Direct assessment of increased pyruvate carboxylase in the hyperammonemic brain using ^{13}C MRS

B. Lanz[1], C. Cudalbu[1], J. M. Duarte[1], and R. Gruetter[1,2]

[1]Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Department of Radiology, Universities of Lausanne and Geneva, Lausanne and Geneva, Switzerland

Introduction:

Dynamic ^{13}C MRS using [1,6-$^{13}C_2$] glucose as a labeled substrate is an interesting tool to analyze brain metabolism non-invasively in vivo. Studying neuroglial metabolism in hyperammonemic conditions may help to understand the mechanism of ammonia toxicity. Past studies using ^{13}C labeled glucose in hyperammonemic rats used [1-^{13}C] glucose to analyze the GluC4 turnover or [2-^{13}C] glucose, which labels GluC3 and GluC2 to assess pyruvate carboxylase (PC) [1,2]. In this study, we extended our previous approach based on ^{15}N MRS using ^{15}N-labeled ammonia [3] by combining ammonium chloride and [1,6-$^{13}C_2$] glucose infusions, in order to investigate the effect of hyperammonemia and anaplerosis on the time courses of the C4 and C3 positions of glutamate and glutamine simultaneously. Since in the [1,6-$^{13}C_2$] glucose infusion case PC is a glial dilution flux only for the labeling position C3, PC can be assessed directly from the different labeling dynamics of the C4 and C3 positions, in a single experiment, limiting in this way the number of working assumptions.

Materials and methods:

Localized ^{13}C spectra were measured on 3 control rats and 3 hyperammonemic rats (Sprague-Dawley, 275±25g, VOI=5x8x8mm^3) fasted overnight and artificially ventilated. The femoral artery and vein were catheterized for monitoring blood gases, blood pressure, glucose concentration, and for infusion of α-chloralose and glucose. An exponentially decaying bolus of 99%-enriched [1,6-$^{13}C_2$] glucose was administrated over 5 min, followed by a continuous infusion of 70%-enriched glucose for 6h. Glucose was infused at a rate adjustable to the concomitantly measured plasma glucose concentrations to maintain the desired glycemia level (around 300 mg/dl). To create hyperammonemic conditions, ammonium chloride was infused continuously at a stable rate (4.5mmmol/h/kg) after a bolus over 1 min [4], starting 3 hours before the glucose injection. All data were acquired on a 9.4T system (Varian/Magnex Scientific) using a home-built 10mm (^{13}C)/13mm (^{1}H quad) surface coil as RF transceiver and the semi-adiabatic DEPT polarization transfer sequence (TR=2.5s, interpulse delay 3.8ms (J_{CH}=130Hz), 45° for last ^{1}H pulse to simultaneously measure signals from CH, CH_2, CH_3 groups) [5]. In vivo ^{13}C spectra were quantified using LCModel [6]. The ^{1}H spectra were acquired before the glucose injection using an ultra-short-TE localized SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 128 scans), in the same VOI. For the hyperammonemic rats, the evolution of glutamate and glutamine concentrations was followed over the ammonia infusion period preceding the glucose injection. The ^{13}C spectra were scaled using the FE of GluC3 at the end of the experiment, calculated from the ratio of the multiplets to the singlet of GluC4.

FE	*GluC4*	*GlnC4*	*GluC3*	*GlnC3*
Control	*0.54±0.03*	*0.35±0.06*	*0.46±0.01*	*0.32±0.05*
Hyper-ammonemia	*0.52±0.05*	*0.43±0.02*	*0.43±0.04*	*0.20±0.01*

Table 1:*fractional enrichment levels at steady-state*

Results and Discussion:

While [Glu] remained stable during hyperammonemia (Fig.1), [Gln] was linearly increasing. Using both the preinfusion ^{1}H MRS data and the measurement of [Gln] derived from the GlnC4 multiplets at steady-state, a net glutamine synthesis rate of 0.04±0.02 µmol/g/min (mean±SEM) was calculated, consistent with our previous results [3]. The constant [Glu] and linearly increasing [Gln] were further used to calculate the FE of GluC4, GluC3, GlnC4 and GlnC3.

Fig.3 shows that even if the total glutamine is steadily increasing under hyperammonemia, the FE of GlnC4 and GlnC3 are reaching a dynamic steady-state level. However, their steady-state level is different from the control, especially for GlnC3 (FE=20±1% vs 32±5% in controls (mean±SEM))(see table 1). This tendency is also clearly visible on the ^{13}C spectrum (Fig.2), where GlnC3 FE can be calculated by the ratio of the peak area of the GlnC4 doublet relative to the GlnC4 doublet+singlet area. This suggests a significant increase in pyruvate carboxylase, responsible for the net glutamine synthesis. Since most of the glutamine is located in the glial cells while glutamate is mainly neuronal, this explains why the glutamine steady-state enrichments are significantly affected by hyperammonemia but not the GluC4 and GluC3 FE. The slightly higher level of GlnC4 at the end of the experiment suggests a lower dilution at the level of glutamine through extracellular glutamine.

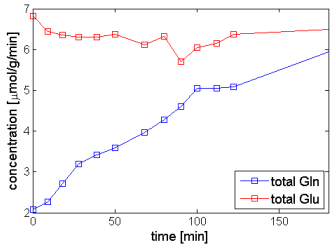

Fig.1: *Typical evolution of the total glutamate and glutamine concentration in a hyperammonemic rat*

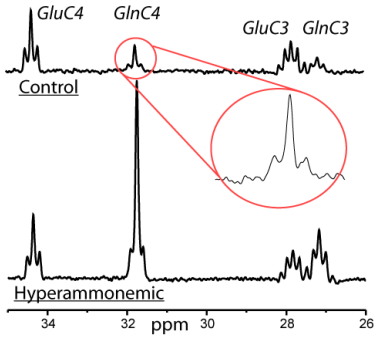

Fig.2: *typical spectra obtained at labeling steady-state. Note the strong increase in the glutamine peaks.*

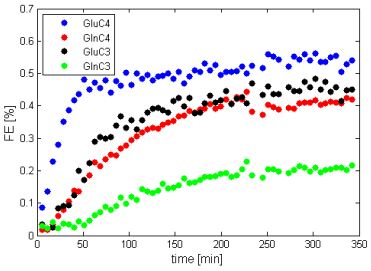

Fig.3: *time course of the FE of the metabolites in hyperammonemic rats (n=3)*

We conclude that the effect of hyperammonemia on glial metabolism is clearly visible on the FE curves obtained using [1,6-$^{13}C_2$] glucose infusion, as reflected by the glutamine enrichment curves. Anaplerosis appear to be the major ammonia detoxification pathway, like measured in our previous ^{15}N ammonia study [3], while neuronal metabolism appears less affected, as reflected by GluC4 and GluC3 FE. The linearly increasing glutamine concentration over several hours leads to a dynamic steady-state of the FE in the glutamine C4 and C3 positions, reflecting constant metabolic fluxes during the infusion. Future modeling of the turnover curves can therefore be based on a two-compartment approach with constant fluxes. Co-infusion of ammonia and [1,6-$^{13}C_2$] glucose enables the simultaneous assessment of the TCA cycle activity with the C4 positions and PC with the dilution of the C3 positions in a single experiment, with minimal hypothesis.

References: **1.** N. Sibson et al., *J.Neurochem.* 76: 975 (2001) **2.** N. Sibson et al., *Proc. Natl. Acad. Sci. U S A.*94(6): 2699 (1997) **3.** C. Cudalbu et al., *Proc. Intl. Soc. Mag. Reson. Med.* 18: 314 (2010) **4.** K. Kanamori et al., *NMR Biomed.* 6:21(1993) **5.** P.G. Henry et al., *Magn. Reson. Med.* 50(4):684(2003) **6.** S.W. Provencher, *Magn. Reson. Med.* 30:672(1993)

Acknowledgments: Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations; SNF grant No. 131087.