Measurements of glial metabolic fluxes with $^{13}$C-acetate using positron emission and $^{1}$H($^{13}$C) NMR spectroscopy

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Introduction:
Nuclear imaging and NMR are two ways to study the brain metabolism. Acetate, which is taken up only by the glia, in combination with $^{13}$C MRS and two-compartment modeling allows the determination of the Krebs cycle fluxes of the glia and the neurons as well as their interaction through the neurotransmission cycle [1]. NMR is able to distinguish different labeled chemical groups, while methods based on radioactive tracers allows a highly sensitive detection and a better time resolution, however without chemical differentiation. In this study, we report the results obtained by using an adapted NMR acetate metabolism model to analyse beta-probe data following $[1-^{13}]$C-acetate infusion in rats and compare it to the results of $[^{1}$H($^{13}$C)] NMR spectroscopy following $[2-^{13}]$C-acetate infusion.

Materials and methods:
Brain acetate infusion experiments were modeled using a two compartments model [3] and a simplification of the Krebs cycle modeling recently proposed [4]. The same brain metabolism model was applied to beta-probe and MRS acetate infusion experiments, using respectively radioactive $[1-^{13}]$C-acetate and stable $[2-^{13}]$C-acetate as precursor. In the first case, the signal measured with a beta-probe inserted in the brain [5,6] represents the time evolution of the total amount of labeled metabolites (glutamine and glutamate at position C5 or C1). Total blood radioactivity was continuously recorded using an arterio-venous shunt and a coincidence counter [7] to obtain the arterial input function, following the bolus infusion. All the measurements were corrected for radioactive decay. A portion of the measured signal is reflecting the blood radioactivity due to blood partial volume in the brain (around 5% of the volume in rats). Since with bolus injection the blood radioactivity was slowly varying and negligible after 2 minutes, we omitted the first 2 minutes for the fit. In the second case, $[^{1}$H($^{13}$C)] NMR spectroscopy was used to measure $^{13}$C labeling of glutamate and glutamine at the positions C4 and C3. The infusion protocol was adjusted to obtain a constant $^{13}$C plasma acetate FE. The model was adapted to the labeled carbon positions of the infused acetate. As shown in the past [4] for single-compartment models, the use of the composite flux $V_{gt} = V_x + V_{nt}$ is expedient to describe the turnover of the first labeled position of glutamate (and thus of glutamine). This still holds in the two-compartment model for glial and neuronal TCA cycles. Thus, in the $[2-^{13}]$C-acetate case, the apparent glial Krebs-cycle rate ($V_{gt}$) and the neuronal one ($V_{nt}$) as well as the apparent neurotransmission flux $V_{nt}$ were fitted using the signal of glutamine and glutamate labeled at position 4 and 3. In addition to previous models, we found that the separate measurement of glutamate and glutamine C3 allowed to assess the glutamine Krebs-cycle flux $V_{nt}$. In the $[1-^{13}]$C-acetate case, the total tissue radioactivity curve is dominated by the signal of the first labeled position C5 of glial glutamate and glutamine essentially reflecting $V_{gt}$ and $V_{nt}$. The small FE of the neuronal pools makes the estimation of neuronal $V_{nt}$ impossible. So, $V_{gt}$ and $V_{nt}$ were fitted, while the $V_{nt}$ was fixed to the average value found in the NMR study.

Results and Discussion:
1. $[^{1}$H($^{13}$C)] MRS data were averaged from 5 rats and the isotropic enrichment turnover curves fitted for glutamate and glutamine C4 and C3 result in values of glial $V_{gt} = 0.061±0.003 \ \mu$mol/g/min, neuronal $V_{nt} = 0.21±0.02 \ \mu$mol/g/min and Vnt = 0.16±0.01 \ \mu$mol/g/min. A preliminary fit of the C3 positions enabled an insight in the Krebs cycle modeling recently proposed [4]. The same brain metabolism model was applied to beta-probe MRS acetate infusion experiments, using radioactive [1-$^{13}$C]-acetate infusion, the model was fitted successfully to the different tissue activity curves of 6 animals, using the model shown in figure 1. Another metabolite entering in consideration when using radioactive $^{13}$C measurements is the production of $^{13}$C02. Based on the high diffusivity of $^{13}$C02 across the blood-brain barrier, the model predicts small amount of $^{13}$C02 below 5% of the total tissue activity after the two first minutes. The initial peak is essentially due to the direct degradation of acetate into $^{13}$C02 through the TCA cycle (i.e. $^{13}$C02 flowing directly from acetate to $^{13}$CO2 without passing through the glutamate pools). The height of this initial peak is thus dependent on the value of the vgl as relative to $^{13}$CO2.

We conclude that the two-compartment model presented here is able to fit data of two intrinsically different measurement modalities of brain metabolism whereas $[^{1}$H($^{13}$C)] MRS following $[2-^{13}]$C-acetate infusion is able to give a precise insight of the respective activity of the glial and neuronal mitochondrial fluxes as well as of the apparent neurotransmission. $^{13}$C PET presents a faster alternative to the glial Krebs cycle measurement, potentially applicable to human PET imaging.

References:

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Fig.1: Model used for $[2-^{13}]$C acetate infusion (NMR study). A similar model [2] was used for $^{13}$C studies, with adapted label positions (i.e. glutamate and glutamine C5 followed by C1)

Fig.2: Fits of the MRS data averaged on 5 animals, using the model shown in figure 1.

Fig.3: Typical fit of beta-probe data, using a similar model than in figure 1, adapted to the different labeled carbon positions.