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

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Introduction:
Dynamic $^{13}$C MRS using [1,6-$^{13}$C] glucose as a labeled substrate is an interesting tool to analyze brain metabolism non-invasively in vivo. Studying neuronal 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 $^{13}$N MRS using $^{13}$N-labeled ammonia [3] by combining ammonium chloride and [1,6-$^{13}$C] 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] glucose infusion case PC is a gial 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=5x8x8mm³) 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] glucose was administered 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 glyemia level (around 300 mg/dl). To create hyperammonemic conditions, ammonium chloride was infused continuously at a stable rate (4.5mmol/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 (¹H) quadrature coil as RF receiver and the semi-adiabatic DEPT polarization transfer sequence (TR=2.5s, interpulse delay 3.8ms (J=300Hz), 45° for last ¹H pulse to simultaneously measure signals from CH2, CH2, CH3 groups) [5]. In vivo $^{13}$C spectra were quantified using LCMs1 [6]. The ¹H 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 GluC4 at the end of the experiment, calculated from the ratio of the multiplets to the singlet of GluC4.

Results and Discussion:

<table>
<thead>
<tr>
<th>FE</th>
<th>GluC4</th>
<th>GluC4</th>
<th>GluC3</th>
<th>GlnC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.45±0.03</td>
<td>0.35±0.06</td>
<td>0.46±0.01</td>
<td>0.32±0.05</td>
</tr>
<tr>
<td>Hyper-ammonia</td>
<td>0.52±0.05</td>
<td>0.43±0.02</td>
<td>0.43±0.04</td>
<td>0.20±0.01</td>
</tr>
</tbody>
</table>

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 glut metabolism is clearly visible on the FE curves obtained using [1,6-$^{13}$C] 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 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] 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.


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