. Inherit. Metab. Dis. 36 (2013) 595–612, https://doi.org/10.1007/s10545-012-9546-2.
[33] L. Hertz, G. Kala, Energy metabolism in brain cells: effects of elevated ammonia concentrations, Metab. Brain Dis. 22 (2007) 199–218, https://doi.org/10.1007/s11011-007-9068-z.
[34] K.V. Rama Rao, M.D. Norenberg, Brain energy metabolism and mitochondrial dysfunction in acute and chronic hepatic encephalopathy, Neurochem. Int. 60 (2012) 697–706, https://doi.org/10.1016/j.neuint.2011.09.007.
[35] K.V.R. Rao, M.D. Norenberg, Cerebral energy metabolism in hepatic encephalopathy and hyperammonemia, Metab. Brain Dis. 16 (2001) 67–78, https://doi.org/10.1023/A:1011666612822.
[36] N. Katunuma, M. Okada, Y. Nishii, Regulation of the urea cycle and TCA cycle by ammonia, Adv. Enzym. Regul. 4 (1966) 317–335, https://doi.org/10.1016/0065-2571(66)90025-2.
[37] J.C.K. Lai, A.J.L. Cooper, Neurotoxicity of ammonia and fatty acids: differential inhibition of mitochondrial dehydrogenases by ammonia and fatty acyl coenzyme a derivatives, Neurochem. Res. 16 (1991) 795–803, https://doi.org/10.1007/BF00965689.
[38] B. Hindfelt, F. Plum, T.E. Duffy, Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts, J. Clin. Invest. 59 (1977) 386–396, https://doi.org/10.1172/JCI108651.
[39] C. Zwingmann, N. Chatauret, D. Leibfritz, R.F. Butterworth, Selective increase of brain lactate synthesis in experimental acute liver failure: results of a [1H-13C] nuclear magnetic resonance study, Hepatology 37 (2003) 420–428, https://doi.org/10.1053/jhep.2003.50052.
[40] Q. Wang, Y. Wang, Z. Yu, D. Li, B. Jia, J. Li, K. Guan, Y. Zhou, Y. Chen, Q. Kan, Ammonia-induced energy disorders interfere with bilirubin metabolism in hepatocytes, Arch. Biochem. Biophys. 555–556 (2014) 16–22, https://doi.org/10.1016/j.abb.2014.05.019.
[41] S. Dasarathy, R.P. Mookerjee, V. Rackayova, V. Rangroo Thrane, B. Vairappan, P. Ott, C.F. Rose, Ammonia toxicity: from head to toe? Metab. Brain Dis. 32 (2017) 529–538, https://doi.org/10.1007/s11011-016-9938-3.
[42] R.A. Hawkins, A.M. Mans, Cerebral function in hepatic encephalopathy, Cirrhosis, Hepatic Encephalopathy, and Ammonium Toxicity, Springer, 1990, pp. 1–22.
[43] J. Lavoie, J.-F. Giguère, G.P. Layrargues, R.F. Butterworth, Activities of neuronal and astrocytic marker enzymes in autopsied brain tissue from patients with hepatic encephalopathy, Metab. Brain Dis. 2 (1987) 283–290, https://doi.org/10.1007/BF00999698.
[44] R.A. Hawkins, J. Jessy, Hyperammonaemia does not impair brain function in the absence of net glutamine synthesis, Biochem. J. 277 (1991) 697–703, https://doi.org/10.1042/bj2770697.

[45] A.H. Lockwood, E.W.H. Yap, H.M. Rhoades, W.-H. Wong, Altered cerebral blood flow and glucose metabolism in patients with liver disease and minimal encephalopathy, J. Cereb. Blood Flow Metab. 11 (1991) 331–336, https://doi.org/10.1038/jcbfm.1991.66.
[46] G. Therrien, J.-F. Giguère, R.F. Butterworth, Increased cerebrospinal fluid lactate reflects deterioration of neurological status in experimental portal-systemic encephalopathy, Metab. Brain Dis. 6 (1991) 225–231, https://doi.org/10.1007/BF00996922.
[47] K. Qureshi, K.V.R. Rao, I.A. Qureshi, Differential inhibition by hyperammonemia of the electron transport chain enzymes in synaptosomes and non-synaptic mitochondria in ornithine transcarbamylase-deficient spf-mice: restoration by acetyl-L-carnitine, Neurochem. Res. 23 (1998) 855–861, https://doi.org/10.1023/A:1022406911604.
[48] L.A. Boer, J.P. Panatto, D.A. Fagundes, C. Bassani, I.C. Jeremias, J.F. Daufenbach, G.T. Rezin, L. Constantino, F. Dal-Pizzol, E.L. Streck, Inhibition of mitochondrial respiratory chain in the brain of rats after hepatic failure induced by carbon tetrachloride is reversed by antioxidants, Brain Res. Bull. 80 (2009) 75–78, https://doi.org/10.1016/j.brainresbull.2009.04.009.
[49] S. Dhanda, A. Sunkaria, A. Halder, R. Sandhir, Mitochondrial dysfunctions contribute to energy deficits in rodent model of hepatic encephalopathy, Metab. Brain Dis. 33 (2018) 209–223, https://doi.org/10.1007/s11011-017-0136-8.
[50] E. Kosenko, V. Felipo, C. Montoliu, S. Grisolía, Y. Kaminsky, Effects of acute hyperammonemia in vivo on oxidative metabolism in nonsynaptic rat brain mitochondria, Metab. Brain Dis. 12 (1997) 69–82, https://doi.org/10.1007/BF02676355.
[51] K.V. Rama Rao, Y.R. Mawal, I.A. Qureshi, Progressive decrease of cerebral cytochrome C oxidase activity in sparse-fur mice: role of acetyl-L-carnitine in restoring the ammonia-induced cerebral energy depletion, Neurosci. Lett. 224 (1997) 83–86, https://doi.org/10.1016/S0304-3940(97)13476-0.
[52] C.M. Veauvy, Y. Wang, P.J. Walsh, M.A. Pérez-Pinzón, Comparison of the effects of ammonia on brain mitochondrial function in rats and gulf toadfish, Am. J. Phys. 283 (2002) R598–R603, https://doi.org/10.1152/ajpregu.00018.2002.
[53] A. Adlimoghaddam, M.G. Sabbir, B.C. Albensi, Ammonia as a potential neurotoxic factor in Alzheimer's disease, Front. Mol. Neurosci. 9 (2016) 1–11, https://doi.org/10.3389/fnmol.2016.00057.
[54] C.R.K. Murthy, K.V. Rama Rao, G. Bai, M.D. Norenberg, Ammonia-induced production of free radicals in primary cultures of rat astrocytes, J. Neurosci. Res. 66 (2001) 282–288, https://doi.org/10.1002/jnr.1222.
[55] B. Görg, N. Qvartskhava, H.-J. Bidmon, N. Palomero-Gallagher, G. Kircheis, K. Zilles, D. Häussinger, Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy, Hepatology 52 (2010) 256–265, https://doi.org/10.1002/hep.23656.
[56] M.D. Norenberg, Oxidative and nitrosative stress in ammonia neurotoxicity, Hepatology 37 (2003) 245–248, https://doi.org/10.1053/jhep.2003.50087.
[57] M.D. Norenberg, A.R. Jayakumar, K.V. Rama Rao, Oxidative stress in the pathogenesis of hepatic encephalopathy, Metab. Brain Dis. 19 (2004) 313–329, https://doi.org/10.1023/B:MEBR.0000043978.91675.79.
[58] D. Häussinger, F. Schliess, Pathogenetic mechanisms of hepatic encephalopathy, Gut 57 (2008) 1156–1165, https://doi.org/10.1136/gut.2007.122176.
[59] E. Kosenko, M. Kaminsky, A. Kaminsky, M. Valencia, L. Lee, C. Hermenegildo, V. Felipo, Superoxide production and antioxidant enzymes in ammonia intoxication in rats, Free Radic. Res. 27 (1997) 637–644, https://doi.org/10.3109/10715769709097867.
[60] E. Kosenko, Y. Kaminsky, O. Lopata, N. Muravyov, A. Kaminsky, C. Hermenegildo, V. Felipo, Nitroarginine, an inhibitor of nitric oxide synthase, prevents changes in superoxide radical and antioxidant enzymes induced by ammonia intoxication, Metab. Brain Dis. 13 (1998) 29–41, https://doi.org/10.1023/A:1020626928259.
[61] A.S. Bender, W. Reichelt, M.D. Norenberg, Characterization of cystine uptake in cultured astrocytes, Neurochem. Int. 37 (2000) 269–276, https://doi.org/10.1016/S0197-0186(00)00035-8.
[62] E. Kosenko, N. Venediktova, Y. Kaminsky, C. Montoliu, V. Felipo, Sources of oxygen radicals in brain in acute ammonia intoxication in vivo, Brain Res. 981 (2003) 193–200, https://doi.org/10.1016/S0006-8993(03)03035-X.
[63] P.S. Brookes, Y. Yoon, J.L. Robotham, M.W. Anders, S.-S. Sheu, Calcium, ATP, and ROS: a mitochondrial love-hate triangle, Am. J. Phys. Cell Phys. 287 (2004) C817–C833, https://doi.org/10.1152/ajpcell.00139.2004.
[64] G. Ermak, K.J.A. Davies, Calcium and oxidative stress: from cell signaling to cell death, Mol. Immunol. 38 (2002) 713–721, https://doi.org/10.1016/S0161-5890(01)00108-0.
[65] M.R. Duchen, Mitochondria and calcium: from cell signalling to cell death, J. Physiol. 529 (2000) 57–68, https://doi.org/10.1111/j.1469-7793.2000.00057.x.
[66] Z. Liang, R. Liu, D. Zhao, L. Wang, M. Sun, M. Wang, L. Song, Ammonia exposure induces oxidative stress, endoplasmic reticulum stress and apoptosis in hepatopancreas of pacific white shrimp (*Litopenaeus vannamei*), Fish Shellfish Immunol. 54 (2016) 523–528, https://doi.org/10.1016/j.fsi.2016.05.009.
[67] C.R. Bosoi, C.F. Rose, Identifying the direct effects of ammonia on the brain, Metab. Brain Dis. 24 (2008) 95–102, https://doi.org/10.1007/s11011-008-9112-7.
[68] P. Casellas, S. Galiegue, A.S. Basile, Peripheral benzodiazepine receptors and mitochondrial function, Neurochem. Int. 40 (2002) 475–486, https://doi.org/10.1016/S0197-0186(01)00118-8.
[69] J. Albrecht, E.A. Jones, Hepatic encephalopathy: molecular mechanisms underlying the clinical syndrome, J. Neurol. Sci. 170 (1999) 138–146, https://doi.org/10.1016/S0022-510X(99)00169-0.
[70] P. Ghafourifar, U. Bringold, S.D. Klein, C. Richter, Mitochondrial nitric oxide Synthase, oxidative stress and apoptosis, Neurosignals 10 (2001) 57–65, https://doi.org/10.1159/000046875.
[71] V.C. Stewart, S.J.R. Heales, Nitric oxide-induced mitochondrial dysfunction: implications for neurodegeneration, Free Radic. Biol. Med. 34 (2003) 287–303, https://doi.org/10.1016/S0891-5849(02)01327-8.
[72] S. Hortelano, B. Dallaporta, N. Zamzami, T. Hirsch, S.A. Susin, I. Marzo, L. Boscá, G. Kroemer, Nitric oxide induces apoptosis via triggering mitochondrial permeability transition, FEBS Lett. 410 (1997) 373–377, https://doi.org/10.1016/S0014-5793(97)00623-6.
[73] A. Jekabsone, L. Ivanoviene, G.C. Brown, V. Borutaite, Nitric oxide and calcium together inactivate mitochondrial complex I and induce cytochrome c release, J. Mol. Cell. Cardiol. 35 (2003) 803–809, https://doi.org/10.1016/S0022-2828(03)00137-8.
[74] C. Szabó, H. Ischiropoulos, R. Radi, Peroxynitrite: biochemistry, pathophysiology and development of therapeutics, Nat. Rev. Drug Discov. 6 (2007) 662–680, https://doi.org/10.1038/nrd2222.
[75] E. Kosenko, Y. Kaminsky, E. Grau, M.-D. Miñana, S. Grisolia, V. Felipo, Nitroarginine, an inhibitor of nitric oxide synthetase, attenuates ammonia toxicity and ammonia-induced alterations in brain metabolism, Neurochem. Res. 20 (1995) 451–456, https://doi.org/10.1007/BF00973101.
[76] V.L.R. Rao, C.R.K. Murthy, R.F. Butterworth, Glutamatergic synaptic dysfunction in hyperammonemic syndromes, Metab. Brain Dis. 7 (1992) 1–20, https://doi.org/10.1007/BF01000437.
[77] M. Zielińska, R.O. Law, J. Albrecht, Excitotoxic mechanism of cell swelling in rat cerebral cortical slices treated acutely with ammonia, Neurochem. Int. 43 (2003) 299–303, https://doi.org/10.1016/S0197-0186(03)00015-9.
[78] N. ElMlili, J. Boix, H. Ahabrach, R. Rodrigo, M. Errami, V. Felipo, Chronic hyperammonemia induces tonic activation of NMDA receptors in cerebellum, J. Neurochem. 112 (2010) 1005–1014, https://doi.org/10.1111/j.1471-4159.2009.06520.x.
[79] A. Klejman, M.l. Węgrzynowicz, E.M. Szatmari, B. Mioduszewska, M.l. Hetman, J. Albrecht, Mechanisms of ammonia-induced cell death in rat cortical neurons: roles of NMDA receptors and glutathione, Neurochem. Int. 47 (2005) 51–57, https://doi.org/10.1016/j.neuint.2005.04.006.
[80] A. Verkhratsky, M. Nedergaard, L. Hertz, Why are astrocytes important? Neurochem. Res. 40 (2015) 389–401, https://doi.org/10.1007/s11064-014-1403-2.
[81] A. Lemberg, M.A. Fernández, Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress, Ann. Hepatol. 8 (2009) 95–102.
[82] L.D. Bobermin, G. Hansel, E.B.S. Scherer, A.T.S. Wyse, D.O. Souza, A. Quincozes-Santos, C.-A. Gonçalves, Ammonia impairs glutamatergic communication in astroglial cells: protective role of resveratrol, Toxicol. in Vitro 29 (2015) 2022–2029, https://doi.org/10.1016/j.tiv.2015.08.008.
[83] C. Rose, Effect of ammonia on astrocytic glutamate uptake/release mechanisms, J. Neurochem. 97 (2006) 11–15, https://doi.org/10.1111/j.1471-4159.2006.03796.x.
[84] J.J. Lemasters, T.P. Theruvath, Z. Zhong, A.-L. Nieminen, Mitochondrial calcium and the permeability transition in cell death, Biochim. Biophys. Acta 1787 (2009) 1395–1401, https://doi.org/10.1016/j.bbabio.2009.06.009.
[85] C. Hermenegildo, G. Marcaida, C. Montoliu, S. Grisolía, M.-D. Miñana, V. Felipo, NMDA receptor antagonists prevent acute ammonia toxicity in mice, Neurochem. Res. 21 (1996) 1237–1244, https://doi.org/10.1007/BF02532401.
[86] O. Detry, A.D. Roover, P. Honoré, M. Meurisse, Brain edema and intracranial hypertension in fulminant hepatic failure: Pathophysiology and management, World J. Gastroenterol. 12 (2006) 7405–7412, https://doi.org/10.3748/wjg.v12.i46.7405.
[87] J.O. Clemmesen, F.S. Larsen, J. Kondrup, B.A. Hansen, P. Ott, Cerebral herniation in patients with acute E liver failure is correlated with arterial ammonia concentration, Hepatology 29 (1999) 648–653, https://doi.org/10.1002/hep.510290309.
[88] K. Rama Rao, M. Chen, J. Simard, M.D. Norenberg, Suppression of ammonia-induced astrocyte swelling by cyclosporin A, J. Neurosci. Res. 74 (2003) 891–897, https://doi.org/10.1002/jnr.10755.
[89] M. Norenberg, A. Jayakumar, K.R. Rao, K. Panickar, New concepts in the mechanism of ammonia-induced astrocyte swelling, Metab. Brain Dis. 22 (2007) 219–234, https://doi.org/10.1007/s11011-007-9062-5.
[90] P.N. Bjerring, M. Eefsen, B.A. Hansen, F.S. Larsen, The brain in acute liver failure. A tortuous path from hyperammonemia to cerebral edema, Metab. Brain Dis. 24 (2009) 5, https://doi.org/10.1007/s11011-008-9116-3.
[91] A.T. Blei, F.S. Larsen, Pathophysiology of cerebral edema in fulminant hepatic failure, J. Hepatol. 31 (1999) 771–776, https://doi.org/10.1016/S0168-8278(99)80361-4.
[92] K.V. Rama Rao, P.V.B. Reddy, X. Tong, M.D. Norenberg, Brain edema in acute liver failure: Inhibition by l-histidine, Am. J. Pathol. 176 (2010) 1400–1408, https://doi.org/10.2353/ajpath.2010.090756.
[93] R.F. Butterworth, Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure, J. Clin. Exp. Hepatol. 5 (2015) S96–S103, https://doi.org/10.1016/j.jceh.2014.02.004.
[94] A.T. Blei, The pathophysiology of brain edema in acute liver failure, Neurochem. Int. 47 (2005) 71–77, https://doi.org/10.3748/wjg.v19.i48.9240.
[95] D. Shawcross, R. Jalan, The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation, Cell. Mol. Life Sci. 62 (2005) 2295–2304, https://doi.org/10.1007/s00018-005-5089-0.
[96] F.S. Larsen, J. Gottstein, A.T. Blei, Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema, J. Hepatol. 34 (2001) 548–554, https://doi.org/10.1016/S0168-8278(00)00069-6.
[97] P.V.B. Reddy, K.V. Rama Rao, M.D. Norenberg, Inhibitors of the mitochondrial

permeability transition reduce ammonia-induced cell swelling in cultured astrocytes, J. Neurosci. Res. 87 (2009) 2677–2685, https://doi.org/10.1002/jnr.22097.
[98] M.D. Norenberg, K.V.R. Rao, The mitochondrial permeability transition in neurologic disease, Neurochem. Int. 50 (2007) 983–997, https://doi.org/10.1016/j.neuint.2007.02.008.
[99] A.R. Jayakumar, K.V. Rama Rao, X.Y. Tong, M.D. Norenberg, Calcium in the mechanism of ammonia-induced astrocyte swelling, J. Neurochem. 109 (2009) 252–257, https://doi.org/10.1111/j.1471-4159.2009.05842.x.
[100] G. Bai, K.v. Rama Rao, C.R.K. Murthy, K.s. Panickar, A.r. Jayakumar, M.d. Norenberg, Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes, J. Neurosci. Res. 66 (2001) 981–991, https://doi.org/10.1002/jnr.10056.
[101] K.V. Rama Rao, A.R. Jayakumar, M.D. Norenberg, Role of oxidative stress in the ammonia-induced mitochondrial permeability transition in cultured astrocytes, Neurochem. Int. 47 (2005) 31–38, https://doi.org/10.1016/j.neuint.2005.04.004.
[102] D. Häussinger, B. Görg, Interaction of oxidative stress, astrocyte swelling and cerebral ammonia toxicity, Curr. Opin. Clin. Nutr. Metab. Care 13 (2010) 87, https://doi.org/10.1097/MCO.0b013e328333b829.
[103] H. Tanigami, A. Rebel, L.J. Martin, T.Y. Chen, S.W. Brusilow, R.J. Traystman, R.C. Koehler, Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonemia in rats, Neuroscience 131 (2005) 437–449, https://doi.org/10.1016/j.neuroscience.2004.10.045.
[104] R. Rai, V.A. Saraswat, R.K. Dhiman, Gut microbiota: its role in hepatic encephalopathy, J. Clin. Exp. Hepatol. 5 (2015) S29–S36, https://doi.org/10.1016/j.jceh.2014.12.003.
[105] B. Görg, A. Karababa, D. Häussinger, Hepatic Encephalopathy and astrocyte senescence, J. Clin. Exp. Hepatol. 8 (2018) 294–300, https://doi.org/10.1016/j.jceh.2018.05.003.
[106] A.J. Kowaltowski, A.E. Vercesi, Mitochondrial damage induced by conditions of oxidative stress, Free Radic. Biol. Med. 26 (1999) 463–471, https://doi.org/10.1016/S0891-5849(98)00216-0.
[107] R.F. Butterworth, Hepatic encephalopathy: a central neuroinflammatory disorder? Hepatology 53 (2011) 1372–1376, https://doi.org/10.1002/hep.24228.
[108] R. Rodrigo, O. Cauli, U. Gomez-Pinedo, A. Agusti, V. Hernandez-Rabaza, J.-M. Garcia-Verdugo, V. Felipo, Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy, Gastroenterology 139 (2010) 675–684, https://doi.org/10.1053/j.gastro.2010.03.040.
[109] O. Cauli, R. Rodrigo, B. Piedrafita, M. Llansola, M.T. Mansouri, V. Felipo, Neuroinflammation contributes to hypokinesia in rats with hepatic encephalopathy: ibuprofen restores its motor activity, J. Neurosci. Res. 87 (2009) 1369–1374, https://doi.org/10.1002/jnr.21947.
[110] M. Luo, H. Liu, S.-J. Hu, F.-H. Bai, Potential targeted therapies for the inflammatory pathogenesis of hepatic encephalopathy, Clin. Res. Hepatol. Gastroenterol. 39 (2015) 665–673, https://doi.org/10.1016/j.clinre.2015.06.020.
[111] W. Jiang, P. Desjardins, R.F. Butterworth, Cerebral inflammation contributes to encephalopathy and brain edema in acute liver failure: protective effect of minocycline, J. Neurochem. 109 (2009) 485–493, https://doi.org/10.1111/j.1471-4159.2009.05981.x.
[112] A. Chastre, M. Bélanger, E. Beauchesne, B.N. Nguyen, P. Desjardins, R.F. Butterworth, Inflammatory cascades driven by tumor necrosis factor-alpha play a major role in the progression of acute liver failure and its neurological complications, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0049670.
[113] R. Jover, R. Rodrigo, V. Felipo, R. Insausti, J. Sáez-Valero, M.S. García-Ayllón, I. Suárez, A. Candela, A. Compañ, A. Esteban, O. Cauli, E. Ausó, E. Rodríguez, A. Gutiérrez, E. Girona, S. Erceg, P. Berbel, M. Pérez-Mateo, Brain edema and inflammatory activation in bile duct ligated rats with diet-induced hyperammonemia: A model of hepatic encephalopathy in cirrhosis, Hepatology 43 (2006) 1257–1266, https://doi.org/10.1002/hep.21180.
[114] V.L. Pearson, N.J. Rothwell, S. Toulmond, Excitotoxic brain damage in the rat induces interleukin-1β protein in microglia and astrocytes: correlation with the progression of cell death, Glia 25 (1999) 311–323, https://doi.org/10.1002/(SICI)1098-1136(19990215)25:4<311::AID-GLIA1>3.0.CO;2-E.
[115] I. Zemtsova, B. Görg, V. Keitel, H.-J. Bidmon, K. Schrör, D. Häussinger, Microglia activation in hepatic encephalopathy in rats and humans, Hepatology 54 (2011) 204–215, https://doi.org/10.1002/hep.24326.
[116] H. Cichoż-Lach, A. Michalak, Current pathogenetic aspects of hepatic encephalopathy and noncirrhotic hyperammonemic encephalopathy, World J. Gastroenterol. 19 (2013) 26–34, https://doi.org/10.3748/wjg.v19.i1.26.
[117] W. Jiang, P. Desjardins, R.F. Butterworth, Minocycline attenuates oxidative/nitrosative stress and cerebral complications of acute liver failure in rats, Neurochem. Int. 55 (2009) 601–605, https://doi.org/10.1016/j.neuint.2009.06.001.
[118] W. Jiang, P. Desjardins, R.F. Butterworth, Direct evidence for central proinflammatory mechanisms in rats with experimental acute liver failure: protective effect of hypothermia, J. Cereb. Blood Flow Metab. 29 (2009) 944–952, https://doi.org/10.1038/jcbfm.2009.18.
[119] R.F. Butterworth, Neuroinflammation in acute liver failure: mechanisms and novel therapeutic targets, Neurochem. Int. 59 (2011) 830–836, https://doi.org/10.1016/j.neuint.2011.07.014.
[120] B. Liu, J.-S. Hong, Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention, J. Pharmacol. Exp. Ther. 304 (2003) 1–7, https://doi.org/10.1124/jpet.102.035048.
[121] M.A. Yenari, T.M. Kauppinen, R.A. Swanson, Microglial activation in stroke: therapeutic targets, Neurotherapeutics 7 (2010) 378–391, https://doi.org/10.1016/j.nurt.2010.07.005.
[122] W.J. Streit, R.E. Mrak, W.S.T. Griffin, Microglia and neuroinflammation: a pathological perspective, J. Neuroinflammation 1 (2004) 14, https://doi.org/10.1186/1742-2094-1-14.
[123] J.V. Welser-Alves, R. Milner, Microglia are the major source of TNF-α and TGF-β in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin, Neurochem. Int. 63 (2013), https://doi.org/10.1016/j.neuint.2013.04.007.
[124] V. Hernández-Rabaza, A. Cabrera-Pastor, L. Taoro-González, M. Malaguarnera, A. Agustí, M. Llansola, V. Felipo, Hyperammonemia induces glial activation, neuroinflammation and alters neurotransmitter receptors in hippocampus, impairing spatial learning: reversal by sulforaphane, J. Neuroinflammation 13 (2016), https://doi.org/10.1186/s12974-016-0505-y.
[125] A.K. Andersson, L. Rönnbäck, E. Hansson, Lactate induces tumour necrosis factor-alpha, interleukin-6 and interleukin-1beta release in microglial- and astroglial-enriched primary cultures, J. Neurochem. 93 (2005) 1327–1333, https://doi.org/10.1111/j.1471-4159.2005.03132.x.
[126] P. Agostinho, R. A Cunha, C. Oliveira, Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease, Curr. Pharm. Des. 16 (2010) 2766–2778, https://doi.org/10.2174/138161210793176572.
[127] R.L. Mosley, E.J. Benner, I. Kadiu, M. Thomas, M.D. Boska, K. Hasan, C. Laurie, H.E. Gendelman, Neuroinflammation, oxidative stress, and the pathogenesis of Parkinson's disease, Clin. Neurosci. Res. 6 (2006) 261–281, https://doi.org/10.1016/j.cnr.2006.09.006.
[128] J.A. Smith, Neutrophils, host defense, and inflammation: a double-edged sword, J. Leukoc. Biol. 56 (1994) 672–686, https://doi.org/10.1002/jlb.56.6.672.
[129] T. Tatsumi, S. Matoba, A. Kawahara, N. Keira, J. Shiraishi, K. Akashi, M. Kobara, T. Tanaka, M. Katamura, C. Nakagawa, B. Ohta, T. Shirayama, K. Takeda, J. Asayama, H. Fliss, M. Nakagawa, Cytokine-induced nitric oxide production inhibits mitochondrial energy production and impairs contractile function in rat cardiac myocytes, J. Am. Coll. Cardiol. 35 (2000) 1338–1346, https://doi.org/10.1016/S0735-1097(00)00526-X.
[130] F.R. Ponziani, M.A. Zocco, L. Cerrito, A. Gasbarrini, M. Pompili, Bacterial translocation in patients with liver cirrhosis: physiology, clinical consequences, and practical implications, Expert Rev. Gastroenterol. Hepatol. 12 (2018) 641–656, https://doi.org/10.1080/17474124.2018.1481747.
[131] D.L. Shawcross, S.S. Shabbir, N.J. Taylor, R.D. Hughes, Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis, Hepatology 51 (2010) 1062–1069, https://doi.org/10.1002/hep.23367.
[132] A.S. Seyan, R.D. Hughes, D.L. Shawcross, Changing face of hepatic encephalopathy: role of inflammation and oxidative stress, World J. Gastroenterol. 16 (2010) 3347–3357, https://doi.org/10.3748/wjg.v16.i27.3347.
[133] A.M. Espinosa-Oliva, R.M. De Pablos, R.F. Villarán, S. Argüelles, J.L. Venero, A. Machado, J. Cano, Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus, Neurobiol. Aging 32 (2011) 85–102, https://doi.org/10.1016/j.neurobiolaging.2009.01.012.
[134] N. Rolando, J.I.M. Wade, M. Davalos, J. Wendon, J. Philpott-Howard, R. Williams, The systemic inflammatory response syndrome in acute liver failure, Hepatology 32 (2000) 734–739, https://doi.org/10.1053/jhep.2000.17687.
[135] C. Solé, E. Solà, M. Morales-Ruiz, G. Fernàndez, P. Huelin, I. Graupera, R. Moreira, G. de Prada, X. Ariza, E. Pose, N. Fabrellas, S.G. Kalko, W. Jiménez, P. Ginès, Characterization of inflammatory response in acute-on-chronic liver failure and relationship with prognosis, Sci. Rep. 6 (2016) 32341, , https://doi.org/10.1038/srep32341.
[136] S. Tilleux, E. Hermans, Neuroinflammation and regulation of glial glutamate uptake in neurological disorders, J. Neurosci. Res. 85 (2007) 2059–2070, https://doi.org/10.1002/jnr.21325.
[137] W. Sheng, Y. Zong, A. Mohammad, D. Ajit, J. Cui, D. Han, J.L. Hamilton, A. Simonyi, A.Y. Sun, Z. Gu, J.-S. Hong, G.A. Weisman, G.Y. Sun, Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, iNOS and sPLA2-IIA expression in astrocytes and microglia, J. Neuroinflammation 8 (2011) 121, https://doi.org/10.1186/1742-2094-8-121.
[138] A. Fernandes, R.F.M. Silva, A.S. Falcão, M.A. Brito, D. Brites, Cytokine production, glutamate release and cell death in rat cultured astrocytes treated with unconjugated bilirubin and LPS, J. Neuroimmunol. 153 (2004) 64–75, https://doi.org/10.1016/j.jneuroim.2004.04.007.
[139] S. Dadsetan, T. Balzano, J. Forteza, A. Cabrera-Pastor, L. Taoro-Gonzalez, V. Hernandez-Rabaza, S. Gil-Perotín, L. Cubas-Núñez, J.-M. García-Verdugo, A. Agusti, Reducing peripheral inflammation with infliximab reduces neuroinflammation and improves cognition in rats with hepatic encephalopathy, Front. Mol. Neurosci. 9 (2016) 106, , https://doi.org/10.3389/fnmol.2016.00106.
[140] C.F. Rose, Increase brain lactate in hepatic encephalopathy: cause or consequence? Neurochem. Int. 57 (2010) 389–394, https://doi.org/10.1016/j.neuint.2010.06.012.
[141] L. Riske, R.K. Thomas, G.B. Baker, S.M. Dursun, Lactate in the brain: an update on its relevance to brain energy, neurons, glia and panic disorder, Therap. Adv. Psychopharmacol. 7 (2017) 85–89, https://doi.org/10.1177/2045125316675579.
[142] A.B. Santamaria, Manganese exposure, essentiality & toxicity, Indian J. Med. Res. 128 (2008) 484.
[143] A.B. Bowman, G.F. Kwakye, E. Herrero Hernández, M. Aschner, Role of manganese in neurodegenerative diseases, J. Trace Elem. Med. Biol. 25 (2011) 191–203, https://doi.org/10.1016/j.jtemb.2011.08.144.
[144] C. Rose, R.F. Butterworth, J. Zayed, L. Normandin, K. Todd, A. Michalak, L. Spahr, P.M. Huet, G. Pomier-Layrargues, Manganese deposition in basal ganglia

structures results from both portal-systemic shunting and liver dysfunction, Gastroenterology 117 (1999) 640–644, https://doi.org/10.1016/S0016-5085(99)70457-9.
[145] R.F. Butterworth, L. Spahr, S. Fontaine, G.P. Layrargues, Manganese toxicity, dopaminergic dysfunction and hepatic encephalopathy, Metab. Brain Dis. 10 (1995) 259–267, https://doi.org/10.1007/BF02109357.
[146] L. Spahr, R.F. Butterworth, S. Fontaine, L. Bui, G. Therrien, P.C. Milette, L.H. Lebrun, J. Zayed, A. Leblanc, G. Pomier-Layrargues, Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms, Hepatology 24 (1996) 1116–1120, https://doi.org/10.1002/hep.510240523.
[147] S. Montes, M. Alcaraz-Zubeldia, P. Muriel, C. Ríos, Striatal manganese accumulation induces changes in dopamine metabolism in the cirrhotic rat, Brain Res. 891 (2001) 123–129, https://doi.org/10.1016/S0006-8993(00)03208-X.
[148] J.L. Aschner, M. Aschner, Nutritional aspects of manganese homeostasis, Mol. Asp. Med. 26 (2005) 353–362, https://doi.org/10.1016/j.mam.2005.07.003.
[149] D. Krieger, S. Krieger, L. Theilmann, O. Jansen, P. Gass, H. Lichtnecker, Manganese and chronic hepatic encephalopathy, Lancet 346 (1995) 270–274, https://doi.org/10.1016/S0140-6736(95)92164-8.
[150] K. Prabhakaran, D. Ghosh, G.D. Chapman, P.G. Gunasekar, Molecular mechanism of manganese exposure-induced dopaminergic toxicity, Brain Res. Bull. 76 (2008) 361–367, https://doi.org/10.1016/j.brainresbull.2008.03.004.
[151] J.S. Crossgrove, D.D. Allen, B.L. Bukaveckas, S.S. Rhineheimer, R.A. Yokel, Manganese distribution across the blood–brain barrier: I. evidence for carrier-mediated influx of managanese citrate as well as manganese and manganese transferrin, Neurotoxicology 24 (2003) 3–13, https://doi.org/10.1016/S0161-813X(02)00089-X.
[152] M. Aschner, J.L. Aschner, Manganese neurotoxicity: cellular effects and blood-brain barrier transport, Neurosci. Biobehav. Rev. 15 (1991) 333–340, https://doi.org/10.1016/S0149-7634(05)80026-0.
[153] G.P. Layrargues, C. Rose, L. Spahr, J. Zayed, L. Normandin, R.F. Butterworth, Role of manganese in the pathogenesis of portal-systemic encephalopathy, Metab. Brain Dis. 13 (1998) 311–317, https://doi.org/10.1023/A:1020636809063.
[154] K.J. Klos, J.E. Ahlskog, N. Kumar, S. Cambern, J. Butz, M. Burritt, R.D. Fealey, C.T. Cowl, J.E. Parisi, K.A. Josephs, Brain metal concentrations in chronic liver failure patients with pallidal T1 MRI hyperintensity, Neurology 67 (2006) 1984–1989, https://doi.org/10.1212/01.wnl.0000247037.37807.76.
[155] L. Spahr, P.R. Burkhard, H. Grötzsch, A. Hadengue, Clinical significance of basal ganglia alterations at brain MRI and 1H MRS in cirrhosis and role in the pathogenesis of hepatic encephalopathy, Metab. Brain Dis. 17 (2002) 399–413, https://doi.org/10.1023/A:1021974321874.
[156] K.J. Klos, J.E. Ahlskog, K.A. Josephs, R.D. Fealey, C.T. Cowl, N. Kumar, Neurologic spectrum of chronic liver failure and basal ganglia T1 hyperintensity on magnetic resonance imaging: probable manganese neurotoxicity, Arch. Neurol. 62 (2005) 1385–1390, https://doi.org/10.1001/archneur.62.9.1385.
[157] R. Jover, L. Compañy, A. Gutiérrez, P. Zapater, J. Pérez-Serra, E. Girona, J.R. Aparicio, M. Pérez-Mateo, Minimal hepatic encephalopathy and extrapyramidal signs in patients with cirrhosis, Am. J. Gastroenterol. 98 (2003) 1599–1604, https://doi.org/10.1111/j.1572-0241.2003.07528.x.
[158] K.J. Klos, M. Chandler, N. Kumar, J.E. Ahlskog, K.A. Josephs, Neuropsychological profiles of manganese neurotoxicity, Eur. J. Neurol. 13 (2006) 1139–1141, https://doi.org/10.1111/j.1468-1331.2006.01407.x.
[159] C.W. Olanow, Manganese-induced parkinsonism and Parkinson's disease, Ann. N. Y. Acad. Sci. 1012 (2004) 209–223, https://doi.org/10.1196/annals.1306.018.
[160] W.N. Sloot, J.-B.P. Gramsbergen, Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia, Brain Res. 657 (1994) 124–132, https://doi.org/10.1016/0006-8993(94)90959-8.
[161] A.W. Dobson, K.M. Erikson, M. Aschner, Manganese neurotoxicity, Ann. N. Y. Acad. Sci. 1012 (2004) 115–128, https://doi.org/10.1196/annals.1306.009.
[162] C.-J. Chen, S.-L. Liao, Oxidative stress involves in astrocytic alterations induced by manganese, Exp. Neurol. 175 (2002) 216–225, https://doi.org/10.1006/exnr.2002.7894.
[163] E.P. Brouillet, L. Shinobu, U. McGarvey, F. Hochberg, M.F. Beal, Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism, Exp. Neurol. 120 (1993) 89–94, https://doi.org/10.1006/exnr.1993.1042.
[164] K.M. Erikson, M. Aschner, Manganese neurotoxicity and glutamate-GABA interaction, Neurochem. Int. 43 (2003) 475–480, https://doi.org/10.1016/S0197-0186(03)00037-8.
[165] M. Sidoryk-Wegrzynowicz, M. Aschner, Role of astrocytes in manganese mediated neurotoxicity, BMC Pharmacol. Toxicol. 14 (2013) 23, , https://doi.org/10.1186/2050-6511-14-23.
[166] V.A. Fitsanakis, C. Au, K.M. Erikson, M. Aschner, The effects of manganese on glutamate, dopamine and γ-aminobutyric acid regulation, Neurochem. Int. 48 (2006) 426–433, https://doi.org/10.1016/j.neuint.2005.10.012.
[167] A. Takeda, N. Sotogaku, N. Oku, Manganese influences the levels of neurotransmitters in synapses in rat brain, Neuroscience 114 (2002) 669–674, https://doi.org/10.1016/S0306-4522(02)00353-6.
[168] F. Zhang, Z. Xu, J. Gao, B. Xu, Y. Deng, In vitro effect of manganese chloride exposure on energy metabolism and oxidative damage of mitochondria isolated from rat brain, Environ. Toxicol. Pharmacol. 26 (2008) 232–236, https://doi.org/10.1016/j.etap.2008.04.003.
[169] J. Jiao, Y. Qi, J. Fu, Z. Zhou, Manganese-induced single strand breaks of mitochondrial DNA in vitro and in vivo, Environ. Toxicol. Pharmacol. 26 (2008) 123–127, https://doi.org/10.1016/j.etap.2007.12.009.
[170] D. Milatovic, S. Zaja-Milatovic, R.C. Gupta, Y. Yu, M. Aschner, Oxidative damage and neurodegeneration in manganese-induced neurotoxicity, Toxicol. Appl. Pharmacol. 240 (2009) 219–225, https://doi.org/10.1016/j.taap.2009.07.004.
[171] K.M. Erikson, A.W. Dobson, D.C. Dorman, M. Aschner, Manganese exposure and induced oxidative stress in the rat brain, Sci. Total Environ. 334–335 (2004) 409–416, https://doi.org/10.1016/j.scitotenv.2004.04.044.
[172] S. Weber, D.C. Dorman, L.H. Lash, K. Erikson, K.E. Vrana, M. Aschner, Effects of manganese (Mn) on the developing rat brain: oxidative-stress related endpoints, Neurotoxicology 23 (2002) 169–175, https://doi.org/10.1016/S0161-813X(02)00014-1.
[173] F. Zhang, Z. Xu, J. Gao, B. Xu, Y. Deng, In vitro effect of manganese chloride exposure on reactive oxygen species generation and respiratory chain complexes activities of mitochondria isolated from rat brain, Toxicol. in Vitro 18 (2004) 71–77, https://doi.org/10.1016/j.tiv.2003.09.002.
[174] P. Karki, E. Lee, M. Aschner, Manganese neurotoxicity: A focus on glutamate transporters, Ann. Occup. Environ. Med. 25 (2013) 4, https://doi.org/10.1186/2052-4374-25-4.
[175] I.J. Yu, J.D. Park, E.S. Park, K.S. Song, K.T. Han, J.H. Han, Y.H. Chung, B.S. Choi, K.H. Chung, M.H. Cho, Manganese distribution in brains of Sprague–Dawley rats after 60 days of stainless steel welding-fume exposure, Neurotoxicology 24 (2003) 777–785, https://doi.org/10.1016/S0161-813X(03)00046-9.
[176] Z. Yin, J.L. Aschner, A.P.d. Santos, M. Aschner, Mitochondrial-dependent manganese neurotoxicity in rat primary astrocyte cultures, Brain Res. 1203 (2008) 1–11, https://doi.org/10.1016/j.brainres.2008.01.079.
[177] V.C. Culotta, M. Yang, M.D. Hall, Manganese transport and trafficking: lessons learned from Saccharomyces cerevisiae, Eukaryot. Cell 4 (2005) 1159–1165, https://doi.org/10.1128/EC.4.7.1159-1165.2005.
[178] B. Chance, L. Mela, Calcium and manganese interactions in mitochondrial ion accumulation, Biochemistry 5 (1966) 3220–3223, https://doi.org/10.1021/bi00874a022.
[179] C.E. Gavin, K.K. Gunter, T.E. Gunter, Manganese and calcium transport in mitochondria: implications for manganese toxicity, Neurotoxicology 20 (1999) 445–453.
[180] K.V.R. Rao, M.D. Norenberg, Manganese induces the mitochondrial permeability transition in cultured astrocytes, J. Biol. Chem. 279 (2004) 32333–32338, https://doi.org/10.1074/jbc.M402096200.
[181] C.E. Gavin, K.K. Gunter, T.E. Gunter, Mn2+ sequestration by mitochondria and inhibition of oxidative phosphorylation, Toxicol. Appl. Pharmacol. 115 (1992) 1–5, https://doi.org/10.1016/0041-008X(92)90360-5.
[182] M.A. Verity, Manganese neurotoxicity: A mechanistic hypothesis, Neurotoxicology 20 (1999) 489–497.
[183] C. Zwingmann, D. Leibfritz, A.S. Hazell, Brain energy metabolism in a sub-acute rat model of manganese neurotoxicity: an ex vivo nuclear magnetic resonance study using [1-13C]glucose, Neurotoxicology 25 (2004) 573–587, https://doi.org/10.1016/j.neuro.2003.08.002.
[184] C. Zwingmann, D. Leibfritz, A.S. Hazell, Energy metabolism in astrocytes and neurons treated with manganese: relation among cell-specific energy failure, glucose metabolism, and intercellular trafficking using multinuclear NMR-spectroscopic analysis, J. Cereb. Blood Flow Metab. 23 (2003) 756–771, https://doi.org/10.1097/01.WCB.0000056062.25434.4D.
[185] D. Milatovic, R.C. Gupta, Y. Yu, S. Zaja-Milatovic, M. Aschner, Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury, Toxicol. Appl. Pharmacol. 256 (2011) 219–226, https://doi.org/10.1016/j.taap.2011.06.001.
[186] M.R. Al Sibae, B.M. McGuire, Current trends in the treatment of hepatic encephalopathy, Ther. Clin. Risk Manag. 5 (2009) 617–626, https://doi.org/10.2147/TCRM.S4443.
[187] L.D. Bobermin, K.M. Wartchow, M.P. Flores, M.C. Leite, A. Quincozes-Santos, C.-A. Gonçalves, Ammonia-induced oxidative damage in neurons is prevented by resveratrol and lipoic acid with participation of heme oxygenase 1, Neurotoxicology 49 (2015) 28–35, https://doi.org/10.1016/j.neuro.2015.05.005.
[188] R. Heidari, A. Jamshidzadeh, H. Niknahad, E. Mardani, M.M. Ommati, N. Azarpira, F. Khodaei, A. Zarei, M. Ayarzadeh, S. Mousavi, N. Abdoli, B.S. Yeganeh, A. Saeedi, A. Najibi, Effect of taurine on chronic and acute liver injury: focus on blood and brain ammonia, Toxicol. Rep. 3 (2016) 870–879, https://doi.org/10.1016/j.toxrep.2016.04.002.
[189] R.F. Butterworth, M.D. Norenberg, V. Felipo, P. Ferenci, J. Albrecht, A.T. Blei, Experimental models of hepatic encephalopathy: ISHEN guidelines, Liver Int. 29 (2009) 783–788, https://doi.org/10.1111/j.1478-3231.2009.02034.x.
[190] P.V.B. Reddy, C.R.K. Murthy, P. Reddanna, Fulminant hepatic failure induced oxidative stress in nonsynaptic mitochondria of cerebral cortex in rats, Neurosci. Lett. 368 (2004) 15–20, https://doi.org/10.1016/j.neulet.2004.06.046.
[191] K. Chadipiralla, P. Reddanna, R.M. Chinta, P.V.B. Reddy, Thioacetamide-induced fulminant hepatic failure induces cerebral mitochondrial dysfunction by altering the electron transport chain complexes, Neurochem. Res. 37 (2012) 59–68, https://doi.org/10.1007/s11064-011-0583-2.
[192] E. Kosenko, Y. Kaminski, O. Lopata, N. Muravyov, V. Felipo, Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication, Free Radic. Biol. Med. 26 (1999) 1369–1374, https://doi.org/10.1016/S0891-5849(98)00339-6.
[193] A. Jamshidzadeh, N. Abdoli, H. Niknahad, N. Azarpira, S. Mousavi, E. Mardani, M. Abasvali, R. Heidari, Carnosine supplementation mitigates brain tissue markers of oxidative stress in a rat model of fulminant hepatic failure, Trends Pharmacol. Sci. 3 (2017) 149–160, https://doi.org/10.1111/tips.v3i3.149.
[194] A. Jamshidzadeh, R. Heidari, Z. Latifpour, M.M. Ommati, N. Abdoli, S. Mousavi, N. Azarpira, A. Zarei, M. Zarei, B. Asadi, M. Abasvali, Y. Yeganeh, F. Jafari, A. Saeedi, A. Najibi, E. Mardani, Carnosine ameliorates liver fibrosis and

hyperammonemia in cirrhotic rats, Clin. Res. Hepatol. Gastroenterol. 41 (2017) 424–434, https://doi.org/10.1016/j.clinre.2016.12.010.
[195] A. Jamshidzadeh, H. Niknahad, R. Heidari, M. Zarei, M.M. Ommati, F. Khodaei, Carnosine protects brain mitochondria under hyperammonemic conditions: relevance to hepatic encephalopathy treatment, PharmaNutrition 5 (2017) 58–63, https://doi.org/10.1016/j.phanu.2017.02.004.
[196] A. Jamshidzadeh, N. Abdoli, H. Niknahad, N. Azarpira, E. Mardani, S. Mousavi, M. Abasvali, R. Heidari, Taurine alleviates brain tissue markers of oxidative stress in a rat model of hepatic encephalopathy, Trends Pharmacol. Sci. 3 (2017) 181–192, https://doi.org/10.1111/tips.v3i3.150.
[197] R. Heidari, A. Jamshidzadeh, V. Ghanbarinejad, M.M. Ommati, H. Niknahad, Taurine supplementation abates cirrhosis-associated locomotor dysfunction, Clin. Exp. Hepatol. 4 (2018) 72–82, https://doi.org/10.5114/ceh.2018.75956.
[198] J. Albrecht, M. Wegrzynowicz, Endogenous neuroprotectants in ammonia toxicity in the central nervous system: facts and hypotheses, Metab. Brain Dis. 20 (2005) 253–263, https://doi.org/10.1007/s11011-005-7904-6.
[199] C.R. Bosoi, C.F. Rose, Oxidative stress: a systemic factor implicated in the pathogenesis of hepatic encephalopathy, Metab. Brain Dis. 28 (2013) 175–178, https://doi.org/10.1007/s11011-012-9351-5.
[200] A.N. Chepkova, O.A. Sergeeva, H.L. Haas, Taurine rescues hippocampal long-term potentiation from ammonia-induced impairment, Neurobiol. Dis. 23 (2006) 512–521, https://doi.org/10.1016/j.nbd.2006.04.006.
[201] G. Malaguarnera, M. Pennisi, G. Bertino, M. Motta, A.M. Borzì, E. Vicari, R. Bella, F. Drago, M. Malaguarnera, Resveratrol in patients with minimal hepatic encephalopathy, Nutrients 10 (2018) 329, https://doi.org/10.3390/nu10030329.
[202] W. Jiang, P. Desjardins, R.F. Butterworth, Hypothermia attenuates oxidative/nitrosative stress, encephalopathy and brain edema in acute (ischemic) liver failure, Neurochem. Int. 55 (2009) 124–128, https://doi.org/10.1016/j.neuint.2009.02.007.
[203] S.H. Hansen, M.L. Andersen, H. Birkedal, C. Cornett, F. Wibrand, The important role of taurine in oxidative metabolism, Adv. Exp. Med. Biol. 583 (2006) 129–135, https://doi.org/10.1007/978-0-387-33504-9_13.
[204] S.H. Hansen, N. Grunnet, Taurine, glutathione and bioenergetics, in: A.E. Idrissi, W.J. L'Amoreaux (Eds.), Taurine 8, Springer, New York, 2013, pp. 3–12.
[205] M.M. Ommati, A. Jamshidzadeh, R. Heidari, Z. Sun, M.J. Zamiri, F. Khodaei, S. Mousapour, F. Ahmadi, N. Javanmard, B.S. Yeganeh, Carnosine and histidine supplementation blunt lead-induced reproductive toxicity through antioxidative and mitochondria-dependent mechanisms, Biol. Trace Elem. Res. (2018) 1–12, https://doi.org/10.1007/s12011-018-1358-2 (In Press).
[206] C. Corona, V. Frazzini, E. Silvestri, R. Lattanzio, R. La Sorda, M. Piantelli, L.M.T. Canzoniero, D. Ciavardelli, E. Rizzarelli, S.L. Sensi, Effects of dietary supplementation of carnosine on mitochondrial dysfunction, amyloid pathology, and cognitive deficits in 3xTg-AD mice, PLoS One 6 (2011) e17971, , https://doi.org/10.1371/journal.pone.0017971.
[207] M. Llansola, R. Rodrigo, P. Monfort, C. Montoliu, E. Kosenko, O. Cauli, B. Piedrafita, N.E. Mlili, V. Felipo, NMDA receptors in hyperammonemia and hepatic encephalopathy, Metab. Brain Dis. 22 (2007) 321–335, https://doi.org/10.1007/s11011-007-9067-0.
[208] T. Saran, W. Hilgier, T. Kocki, E.M. Urbanska, W.A. Turski, J. Albrecht, Acute ammonia treatment in vitro and in vivo inhibits the synthesis of a neuroprotectant kynurenic acid in rat cerebral cortical slices, Brain Res. 787 (1998) 348–350, https://doi.org/10.1016/S0006-8993(98)00031-6.
[209] G. Marcaida, V. Felipo, C. Hermenegildo, M.D. Miñana, S. Grisolía, Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors, FEBS Lett. 296 (1992) 67–68, https://doi.org/10.1016/0014-5793(92)80404-5.
[210] O. Cauli, R. Rodrigo, J. Boix, B. Piedrafita, A. Agusti, V. Felipo, Acute liver failure-induced death of rats is delayed or prevented by blocking NMDA receptors in brain, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G503–G511, https://doi.org/10.1152/ajpgi.00076.2008.
[211] C.Y. Chan, H.S. Sun, S.M. Shah, M.S. Agovic, E. Friedman, S.P. Banerjee, Modes of direct modulation by taurine of the glutamate NMDA receptor in rat cortex, Eur. J. Pharmacol. 728 (2014) 167–175, https://doi.org/10.1016/j.ejphar.2014.01.025.
[212] C.Y. Chan, H.S. Sun, S.M. Shah, M.S. Agovic, I. Ho, E. Friedman, S.P. Banerjee, Direct interaction of taurine with the NMDA glutamate receptor subtype via multiple mechanisms, Adv. Exp. Med. Biol. 775 (2013) 45–52, https://doi.org/10.1007/978-1-4614-6130-2_4.
[213] C. Chung, J. Gottstein, A.T. Blei, Indomethacin prevents the development of experimental ammonia-induced brain edema in rats after portacaval anastomosis, Hepatology 34 (2001) 249–254, https://doi.org/10.1053/jhep.2001.26383.
[214] F. Tofteng, F.S. Larsen, The effect of indomethacin on intracranial pressure, cerebral perfusion and extracellular lactate and glutamate concentrations in patients with fulminant hepatic failure, J. Cereb. Blood Flow Metab. 24 (2004) 798–804, https://doi.org/10.1097/01.WCB.0000125648.03213.1D.
[215] O. Cauli, R. Rodrigo, B. Piedrafita, J. Boix, V. Felipo, Inflammation and hepatic encephalopathy: ibuprofen restores learning ability in rats with portacaval shunts, Hepatology 46 (2007) 514–519, https://doi.org/10.1002/hep.21734.
[216] J. Das, J. Ghosh, P. Manna, M. Sinha, P.C. Sil, Taurine protects rat testes against NaAsO2-induced oxidative stress and apoptosis via mitochondrial dependent and independent pathways, Toxicol. Lett. 187 (2009) 201–210, https://doi.org/10.1016/j.toxlet.2009.03.001.
[217] C.J. Jong, J. Azuma, S.W. Schaffer, Role of mitochondrial permeability transition in taurine deficiency-induced apoptosis, Exp. Clin. Cardiol. 16 (2011) 125–128.
[218] A. Mignarri, A. Federico, From the liver to the brain: manganese matters: focus on cirrhosis-related parkinsonism, Neurol. Sci. 35 (2014) 521–522, https://doi.org/10.1007/s10072-014-1665-0.
[219] E. Herrero Hernandez, G. Discalzi, C. Valentini, F. Venturi, A. Chiò, C. Carmellino, L. Rossi, A. Sacchetti, E. Pira, Follow-up of patients affected by manganese-induced parkinsonism after treatment with CaNa2EDTA, Neurotoxicology 27 (2006) 333–339, https://doi.org/10.1016/j.neuro.2005.09.003.
[220] K. Tuschl, P.B. Mills, P.T. Clayton, Manganese and the brain, Int. Rev. Neurobiol. (2013) 277–312 Elsevier.
[221] S.S. Oja, P. Saransaari, E.R. Korpi, Neurotoxicity of Ammonia, Neurochem. Res. 42 (2017) 713–720, https://doi.org/10.1007/s11064-016-2014-x.
[222] N. Haghighat, D.W. McCandless, P. Geraminegad, The effect of ammonium chloride on metabolism of primary neurons and neuroblastoma cells in vitro, Metab. Brain Dis. 15 (2000) 151–162, https://doi.org/10.1007/BF02679981.