IDENTIFYING THE DIRECT EFFECTS OF AMMONIA ON THE BRAIN

Cristina R. Bosoi[1] and Christopher F. Rose[1]

Neuroscience Research Unit, Hôpital Saint-Luc (CRCHUM), Université de Montreal, 264, boulevard René Lévesque Est, H2X 1P1, Montréal, Québec, Canada Christopher F. Rose Email**:** christopher.rose@umontreal.ca

ABSTRACT

Elevated concentrations of ammonia in the brain as a result of hyperammonemia leads to cerebral dysfunction involving a spectrum of neuropsychiatric and neurological symptoms (impaired memory, shortened attention span, sleep-wake inversions, brain edema, intracranial hypertension, seizures, ataxia and coma). Many studies have demonstrated ammonia as a major player involved in the neuropathophysiology associated with liver failure and inherited urea cycle enzyme disorders. Ammonia in solution is composed of a gas (NH_3) and an ionic (NH_4^+) component which are both capable of crossing plasma membranes through diffusion, channels and transport mechanisms and as a result have a direct effect on pH. Furthermore, NH_4^+ has similar properties as K^+ and, therefore, competes with K^+ on K^+ transporters and channels resulting in a direct effect on membrane potential. Ammonia is also a product as well as a substrate for many different biochemical reactions and consequently, an increase in brain ammonia accompanies disturbances in cerebral metabolism. These direct effects of elevated ammonia concentrations on the brain will lead to a cascade of secondary effects and encephalopathy.

Keywords

Ammonia Brain Liver failure Hepatic encephalopathy

INTRODUCTION

Elevated concentrations of ammonia[1] in the brain consequently leads to central nervous system dysfunction and encephalopathy. Ammonia accumulates to toxic levels in the brain arising from the blood during hyperammonemic conditions as seen in a large number of diseases including liver failure and inborn errors of the urea cycle (Cooper and Plum *1987*). Ammonia is a metabolite which is mostly produced within the gut during protein digestion and deamination. The urea cycle within the liver regulates the concentration of ammonia in the systemic circulation maintaining blood ammonia levels in the low 50–100 uM range. Therefore, reduced hepatic capacity for ammonia removal leads to hyperammonemia, increased levels of brain ammonia and consequently a spectrum of neuropsychiatric and neurological symptoms including impaired memory, shortened attention span, sleep-wake inversions, brain edema, intracranial hypertension, seizures, ataxia and coma. During liver failure, brain ammonia concentrations can attain 1–5 mM. Many deaths of patients with liver failure occur due to neurological complications such as brain edema, increased intracranial pressure and brain stem herniation, which have been demonstrated to be related to arterial ammonia concentrations in both clinical (Bernal et al. *2007*; Bhatia et al. *2006*; Jalan et al. *2004*; Clemmesen et al. *1999*) and animal studies (Sen et al. *2006*; Jover et al. *2006*; Rose et al. *1999*).

AMMONIA

Ammonia is a unique molecule which can act both as a weak base (NH_3) or weak acid (NH_4^+), has electrolytic conductance, and is a product as well as a substrate for many biochemical reactions. Ammonia is composed of a gaseous (NH_3) and ionic (NH_4^+) component, both of which are transportable across phospholipid bilayers (cell membranes). This in turn alters intracellular as well as extracellular pH. In aqueous solutions, NH_3 is in equilibrium with NH_4^+ ($NH_3 + H^+ \leftrightarrow NH_4^+$) where the ratio of NH_3/NH_4^+ is a function of pH defined by the Henderson-Hasselbach equation:

$$\log_{10}\left[NH_3/NH_4^+\right] = pH - pKa$$

At 37°C the pKa of ammonia is 9.15 (Bromberg et al. *1960*), therefore under normal physiological conditions (pH 7.4), more than 98% of ammonia is present as NH_4^+.

DISTRIBUTION

Since both the gas (NH_3) and the ion (NH_4^+) are capable of entering into the cell, this defines ammonia as a complex molecule in comparison to other weak acids and bases.

Ammonia as a gas (NH_3) is lipid soluble and therefore enters the brain through diffusion. Interestingly, NH_4^+ has very similar ionic properties to those of K^+ with a comparable ionic radius and a similar diffusion coefficient. These parallel properties allow NH_4^+ to compete with K^+ on membrane ion channels such as inward rectifying and voltage-gated K^+ channels. Furthermore, NH_4^+ has been demonstrated to cross cell membranes by substituting K^+ in the ATPase transporters Na^+/K^+ and H^+/K^+ (Moser *1987*). In addition, the NH_4^+/Cl^- and $Na^+/K^+/Cl^-$ cotransporters have also demonstrated to transport NH_4^+ (Aickin et al. *1982*; Kelly et al. *2008*).

In addition to ammonia being capable of crossing cell membranes through diffusion as well as through K^+ ion channels and transporters, there is increasing evidence that a specific ammonia transporter exists in mammals. The human nonerythroid Rhesus (Rh) glycoprotein B (RhBG) and C (RhCG) have been identified as mammalian ammonia transporters (for review see Bakouh et al. *2006*). It has also been demonstrated that ammonia can be transported through aquaporin channels, specifically aquaporin-8 (Saparov et al. *2007*). However, the regulation as well as the specificity (NH_3 vs NH_4^+) of these transporters still needs to be confirmed.

CONSEQUENTIAL EFFECTS OF AMMONIA TOXICITY

Animal models of liver failure develop hyperammonemia and hepatic encephalopathy which are associated with an array of biochemical, physiological and molecular changes (Fig. 1). Similar effects are observed when either cultured neurons or glia cells are treated with pathophysiological concentrations of ammonia (Sánchez-Pérez and Felipo *2006*; Rama Rao et al. *2003*; Chan et al. *2003*). For further readings on the effects of ammonia toxicity refer to reviews by Felipo and Butterworth (*2002*) and Cooper and Lai (*1987*).
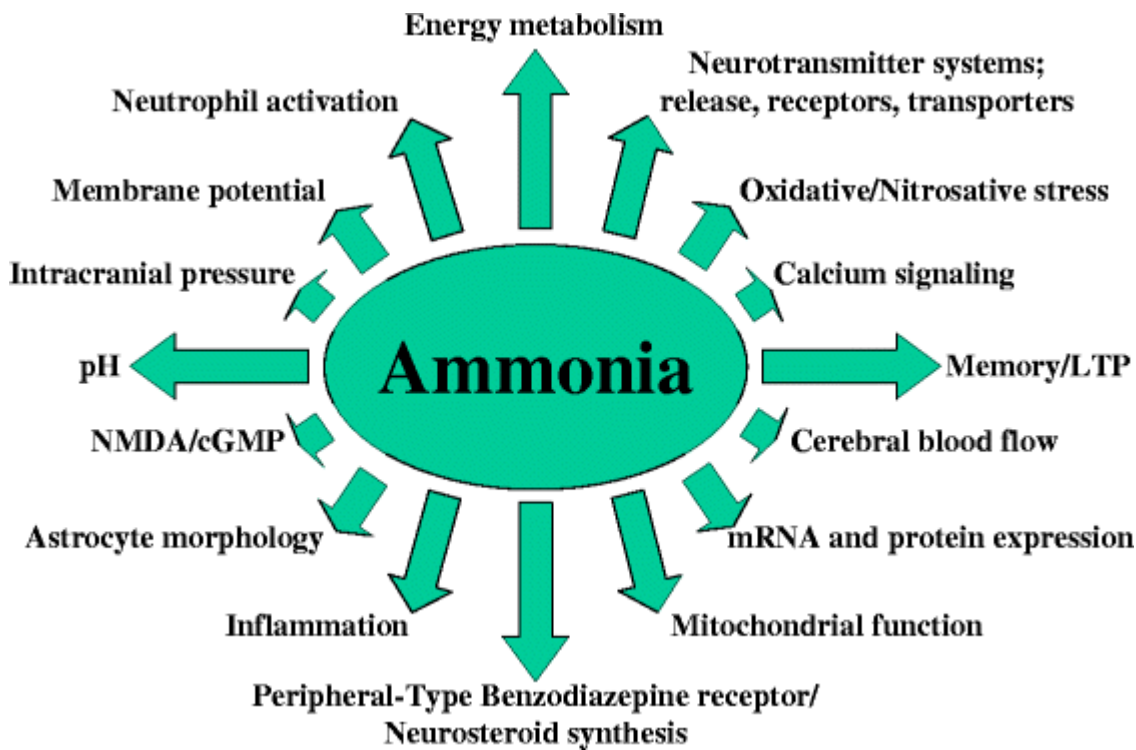
Fig. 1 Cerebral effects due to ammonia toxicity

DIRECT EFFECTS OF AMMONIA

Ammonia has *direct* effects on cell function which can consequently initiate a cascade of pathophysiological pathways as mechanisms as demonstrated in Fig. 1.

PH

When ammonia (pH 7.4) is applied to cultured cells (ex. astrocytes), NH_3 (<2%) rapidly diffuses across the plasma membrane and establishes an equilibrium inside the cell ($NH_3 + H^+ \leftrightarrow NH_4^+$) by combining with cytosolic H^+ to form NH_4^+. Consequently intracellular pH $[pH]_i$ increases leading to intracellular alkalinization (Fig. 2). Similar results are observed when other weak bases are applied to cultured cell preparations such as trimethylamine (Rose et al. *2005*). However, the ammonia-induced transient increase in $[pH]_i$ recovers towards baseline since NH_4^+ (>98%) is capable of crossing cell membranes (at a slower rate than NH_3 diffusion) through different channels and transporters (see above). The entry of NH_4^+ re-establishes a new equilibrium inside the cell ($NH_3 + H^+ \leftrightarrow NH_4^+$), releasing H^+ and subsequently lowering $[pH]_i$. The amplitude of increased $[pH]_i$ (degree of alkalinization) upon ammonia application is dependent upon a) the rate of entry of NH_3 vs NH_4^+ into the cell b) the concentration and ratio of NH_3 vs NH_4^+ (pH and temperature dependent) and c) the quantitative expression of ammonia "transporters and channels" permitting NH_4^+ to enter the cell. These will also reflect time to recovery of $[pH]_i$ and degree of acidification. For example, ammonia applied to cultured cells with an over-expression of K^+-channels/transporters (or ammonia transporters) will result in a smaller amplitude of increased $[pH]_i$ and a faster recovery of $[pH]_i$, leading to intracellular acidification. Since the ionic component of other weak acids is not capable of crossing cell membranes, the initial alkalinization upon application of trimethylamine, does not fully recover. This is supported with experiments by co-applying barium chloride (a blocker of inwardly-rectifying K^+ channels) along with ammonia and observing an initial alkalinization followed by a slow recovery to baseline. In conclusion, ammonia due to its similarities with K^+, alters both intracellular and extracellular pH distinctively from other weak acids and bases.
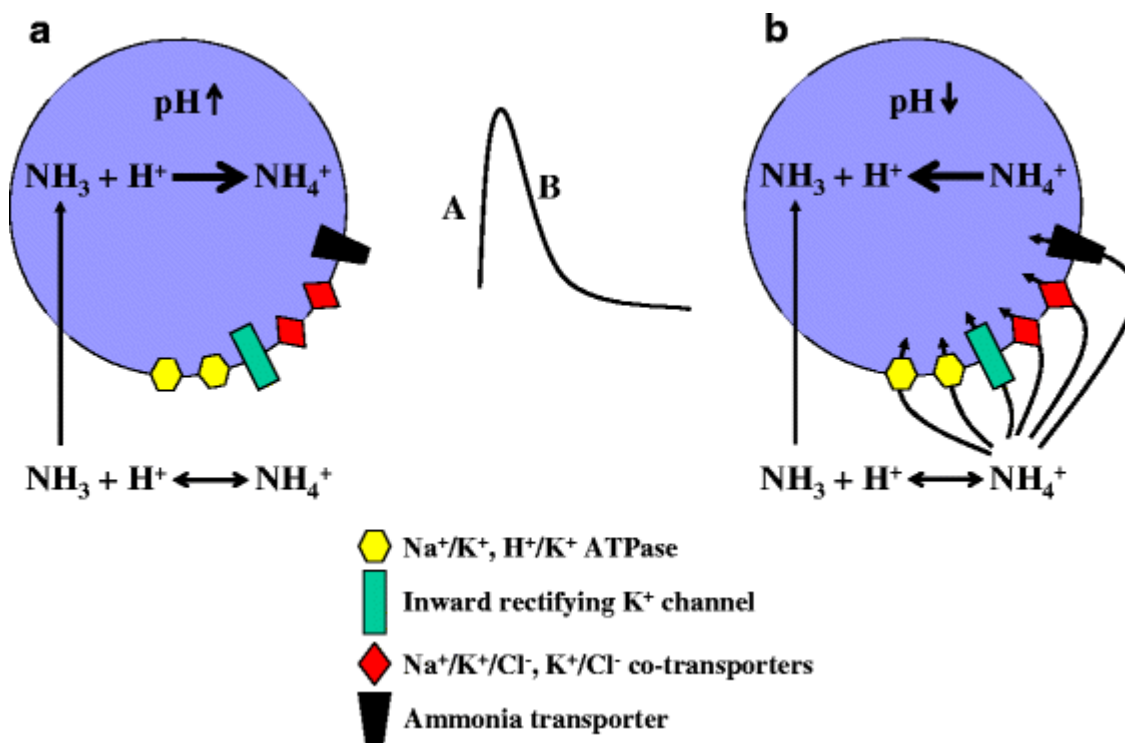
Fig. 2 The effect of ammonia on intracellular pH. Transmembrane fluxes of NH_3 exceed fluxes of NH_4^+ therefore resulting in intracellular alkalinization (A). The amplitude of increased $[pH]_i$ (degree of alkalinization) as well as degree of secondary acidification (B) upon ammonia application is dependent upon the rate of entry and concentration of NH_3 and NH_4^+ as well as the quantitative expression of ammonia "transporters and channels" which allow NH_4^+ to enter the cell

MEMBRANE POTENTIAL

Since K^+ and NH_4^+ ions are comparable in many aspects, increased concentrations of NH_4^+ may affect K^+ homeostasis and therefore compete with K^+ on Na^+/K^+ and H^+/K^+ ATPase exchangers as well as $Na^+/K^+/Cl^-$ and K^+/Cl^- co-transporters. This in turn may have an impact on the Nernst potential for potassium and resting membrane potential.

High concentrations of ammonia can raise the membrane potential, depolarizing both neurons and astrocytes. It has been demonstrated that 2 mM NH_4Cl can depolarize hippocampal neurons by approximately 10 mV. An increase in positive ions (NH_4^+)/positive charge inside the cell depolarizes the membrane potential (Fig. 3a) but not sufficient enough to generate an action potential (Fan and Szerb *1993*). Astrocytes, who have a lower resting membrane potential than neurons, exhibit a concentration-dependent increase in membrane potential following application of 5, 10 and 20 mM of NH_4Cl (Fig. 3b) (Allert et al. *1998*). Ammonia (2 mM) added to the superfusate caused a very small depolarization (1.6 mV) of the glial cells in a slice of the retina of the honey-bee drone (Coles et al. *1996*). Activation of the glutamate ionotropic receptor N-methyl-D-aspartate (NMDA) has been demonstrated to play an important role in the pathophysiology of hepatic encephalopathy (Llansola et al. *2007*). The opening of the channel is controlled by a powerful voltage-dependent block by external magnesium ions (Mayer et al. *1984*; Nowak et al. *1984*). It is believed ammonia, by raising the membrane potential, removes the magnesium block rendering NMDA receptors susceptible to activation. To which degree the magnesium block is removed due to ammonia needs to be clarified as it has been demonstrated half the magnesium block is removed upon raising the membrane potential to −20 mV (Mayer et al. *1984*). In conclusion, pathophysiological concentrations of ammonia directly raise the membrane potential in both astrocytes and neurons, however, not sufficiently to activate voltage-gated channels or generate an action potential in neurons.
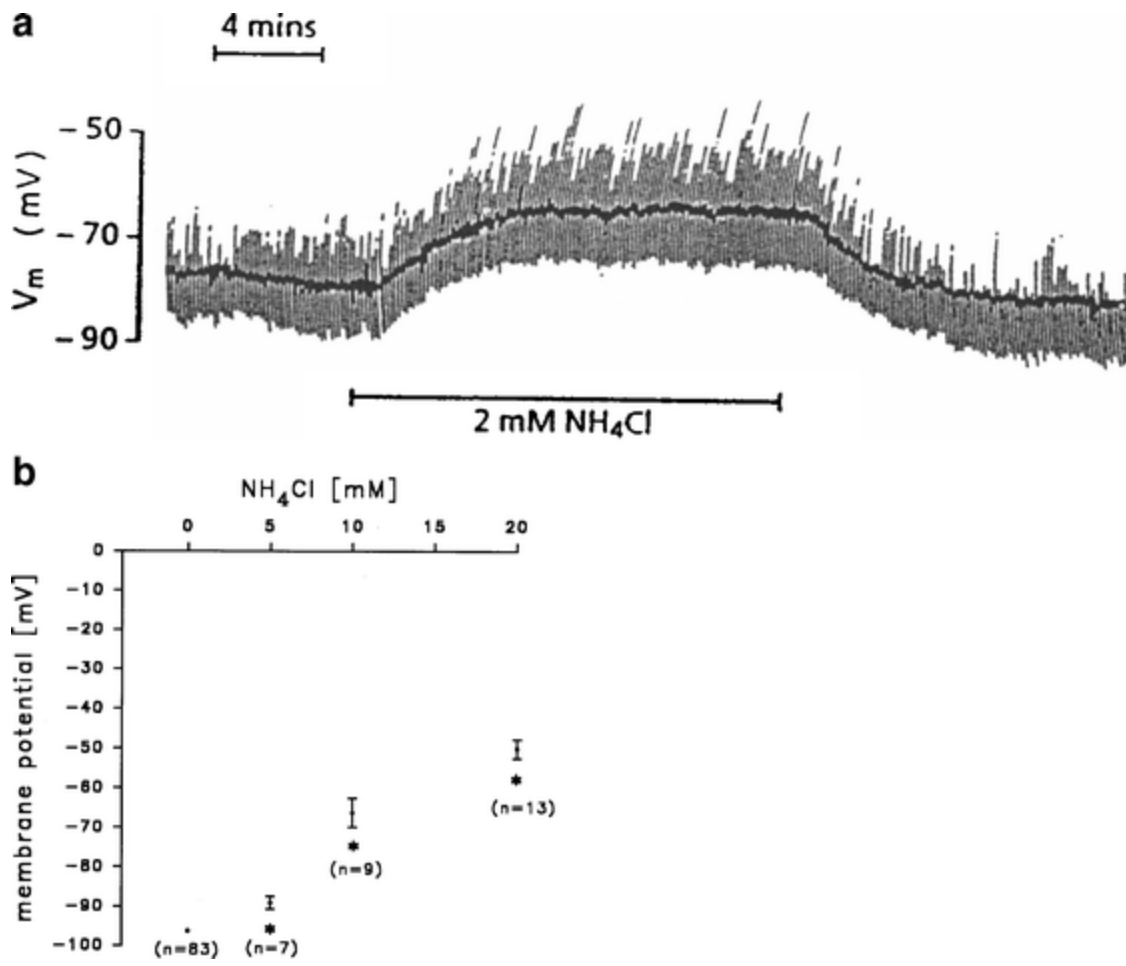
Fig. 3 Effect of ammonia on membrane potential in **a)** neurons (modified from Fan and Szerb *1993*) and **b)** astrocytes (modified from Allert et al. *1998*)
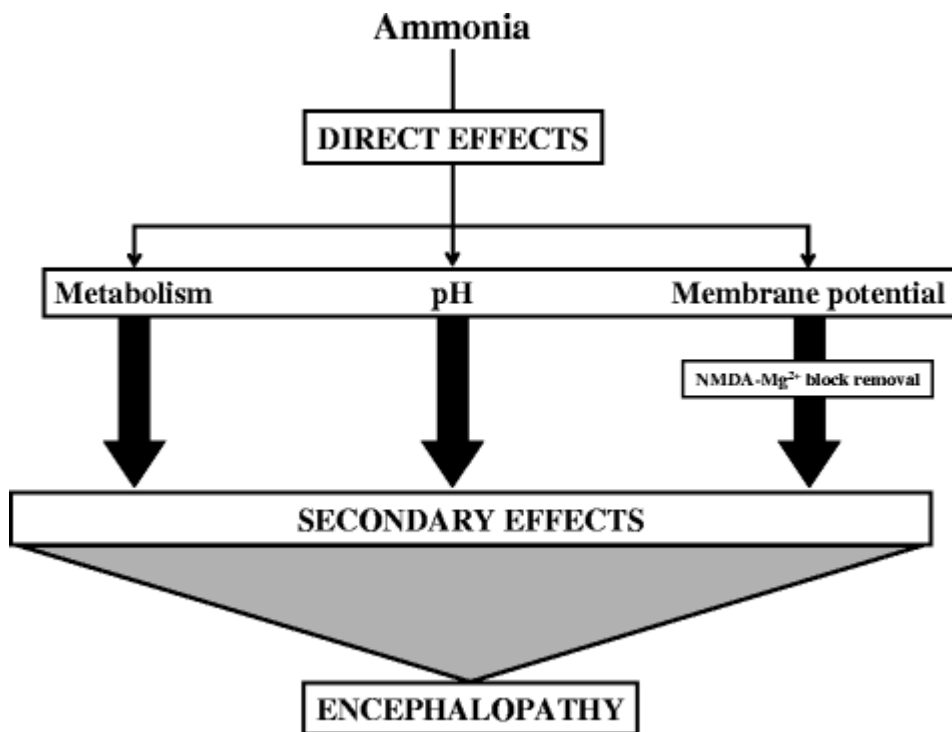
Ammonia

DIRECT EFFECTS

| Metabolism | pH | Membrane potential |

NMDA-Mg²⁺ block removal

SECONDARY EFFECTS

ENCEPHALOPATHY

Fig. 4 Direct effects of ammonia consequently lead to secondary effects

METABOLISM

Ammonia is an important substrate as well as a product for at least 16 different enzymatic reactions in the brain (Cooper and Plum *1987*). Increased concentrations of ammonia in the brain results in alterations in metabolism affecting regulatory activities of important enzymes such as glutaminase, glutamine synthetase and glutamate dehydrogenase.

SUMMARY

Ammonia plays a major role in the pathogenesis of hepatic encephalopathy as well as other hyperammonemia-induced encephalopathies. Ammonia-lowering strategies remain the key therapeutic approach. Ammonia neurotoxicity has been demonstrated to be associated with a number of physiological, biochemical and molecular changes in the brain. It is the initiating/direct effects of ammonia on cell function which consequentially leads to additional secondary effects and stimulates a cascade of pathophysiological pathways leading to cerebral dysfunction (Fig. 4).

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