Ammonia is diffused and transported across all plasma membranes. This entails that hyperammonemia leads to an increase in ammonia in all organs and tissues. It is known that the toxic ramifications of ammonia primarily touch the brain and cause neurological impairment. However, the deleterious effects of ammonia are not specific to the brain, as the direct effect of increased ammonia (change in pH, membrane potential, metabolism) can occur in any type of cell. Therefore, in the setting of chronic liver disease where multi-organ dysfunction is common, the role of ammonia is challenged. This review provides insights and evidence that increased ammonia can disturb many organ and cell types and hence lead to dysfunction.
Ammonia toxicity: from head to toe?

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Abstract

Ammonia is diffused and transported across all plasma membranes. This entails that hyperammonemia leads to an increase in ammonia in all organs and tissues. It is known that the toxic ramifications of ammonia primarily touch the brain and cause neurological impairment. However, the deleterious effects of ammonia are not specific to the brain, as the direct effect of increased ammonia (change in pH, membrane potential, metabolism) can occur in any type of cell. Therefore, in the setting of chronic liver disease where multi-organ dysfunction is common, the role of ammonia is challenged. This review provides insights and evidence that increased ammonia can disturb many organ and cell types and hence lead to dysfunction.
Effect of ammonia on cell function

Ammonia is used for a number of different metabolic reactions (including the synthesis of non-essential amino acids). However it is primarily a waste product of cellular metabolism and due to its harmful properties at higher concentrations, ammonia is quickly detoxified and transformed into less toxic compounds (Cooper and Plum, 1987; Butterworth, 2002). Under physiological conditions, blood levels of ammonia (in systemic circulation) are carefully maintained at concentrations <50µM in adults, 50-75µM in term neonates and 50-150µM in preterm neonates (Braissant et al., 2013). Here, the liver plays a vital role in regulating the levels of ammonia via the production of urea (a cycle of enzymes which together are solely found in the liver). Ammonia is also removed in extrahepatic organs, including the muscle and brain (astrocytes), through the amidation of glutamate to glutamine via the enzyme glutamine synthesis (GS) (Cooper and Plum, 1987).

The main source of ammonia generation occurs in the intestines; from lysis of blood-borne urea and also from protein digestion/deamination by urease-positive bacteria and microbial deaminase (Jones et al., 1969). A large amount of metabolically-generated ammonia is absorbed into the blood and via the portal vein is detoxified through the liver (Cooper and Plum, 1987; Brusilow et al., 2010). The concentration of ammonia in portal vein can be 5-10x higher than in the systemic circulation (Abdo, 2006) and during conditions of liver disease or failure, ammonia is poorly removed (due to impaired ureagenesis from hepatocellular dysfunction and portosystemic shunting) and is liberated into the systemic circulation and exposed to all organs (Rovira et al., 2008). Glutaminase, an enzyme which generates glutamate and ammonia from glutamine and which is found in the intestine, kidney and brain (neurons), also plays a contributing role to the development of hyperammonemia. Diseases causing hyperammonemia include acute liver failure (ALF), chronic liver disease (CLD) and porto-systemic shunting (Felipo and Butterworth, 2002). Hyperammonemia also develops in children with inborn errors of the urea cycle and tend to present with higher blood ammonia concentrations (up to 5 mM) compared to acquired causes (such ALF and CLD) which usually result in hyperammonemia levels between 0.2-1 mM (Ratnakumari et al., 1992; Matkowskyj et al., 1999; Butterworth, 2002; Lichter-Konecki et al., 2008).

Ammonia in solution (i.e blood) is present as NH$_3$ and NH$_4^+$ with the ratio NH$_3$/NH$_4^+$ depending on the pH as defined by the Henderson-Hasselbach equation. Under physiological conditions with a blood pH of 7.4, more than 98% of ammonia is in NH$_4^+$ form (Bromberg et al., 1960). Both forms affect pH, electrolytic, acid-base and ion equilibrium. NH$_3$ is a weak base in gaseous form and uncharged, therefore lipid soluble and capable to cross cell membranes through diffusion. Conversely, NH$_4^+$ is a weak acid, water soluble and because of its ionic properties crosses cell membranes at a less rapid rate through various transport channels (Bosoi and Rose, 2009). Due to almost identical hydration radius with K$^+$ (Kikeri et al., 1989), NH$_4^+$ can substitute K$^+$ in its channels and transporters (ATPase transporters Na$^+$/K$^+$ and H$^+$/K$^+$ (Moser, 1987) and Na$^+$/K$^+$/Cl$^-$ co-transporters (Kelly et al., 2009)). This NH$_4^+$ substitution in K$^+$ channels mediates membrane depolarization in vitro (Allert et al., 1998; Norenberg, 1998), but putatively not in situ (Rangroo Thrane et al., 2013). Ammonia can also enter cells through aquaporin-8 channel (Liu et al., 2006; Saparov et al., 2007) and ammonia specific transporters (human non-erythroid Rhesus glycoprotein B and C (Bakouh et al., 2006)). There is also growing
Ammonia alters both intracellular and extracellular pH and can cause intracellular acidification as well as alkalisation, all depending on ammonium concentration, pH and rate of NH₃ vs. NH₄⁺ transport through the cell membrane (Norenberg, 1998; Bosoi and Rose, 2009; Rangroo Thrane et al., 2013). Ammonia has also be shown to cause a rise in intracellular Ca²⁺ primarily due to cytosolic alkalisation in vitro (Rose et al., 2005) and through less well characterized mechanisms lead to increased intracellular Ca²⁺ signalling in vivo (Rangroo Thrane et al., 2013). Ammonia-induced changes in pH, membrane potential as well as alterations in cellular metabolism negatively impacts cell function by influencing signalling transduction pathways, activities of many enzymes, alteration in protein phosphorylation and the state of various other ion channels and transporters (Busa and Nuccitelli, 1984; Norenberg, 1998; Cudalbu, 2013). Furthermore, increased ammonia metabolism will lead to metabolic disturbances. NH₄⁺ detoxification by converting α-ketoglutarate to glutamate and glutamate to glutamine leads to α-ketoglutarate depletion consequently stressing the tricarboxylic acid cycle (Braissant et al., 2013).

Elevated concentrations of ammonia have been shown to generate free radicals (Kosenko et al., 1997; Murthy et al., 2001; Sinke et al., 2008; Norenberg et al., 2009) and leads to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle (Braissant et al., 1999; Bachmann et al., 2004; Zielinska et al., 2011). Alteration of NO synthesis and oxidative stress can result in the induction of mitochondrial permeability transition (MPT) (Halestrap et al., 1997; Kowaltowski et al., 2001), alterations of BBB permeability (in some disease models) (Rangroo Thrane et al., 2012; Skowronska and Albrecht, 2012; Braissant et al., 2013) and activation of MAPKs (Cagnon and Braissant, 2009). Ammonia also activates the transcription factor NF-κB, involved in immune and inflammatory reactions, (Sinke et al., 2008) probably as a result of increased oxidative stress, activated MAPKs and induced MPT (Marchetti et al., 1996; Bowie and O’Neill, 2000; Kyriakis and Avruch, 2001).

In addition, liver function impairment independently provokes deviation from physiological values of over 20 different compounds in the circulation (Zieve, 1987) as well as many other factors including inflammation and oxidative stress. In some cases it can be very difficult to distinguish the independent effect of ammonia and therefore other factors (synergistic effects) should be considered (Butterworth, 2008; Bosoi et al., 2012; DeMorrow, 2013; Aldridge et al., 2015).

The effects of ammonia toxicity on the brain

In the brain, ammonia homeostasis is tightly regulated and linked to recycling of the major excitatory and inhibitory neurotransmitters glutamate and γ-aminobutyric acid (GABA) (Paulsen et al., 1987; Bak et al., 2006; Hertz and Kala, 2007). Interestingly, GS in astrocytes has a higher affinity for ammonia than glutamate, indicating that the removal of ammonia may be more important than that of the principle excitatory neurotransmitter.
Ammonia neurotoxicity is a phenomenon that affects all cerebrate species, from fish to humans (Ip and Chew, 2010). Gross neurological dysfunction occurs in conditions that lead to excess blood and therefore brain ammonia, including encephalopathy, seizures, ataxia and coma (Butterworth, 2002).

Toxic levels of ammonia and alterations in pH, electrolyte disturbances, membrane potential depolarization, are thought to lead to neurological dysfunction primarily by causing cellular swelling accompanied by brain edema and metabolic dysfunction (Bosoi and Rose, 2009). Ammonia is likely to be particularly toxic to astrocytes as they are the only cells that possess the enzyme GS responsible for detoxifying ammonia in the brain through condensation with glutamate (Martinez-Hernandez et al., 1977; Hertz and Zielke, 2004). In fact, astrocytes are likely to take up four times more ammonia than any other cell type in the brain (Cooper et al., 1979). Moreover, one of the early pathophysiological findings in HE was the presence of brain edema, and specifically astrocyte swelling in histological specimens and in vitro. Astrocyte swelling is also found in the later stages of HE in most animal models of liver failure and hyperammonemia (Gregorios et al., 1985a, 1985b; Ganz et al., 1989; Willard-Mack et al., 1996; Tanigami et al., 2005; Cagnon and Braissant, 2007; Jayakumar et al., 2008; Butterworth et al., 2009; Rangroo Thrane et al., 2012; Rao et al., 2014). These findings correlated with increased ammonia and glutamine levels and it was therefore hypothesized that ammonia exerted its neurotoxic effects by accumulation of glutamine causing astrocyte swelling (termed osmotic gliopathy). However, astrocyte swelling in the context of ammonia neurotoxicity had until recently not been directly visualized in living tissue. In a hyperacute model of isolated hyperammonemia (urea cycle deficiency), doses of ammonia sufficient to cause stupor, seizures and ataxia did not induce astrocyte swelling in vivo (Rangroo Thrane et al., 2013). Additionally, deleting the main astrocyte water channel, aquaporin 4 (AQP4), did not affect disease outcome. However, lethal doses of parenteral ammonia did cause brain swelling in vivo and astrocyte swelling in situ (Rangroo Thrane et al., 2013), and AQP4 deletion does appear to protect against brain edema in the context of HE (Rao et al., 2014). Therefore, although brain edema is a prominent and widely acknowledged clinical feature of the later stages of HE and contributes to mortality, it does not explain all the neurotoxic effects of ammonia (Joshi et al., 2014).

Several alternate and/or synergistic neurotoxic effects of ammonia on astrocytes have previously been studied. These include impairment of oxidative metabolism; with a consequent increase glycolysis (mainly in astrocytes) and dangerously elevated brain lactate levels (Ott et al., 2005; Dam et al., 2013; Bosoi et al., 2014). Ammonia itself is also widely known to cause pH changes that might affect cellular function due to its ability to act both as a weak acid and base. However, in vivo studies using liver failure models, pH electrodes and NMR show either an overall increase of intracellular pH ranging from 0.1 to 0.4 or no change (Fitzpatrick et al., 1989; Swain et al., 1991; Kanamori and Ross, 1997; Rangroo Thrane et al., 2013). The main limitations of these studies are the indirect measures of pH and the inability to accurately distinguish intra- from extracellular pH. Ammonia has also recently been shown to impair astrocytic calcium signaling in vivo, which is closely linked to many astrocytic housekeeping functions, such as K⁺ homeostasis (Rose et al., 2005; Wang et al., 2012; Rangroo Thrane et al., 2013). Additionally, ammonia appears to directly disrupt astrocyte potassium buffering in vivo by competing for transport via either the Na⁺-K⁺-ATPase, K⁺ channels or Na⁺-K⁺-Cl⁻ cotransporters (Alger and Nicoll,
1983; Brookes and Turner, 1993; Marcaggi et al., 2004; Rangroo Thrane et al., 2013). An acute increase in both extracellular ammonia and potassium has been shown to cause a depolarizing shift in the GABA equilibrium potential (E_GABA), thus leading to decreased neuronal inhibition in vivo (Lux, 1971; Benjamin et al., 1978; Rangroo Thrane et al., 2013). This effect likely explains the seizure phenotype seen in models of urea cycle deficiencies (Cagnon and Braissant, 2007). Additionally, ammonia has been found to contribute to seizure generation in epileptic children with normal liver function (Yamamoto et al., 2013), and reactive gliosis seen in temporal lobe epilepsy may induce seizures via an almost identical mechanism (Robel et al., 2015).

More recently, a contribution of other cell types than astrocytes to the pathophysiology of HE and hyperammonemia has gained greater recognition (Butterworth, 2011). Microglia are the resident immune cells in the brain, and known to survey the brain microenvironment for signs of pathogens and inflammation with their fine processes (Nimmerjahn, 2005). When activated, they become more amoeboid in structure, facilitating phagocytosis and release multiple pro-inflammatory mediators (Ransohoff and Cardona, 2010). In the late stages of HE, histological studies have shown that microglia are activated in animal models of CLD, ALF, portocaval shunting and in post mortem tissue from patients (Jiang et al., 2009a, 2009b; Rodrigo et al., 2010; Agusti et al., 2011; Zemtsova et al., 2011). Several studies indicate that this microglial activation is correlated with BBB opening and brain edema (Jiang et al., 2009a, 2009b; Rangroo Thrane et al., 2012). However, microglia were not found to be activated in a model of portal vein ligation (Brück et al., 2011) and acute isolated hyperammonemic mice (Rangroo Thrane et al., 2012). It is therefore possible that the microglial activation is not a response to ammonia per se, but rather relates to other secondary features of ammonia neurotoxicity such as oxidative stress, cellular distress signals or BBB opening. Finally, ammonia likely has a range of direct and indirect effects on neurons, pericytes and endothelial cells, which have been more extensively reviewed in other publications (Szerb and Butterworth, 1992; Rodrigo and Felipo, 2006; Leke et al., 2011; Thumburu et al., 2012; Shaik et al., 2013).

In summary, the best described deleterious effects of ammonia are associated with the brain as the brain is exquisitely sensitive to even minor increases in the blood ammonia levels. Within the brain, although elevated levels of ammonia are noxious for astrocytes, it also has deleterious effects on all cells of the central nervous system (Figure 1). However, can ammonia be considered solely as a neurotoxin? The toxic effects of ammonia are not specific to the brain and there is evidence depicting ammonia toxicity is diffused to organs/tissues and cells in the body.

The effects of ammonia toxicity on muscle

Sarcopenia or loss of skeletal muscle mass is the most frequent and potentially reversible complication in cirrhosis (Dasarathy, 2012). Despite the universally recognized adverse consequences on survival, development of other complications in cirrhosis, quality of life, and post liver transplant outcomes, there are no effective therapies primarily because the mechanisms of sarcopenia are incompletely understood (Dasarathy, 2012). In addition to the lower muscle mass, muscle strength is also reduced in cirrhosis and contributes to adverse clinical outcomes (Jones et al., 2012). Recent evidence strongly suggests that hyperammonemia is a mediator of the liver-muscle axis (Qiu et al., 2013). It is also important to emphasize that identifying ammonia as a mediator of the liver-muscle
axis is especially relevant because effective therapeutic strategies to reverse hyperammonemia are available (Phongsamran et al., 2010).

Previous studies have reported an increased muscle uptake of ammonia in cirrhosis results in glutamine synthesis that is released into the circulation (Ganda and Ruderman, 1976; Lockwood et al., 1979; Holecek, 2013). Muscle uptake and metabolism of ammonia is however not a benign process since a number of molecular and metabolic perturbations have been reported during muscle hyperammonemia (Qiu et al., 2012, 2013). The consequences of increased muscle uptake of ammonia include both a reduction in muscle mass and muscle strength (Shanely and Coast, 2002; Qiu et al., 2012). Both metabolic and molecular responses to muscle hyperammonemia contribute to muscle loss and weakness.

Metabolic perturbations during hyperammonemia

Since ureagenesis in the liver is impaired and muscle cannot generate urea, ammonia removal by the muscle utilizes a cataplerotic conversion of α-ketoglutarate, a critical tricarboxylic acid cycle intermediate to generate glutamate and glutamine thereby removing 2 moles of ammonia for each mole of α-ketoglutarate. However, the conversion of α-ketoglutarate to glutamate, the first step in this reaction is catalyzed by glutamate dehydrogenase that has a low affinity for ammonia promoting the anaplerotic direction generating α-ketoglutarate from glutamate rather than glutamate from α-ketoglutarate (Wootton, 1983). Under most physiological conditions, since muscle ammonia concentrations do not reach the $K_m$ of this enzyme (~1 mM), there is not net cataplerosis of α-ketoglutarate. In cirrhosis, we have consistently reported tissue concentrations of ammonia ~4 mM that favors cataplerosis of α-ketoglutarate to glutamate. This is consistent with our observations of lower TCA cycle intermediates in the skeletal muscle during hyperammonemia. Impaired muscle mitochondrial function and ATP generation accompany lower TCA cycle intermediates. These observations provide a mechanistic basis for the previously reported impaired skeletal muscle mitochondrial electron chain complex function in cirrhosis. Reduction in muscle ATP also contributes to lower protein synthesis since peptide chain elongation is a high energy-requiring step. Increased autophagy is a homeostatic response and provides amino acids for anaplerotic reactions. Additionally, reduction in ATP generation also contributes to skeletal muscle contractile dysfunction in cirrhosis.

Skeletal muscle molecular abnormalities during hyperammonemia

In addition to skeletal muscle metabolic derangements, ammonia also results in impaired skeletal muscle protein synthesis and increased autophagy. The principal pathway of impaired protein synthesis involves the canonical Akt/mTOR mediated regulation of cap dependent protein synthesis. Myostatin, a TGF-β superfamily member is a potent inhibitor of muscle protein synthesis and inhibits mTOR activation either via an Akt dependent or independent mechanism. Consistently, blocking myostatin increases muscle mass and protein synthesis (Dasarathy et al., 2011).

Hyperammonemia also increases muscle autophagy that in combination with impaired protein synthesis results in loss of muscle mass (Qiu et al., 2012). Both impaired mTOR signaling and activation of AMPK contribute to the increased autophagy. Ammonia also increases protein nitration either directly or by increased generation of reactive oxygen and nitrogen species and autophagy has been shown to contribute to clearance of post
translational modification of muscle proteins (unpublished data). Posttranslational modification of proteins can potentially impair the actomyosin interaction during muscle contraction and result in impaired muscle contractile function.

**Therapeutic relevance of muscle hyperammonemia.**

The metabolic and molecular data support our belief that hyperammonemia induces a state of anabolic resistance because previous strategies to increase muscle mass by nutritional and hormonal strategies have not been consistently been effective (Tsien et al., 2012). Our studies using an integrated molecular-metabolic approach have shown the interaction between signaling abnormalities and metabolic demands during muscle hyperammonemia lay the foundation for novel mechanistic therapeutic strategies. Our model supports the use of long term ammonia lowering, myostatin antagonism, stimulating muscle mTOR, and providing anaplerotic substrates to reverse the adverse consequences of hyperammonemia. Mitochondrial dysfunction and generation of mitochondrial reactive oxygen species and resultant oxidative stress responses are also potential targets to reverse the adverse skeletal muscle responses of hyperammonemia (unpublished data).

Consistently, myostatin blocking using either follistatin or myostatin knockout in mice have been reported to be effective in reversing hyperammonemia mediated impaired muscle protein synthesis and contractile function (Dasarathy et al., 2011; Qiu et al., 2012, 2013). Even though ammonia lowering strategies have been effective in reversing encephalopathy (Phongsamran et al., 2010), there is little data on the impact on the skeletal muscle. One potential reason is that skeletal muscle contractile proteins are long lived and long-term ammonia lowering strategies may be necessary to result in clinically relevant responses.

Interestingly, recent studies have reported that leucine is an anaplerotic substrate generating of α-ketoglutarate (Schachter and Sang, 1997). Additionally, leucine stimulates mTOR directly providing a compelling therapeutic rationale for the use of leucine to reverse muscle hyperammonemia and its consequences. Consistently, human and animal studies in cirrhosis have shown that high doses of leucine have a beneficial effect in reversing impaired skeletal muscle protein synthesis (Tsien et al., 2015). The use of cell permeable esters of α-ketoglutarate also holds promise as a strategy to increase muscle ammonia disposal. Other potential interventions include enhanced non-hepatic ammonia disposal via non-toxic pathways, antioxidants and mitochondrial stabilizers need to be evaluated. On final note, even though hyperammonemia is a mediator of the liver muscle axis, plasma ammonia is elevated in patients with chronic pulmonary disease, severe heart failure and cancers all of which are accompanied by significant skeletal muscle loss (Bessman and Evans, 1955; Chance et al., 1988; Calvert et al., 2010). Therefore, the current approach has broad therapeutic potential across multiple organ dysfunctions.

In conclusion, hyperammonemia occurs in a number of chronic diseases and potentially contributes to sarcopenia and adverse clinical outcomes. Recent advances in our understanding of the molecular and metabolic responses to hyperammonemia in the skeletal muscle provide a mechanistic basis for developing effective therapies (Figure 2).

**The effect of ammonia on other organs**

*Kidney*
It has been shown in healthy volunteers that following exposure to hyperammonemia, the kidneys take up net ammonia from the systemic circulation (Owen et al., 1961). However, it has been demonstrated in both cirrhotic patients and hyperammonemic rodents, that the kidney becomes a primary source of ammonia (Owen et al., 1961; Olde Damink et al., 2003; Ytrebø et al., 2006). Elevated systemic ammonia concentrations following CLD may directly interact with glomerular cells and contribute to glomerular injury (Ling et al., 1998). Hyperammonemia also plays a crucial role in tubulointestinal fibrosis (Ling et al., 1998). Gordon et al. displayed evidence ammonia has the capacity to promote tubulointestinal injury due to the interaction of ammonia with the third component of complement, a potent stimulus to the production of reactive oxygen species by polymorphonuclear leukocytes and monocytes (Gordon et al., 1985). In this context, Nath et al. demonstrated that hyperammonemia induces the progression of renal injury, not only through complement cascade, but also through the stimulatory effects of ammonia on renal growth (Nath et al., 1991). In fact, oxidative stress can also cause renal amoniagenesis which may contribute to progression of renal injury (Dan et al., 2008). By contrast, a very recent study by Satpute et al. observed that subacute exposure to ammonium acetate in rats induces renal necrosis and tubular degeneration, which were not correlated with either oxidative or biochemical changes (Satpute et al., 2014). Indeed, it is apparent that exposure of neutrophils to ammonia impair phagocytosis and promotes increased reactive oxygen species generation that results in oxidative stress at infection sites and collateral damage to host tissue (Shawcross et al., 2008).

During ALF, kidneys are capable of continuous ammonia release into the systemic circulation (Ytrebø et al., 2006). A recent study by Cauli et al. demonstrated in rats with acute liver failure that activation of N-methyl D aspartate (NMDA) receptors contribute to hyperammonemia-induced encephalopathy and kidney damage. Moreover, blocking NMDA receptors with MK-801 improved ammonia elimination and glomerular filtration rate and delayed kidney damage (Cauli et al., 2014), confirming the deleterious effects of high ammonia concentration in kidneys. Furthermore, ammonia administered to rats lead to disturbances in renal sodium handling that was preceded by activation of kidney mitogen activated protein kinases/extracellular signal regulated kinases (MAPK/ERKs) signaling pathways, and consequently renal injury (Bento et al., 2005). It has been shown that kidneys perfused with ammonium salts results in cortical and tubular necrosis, with necrotic kidneys demonstrating depressed creatinine clearance (Orvell and Wesson, 1976). Ammonia exposure also inhibits tubular cell proliferation in primary rabbit proximal tubular epithelial cells, therefore affecting cell replication (Rabkin et al., 1993). Collectively, these findings suggest that hyperammonemia does lead to impaired kidney function and sustaining kidney injury.

Liver

As indicated above, whilst astrocytes are major effectors of brain injury in liver failure, it is of interest that they share many similar markers of activation with hepatic stellate cells (HSC), which are implicated in the development of liver fibrosis and also the increase in intra-hepatic resistance with evolving liver injury (Rockey, 1997). Indeed, HSC contraction is one of the mechanisms that is central to the development of portal hypertension, a serious haemodynamic consequence of cirrhosis. HSC have been shown to possess GS (Bode et al., 1998) and recent data shows that in rodent models of cirrhosis,
high ammonia concentrations are associated with increased HSC contraction and raised portal pressure and conversely, lowering ammonia decreases HSC activation and ameliorates portal hypertension (Jalan et al., 2016).

In addition to the impact on HSC contraction, elevated ammonia has been shown to promote liver injury through up-regulation of toll-like receptor genes, generation of oxidative stress through activation of resident macrophages and neutrophils and activation of NFkB and iNOS. In addition, ammonia promotes increased hepatocyte apoptosis and changes in cell cycle with higher cyclin D1, thereby further promoting liver injury as liver disease evolves (Jia et al., 2014). It is possible that impaired urea cycle metabolism and reduced liver clearance of ammonia primes the liver and other organs to the impact of acute inflammation and further oxidative stress, as occurs in conditions such as acute-on-chronic liver failure, though this assertion requires mechanistic evaluation.

Lung

Several studies have discussed the detrimental effects of ammonia on lungs following exposure to ammonia gas. Ammonia inhalation also can damage respiratory tract leading to acute lung injury and pulmonary edema, which can ultimately lead to death (Ortiz-Pujols et al., 2014). Furthermore, ammonia inhalation to rats causes increased oxidative stress, decreased antioxidant enzymes and acute lung injury which was attenuated following α-ketoglutarate treatment (Ali et al., 2012). Ammonia induced acute lung injury have also been shown in rabbits associated with an elevated airway pressure and a decrease in PaO2 (Sjöblom et al., 1999) and has been shown to induce lung fibrosis (Ohnuna-Koyama et al., 2013). Ammonia and enzymatically active urease released from parasitic cells of Coccidioides posadasii contribute to host tissue damage and exacerbate the severity of coccidioidal lung infections (Mirbod et al., 2002; Mirbod-Donovan et al., 2005; Wise et al., 2013). In this context, it has been concluded that enzymatically active urease is partly responsible for increased extracellular ammonia concentrations at sites of lung infection, contributing to both localized host tissue damage and exacerbation of the respiratory disease (Lichtenstein et al., 1997).

The effect of ammonia on vascular function

Chronic hyperammonemia is believed to decrease cGMP formation and thereby secondary signaling for NO, one of the main regulators of tonic vasodilatation (Cauli et al., 2007). Moreover, as previously mentioned, the promotion of reactive oxygen species generation by ammonia gives rise to further endothelial dysfunction by decreasing expression of dimethylarginine dimethylamino hydrolase (DDAH1), a cytosolic enzyme that metabolizes asymmetric dimethylarginine (ADMA), a key endogenous inhibitor of NO. High ADMA levels have previously been shown to be associated with impaired cerebral perfusion during hyperammonemia (Balasubramaniyan et al., 2012) and also with high intrahepatic resistance in advanced liver disease (Mookerjee et al., 2007). Similarly, under high oxidative stress conditions, there is increased un-coupling of nitric oxide synthase with less NO bioavailability (Xia et al., 1998) and greater nitration of proteins with the generation of peroxynitrite and nitrotyrosine, further promoting organ injury. Furthermore, important cationic transporters such as the y⁺ LAT2 system are also stimulated by high ammonia levels, resulting in arginine efflux from endothelial cells in exchange for glutamine uptake (Zielinska et al., 2011), and thus loss of substrate for nitric
oxide synthase to generate NO. Thus at multiple levels, ammonia appears to control endothelial function and resistance to vascular flow, whilst also promoting further organ injury. It is perhaps, therefore, not surprising that with progression to advanced cirrhosis and ascites formation and greater ammonia generation, resistive indices for the middle cerebral artery and renal vasculature have been shown to increase, and correlate with severity of hyperdynamic circulatory dysfunction as noted through high renin levels (Guevara et al., 1998).

Conclusion

Increases in ammonia can lead to changes in pH, membrane potential (due to similar properties of NH₄⁺ and K⁺) and cell metabolism. While ammonia (hyperammonemia) is unequivocally key in the development of HE, the effects of ammonia toxicity are not specific to the brain. It is therefore possible that the deleterious consequences of increased ammonia can impinge on other organs and to date, there is increasing evidence that ammonia is clearly much more than a neuro-toxin. Evidently the brain is more sensitive to ammonia toxicity and recognizing ammonia tolerance/intolerance in other organs merits to be further investigated. Better understanding of how increased ammonia affects various cells and tissues within the body can help lead to better therapeutic strategies for the management of patients with hyperammonemia.
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Figure 1. Effects of ammonia toxicity in brain. Left, physiological ammonia handling in the brain. Right, some of the proposed neurotoxic effects of excess brain ammonia. Glutamate (Glu), glutamine synthetase (GS), glutamine (Gln), adenosine triphosphate (ATP), sodium-coupled neutral amino acid transporters (SNAT1/2 and 5), glutamate transporter 1 (GLT1), glutaminase (Glnase), glutamate and γ-aminobutyric acid (GABA), glutamate receptor (GluR), GABA receptor (GABA_R), cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO_2), cerebral metabolic rate of glucose (CMRGlc), GABA reversal potential (\(E_{\text{GABA}}\)), cytokines (CK).