

New findings on cerebral ammonia uptake in HE using functional ^{13}N -ammonia PET

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Abstract PET is a functional imaging technique suitable for studies of brain ammonia metabolism. Dynamic ^{13}N -ammonia PET yields time-courses of radioactivity concentrations in brain (PET camera) and blood (samples). Ahl *et al.* (Hepatology 40:73–79, 2004) and Keiding *et al.* (Hepatology 43:42–50, 2006) analysed such data in patients with HE by a kinetic model accounting for transfer of ^{13}N -ammonia across the blood–brain barrier (BBB) and intracellular formation of ^{13}N -glutamine. Initial unidirectional ^{13}N -ammonia transfer across BBB was characterized by the permeability-surface area product PS_{BBB} ($\text{ml blood min}^{-1} \text{ml}^{-1}$ tissue). There was a tendency to lower PS_{BBB} values in patients with cirrhosis and HE than in patients with cirrhosis without HE and healthy controls but the differences were not statistically significant. Keiding *et al.* (Hepatology 43:42–50, 2006) also calculated PS_{met} ($\text{ml blood min}^{-1} \text{ml}^{-1}$ tissue) as a measure of the combined transfer of ^{13}N -ammonia across BBB and subsequent intracellular metabolism of ^{13}N -ammonia; neither did this PS-value show significant difference between the groups of subjects. Net flux of ammonia from blood into intracellular metabolites was linearly correlated to arterial ammonia. *In conclusion*, basic brain ammonia kinetics was not changed significantly in patients with cirrhosis +/- HE compared to healthy controls. Blood ammonia seems to be the more important factor for increased brain ammonia uptake in HE.

Keywords Ammonia · Blood–brain barrier · Brain imaging · Cerebral blood flow · Hepatic encephalopathy · Positron emission tomography

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Introduction

The role of increased blood ammonia in patients with liver impairment in the development of hepatic encephalopathy (HE) has been a puzzle for decades. There is no doubt, however, that ammonia exerts toxic biochemical effects on brain energy metabolism, mitochondrial membrane permeability, glucose metabolism and neurotransmission, as shown in experimental animal studies, cell cultures, and human studies and being discussed in other communications at this 4th International Hannover Conference on Hepatic Encephalopathy.

Positron emission tomography (PET) is a functional imaging technique providing unique possibilities for the study of transport and metabolic processes in awake human subjects. Early ^{13}N -ammonia PET studies indicated that increased permeability of the blood–brain barrier (BBB) for ammonia plays a role for the development of HE in humans (Lockwood *et al.* 1979, 1984, 1991) but recent studies find no increased permeability in patients with cirrhosis and HE (Ahl *et al.* 2004; Keiding *et al.* 2006; Goldbecker *et al.* 2007, this conference).

Dynamic ^{13}N -ammonia PET

In general, PET studies can be performed as static or dynamic recordings. *Static* PET recordings yield 3D images of the mean radioactivity concentrations in a given time interval. The image can be considered as a snapshot of the combined result of all tracer distribution and metabolic processes that have taken place up to and including the time interval of the recordings.

Today many PET centres take advantage of *dynamic* PET recording for state-of-the-art quantification of specific metabolic processes in the intact, living organism (Weissenborn *et al.* 2004). Dynamic recording yields data on the time course of radioactivity concentrations in tissue (PET camera) and blood (arterial blood samples or PET/aorta regions-of-interest (Keiding *et al.* 2000)) in successive short time intervals immediately following tracer injection (Fig. 1). The time course of the radioactivity in brain tissue reflects the time-dependent distribution of the tracer. In the case of ^{13}N -ammonia, tracer distribution in brain tissues is a result of the combination of transfer of ^{13}N -ammonia across the BBB from blood to cell, intracellular metabolism, and possible back-flux from cells to blood of unmetabolized ^{13}N -ammonia and ^{13}N -metabolites. The initial time courses of the brain radioactivity concentrations are mainly determined by transfer across the BBB and the later time courses of the radioactivity concentrations are mainly determined by intracellular metabolic processes and outcome of the metabolites. In the blood, ^{13}N -urea and ^{13}N -glutamine formed from other tissues in the body also start to appear (Fig. 1) (Sørensen and Keiding 2006a). The dynamic ^{13}N -ammonia PET brain study by Phelps *et al.* (1976) showed regional variations in cerebral ^{13}N -ammonia uptake in human subjects; regions with high blood flow, i.e. regions with high vascular density, had high ^{13}N -ammonia uptake, a finding they later reproduced under experimentally changed cerebral flow rates (Phelps *et al.* 1981).

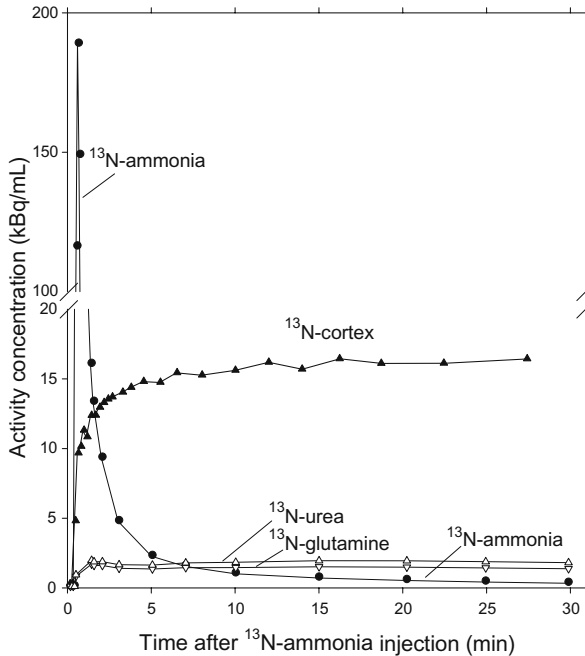


Fig. 1 Dynamic PET recording: Time courses of ^{13}N -radioactivity concentration in cortex (PET camera) and of ^{13}N -ammonia, ^{13}N -urea, and ^{13}N -glutamine in arterial blood (manual blood sampling) in a healthy subject (from Keiding *et al.* 2006)

PET-modelling of brain ammonia metabolism

Kinetic parameters are calculated by fitting mathematical models of tracer distribution and metabolism to the dynamic data, using non-linear or linear regression procedures. The models must include as much physiological realism as possible while keeping the number of parameters as low as possible so that they are estimated with reasonable accuracy. Models thus must be adjusted according to study design, data and current knowledge of physiology of the specific processes studied (Sørensen and Keiding 2006b; Keiding and Sørensen 2007).

Figure 2 illustrates the proposal of a model of brain ^{13}N -ammonia metabolism used in the study of brain ^{13}N -ammonia metabolism by Keiding *et al.* (2006). Within the 30-minute study period, the model assumes irreversible trapping of ^{13}N -ammonia as ^{13}N -metabolites, mainly ^{13}N -glutamine. This assumption is reasonable in view of the findings by Phelps *et al.* (1981) that there is no escape of ^{13}N -metabolites within the first 50 minutes after a bolus injection of ^{13}N -ammonia. The model is quite similar to that used by Ahl *et al.* (Fig. 1A in (Ahl *et al.* 2004)) except they did not include specific ^{13}N -metabolites in their fitting; they subtracted the sum of ^{13}N -metabolites in accordance with data by Rosenspire *et al.* (1990) to obtain a blood ^{13}N -ammonia input function. In our laboratory we have recently developed a simple procedure to separate the quantitatively most important fractions of ^{13}N -metabolites in blood, namely ^{13}N -urea and ^{13}N -glutamine in a larger number of

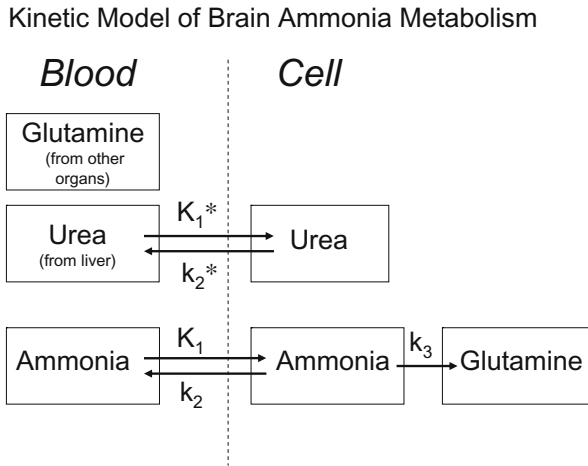


Fig. 2 Compartmental model of ^{13}N -ammonia metabolism in brain: K_1 , unidirectional clearance of ^{13}N -ammonia transfer across the blood–brain barrier ($\text{ml blood min}^{-1} \text{ ml}^{-1} \text{ tissue}$); k_2 , back-flux rate constant (min^{-1}), k_3 , rate constant of enzymatic trapping (min^{-1}). ^{13}N -urea (formed in the liver) enters and leaves the brain cells freely. ^{13}N -glutamine is constrained into two separate compartments: intracellular ^{13}N -glutamine generated from ^{13}N -ammonia and blood ^{13}N -glutamine generated by extra-cerebral organs

blood samples than has hitherto been possible (Keiding *et al.* 2006). This enabled us to take into account not only the blood ^{13}N -ammonia input but also the individual distribution of ^{13}N -urea and ^{13}N -glutamine when fitting the model in Fig. 2 to the data. We assumed that ^{13}N -urea distributes similarly to ^{15}O -water and that ^{13}N -glutamine is present in two separate compartments in the brain: intracellular ^{13}N -glutamine formed in the brain cells from ^{13}N -ammonia and blood ^{13}N -glutamine coming from extra-cerebral formation from ^{13}N -ammonia (Sørensen and Keiding 2006a). The kinetic analyses in the two studies by Ahl *et al.* (2004) and Keiding *et al.* (2006) are examples of appropriate models fitted to experimental data (Sørensen and Keding 2007; Keiding and Sørensen 2007).

The question of possible back-flux of free ammonia from brain to blood has been discussed in various studies but no studies report data that exclude back-flux (Ott and Larsen 2004). Our data analysis yielded low but positive k_2 -values in each subject (Keiding *et al.* 2006). Although we observed a high inter-individual variation, this finding strongly indicates the presence of ^{13}N -ammonia back-flux from brain cells to blood. No k_2 -values were reported in the study by Ahl *et al.* (2004).

Permeability-surface-area products for ^{13}N -ammonia

K_1 ($\text{ml blood min}^{-1} \text{ ml}^{-1} \text{ tissue}$) is the initial unidirectional clearance of ammonia across the BBB (Fig. 2) and is defined as:

$$K_1 = \text{CBF} (1 - e^{-\text{PS}_{\text{BBB}}/\text{CBF}}). \quad (1)$$

According to the Kety–Renkin–Crone relation (Kety 1951; Renkin 1959; Crone 1964) the permeability–surface area product (PS) ($\text{ml blood min}^{-1} \text{ ml}^{-1} \text{ tissue}$)

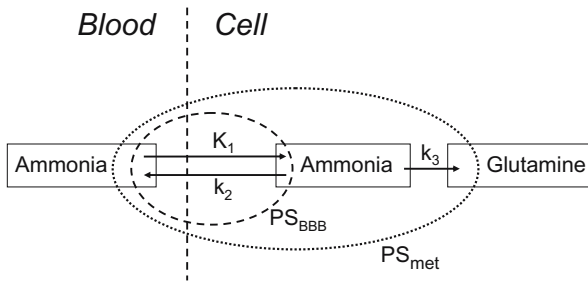


Fig. 3 PS_{BBB} is a measure of the capacity of ammonia transfer across BBB and given as $-CBF \ln(1-K_1/CBF)$. PS_{met} is a measure of the combined transfer of ammonia from blood to cells and the metabolic trapping within the cells. PS_{met} is given as $-CBF \ln(1-K_{met})$ where $K_{met}=K_1 k_3/(k_2+k_3)$ (see Fig. 2)

characterizes the capacity of unidirectional transfer of substrate across the capillary wall. In the present context, PS for ^{13}N -ammonia across the BBB, PS_{BBB} , following a bolus injection of ^{13}N -ammonia (Fig. 3) was calculated as

$$PS_{BBB} = -CBF \ln(1 - K_1/CBF) \tag{2}$$

PS_{BBB} is a measure of the capacity of transfer of ammonia across the BBB from blood to cells. K_1 depends on CBF in such a way that PS_{BBB} is independent of CBF (Eqs. 1 and 2). It may be noted that K_1/CBF equals the extraction fraction of the initial distribution of ^{13}N -ammonia from blood into the cells (E_0).

Both Ahl *et al.* (2004) and Keiding *et al.* (2006) used individual CBF values from state-of-the-art dynamic ^{15}O -water PET measurements to calculate PS_{BBB} (Eq. 2); Ahl *et al.* (2004) found no significant differences between average values of PS_{BBB} between groups of patients with cirrhosis and early HE and patients with cirrhosis without HE (Fig. 4). Similarly Keiding *et al.* (2006) found no significant differences between average values of PS_{BBB} between groups of patients with cirrhosis without HE and healthy subjects but a small reduction of PS_{BBB} in a group of patients with cirrhosis and clinically manifest HE (Fig. 4). At this conference, Goldbecker *et al.*

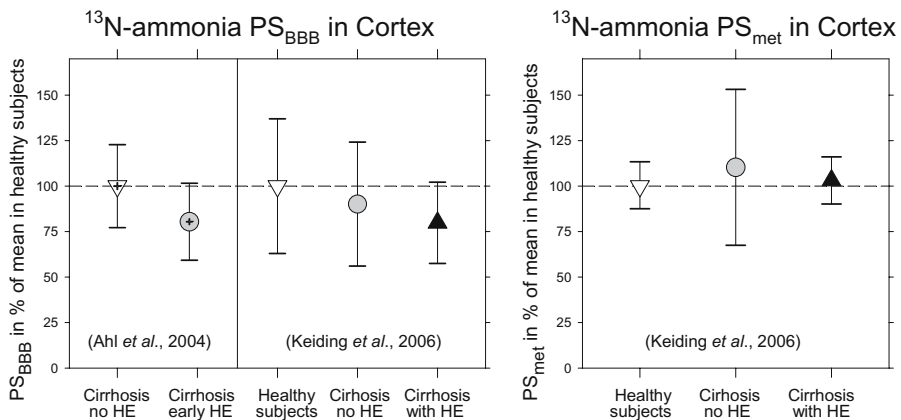


Fig. 4 Cortical PS_{BBB} and PS_{met} (Ahl *et al.* 2004; Keiding *et al.* 2006): mean values \pm standard deviation. Values are relative to the mean values of the group of healthy subjects in each study

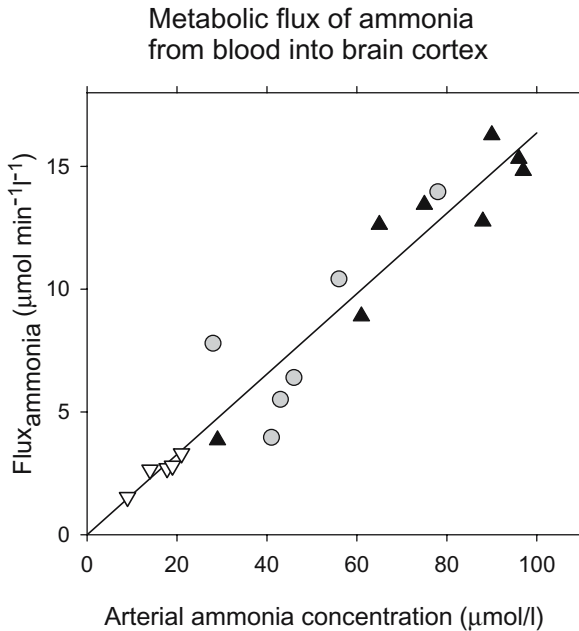


Fig. 5 Net metabolic flux of ammonia from blood to brain cortex in relation to arterial blood ammonia (Keiding *et al.* 2006). Symbols as in Fig. 4

(2007, this conference) similarly reported reductions of the PS_{BBB} in patients with liver fibrosis and minimal HE measured with dynamic ^{13}N -ammonia PET.

Keiding *et al.* (2006) introduced the concept of PS_{met} ($ml\ blood\ min^{-1}\ ml^{-1}\ tissue$) as a PS-value for the combined transfer of ^{13}N -ammonia across the BBB and subsequent intracellular metabolic conversion of ^{13}N -ammonia to ^{13}N -metabolites, i.e. mainly ^{13}N -glutamine (Fig. 3). It is based on K_{met} ($ml\ blood\ min^{-1}\ ml^{-1}\ tissue$),

$$K_{met} = K_1 k_3 / (k_2 + k_3) \quad (3)$$

K_{met} is the unidirectional clearance of blood ^{13}N -ammonia by irreversible trapping of tracer in tissue, and K_1 , k_2 , and k_3 are defined as in Fig. 2.

PS_{met} ($ml\ blood\ min^{-1}\ ml^{-1}\ tissue$) accordingly is calculated as

$$PS_{met} = -CBF \ln(1 - K_{met}/CBF) \quad (4)$$

K_{met}/CBF is the metabolic extraction fraction for the ammonia (E_{met}). As shown in Fig. 4, mean PS_{met} was not significantly different between the three groups of patients with cirrhosis with clinically manifest HE, patients with cirrhosis without HE and healthy subjects (Keiding *et al.* 2006).

In the study by Keiding *et al.* (2006) arterial blood ammonia was on average 73, 50, and 16 $\mu mol/l$ in patients with cirrhosis with overt HE, patients with cirrhosis without HE and healthy subjects, respectively; the levels in the group of patients with cirrhosis without HE corresponds closely to the average venous plasma values found by Ahl *et al.* (2004), being 57 $\mu mol/l$ in patients with cirrhosis without HE and 67 $\mu mol/l$ in patients with cirrhosis with early HE. Net metabolic flux of

ammonia from blood into brain tissue metabolites, $\text{Flux}_{\text{ammonia}}$ ($\mu\text{mol ammonia min}^{-1} \text{ ml}^{-1} \text{ brain tissue}$) equals the product of K_{met} and arterial ammonia concentration, A ($\mu\text{mol/ml}$),

$$\text{Flux}_{\text{ammonia}} = K_{\text{met}}A. \quad (5)$$

Keiding *et al.* (2006) demonstrated that K_{met} was similar in patients with cirrhosis ± HE and healthy subjects. $\text{Flux}_{\text{ammonia}}$ was accordingly found to be linearly correlated to blood ammonia, independently of which group of subjects were studied (Fig. 5). These results are in agreement with early findings by Lockwood *et al.* (1979).

As mentioned, the PS_{BBB} -product is per definition flow-independent. This requires that neither the permeability (P) nor the surface-area of the capillaries (S) is changed during flow changes (Kety 1951; Renkin 1959; Crone 1964). Phelps *et al.* (1981) showed however that regional brain PS -values increased during experimentally increased flow rates, which could be explained by recruitment of capillaries which would increase S . This does not influence the interpretations of the results by Ahl *et al.* (2004) and Keiding *et al.* (2006) since neither of the studies used experimentally induced changes of the CBF. Furthermore in both recent studies there were no significant differences in CBF between the groups of subjects when comparing specific regions.

In summary, increased cerebral trapping of ammonia in patients with cirrhosis seems primarily attributable to an increased blood ammonia concentration. This may make cerebral regions with high perfusion more vulnerable to increased blood ammonia since these areas are predominantly exposed to more ammonia.

Concluding remarks

Use of contemporary PET technology with high spatial and temporal resolution in conjunction with proper mathematical-physiological modelling is an excellent method to address the question of ammonia metabolism in patients with HE. In the most recent ^{13}N -ammonia PET studies of brain ammonia metabolism in liver patients, the basic ammonia kinetics in the brain did not seem to be changed significantly (Ahl *et al.* 2004; Keiding *et al.* 2006; Goldbecker *et al.* 2007, this conference). The blood ammonia concentration seems to be the more important factor for brain ammonia uptake (Lockwood *et al.* 1979; Ahl *et al.* 2004; Keiding *et al.* 2006). There seems to be no or only a slight tendency to a decrease in the BBB's permeability to ammonia (Ahl *et al.* 2004; Keiding *et al.* 2006; Goldbecker *et al.* 2007, this conference). Only one of the studies however included patients with more severe degrees of HE (Keiding *et al.* 2006) and more studies are needed to elucidate this very important issue.

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