Measurements of glial metabolic fluxes with ^{11}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-^{11}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-^{11}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 $Vgt = \frac{Vx\,Vtca}{Vx + Vtca}$ 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 (Vgt^{g}) and the neuronal one (Vgt^{n}) as well as the apparent neurotransmission flux Vnt 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 glial transmitochondrial flux Vx^{g}. In the [1-^{11}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 Vgt^{g} and Vnt. The small FE of the neuronal pools makes the estimation of neuronal Vgt^{n} impossible. So, Vgt^{g} and Vnt were fitted, while the Vgt^{n} 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 isotopic enrichment turnover curves fitted for glutamate and glutamine C4 and C3 result in values of glial Vgt^{g} = 0.061±0.003 µmol/g/min, neuronal Vgt^{n} = 0.21±0.02 µmol/g/min and Vnt = 0.16±0.01 µmol/g/min. A preliminary fit of the C3 positions enabled an insight in the glial transmitochondrial flux Vx^{g}. Note the excellent fit in figure 2 found with Vx^{g} on the order of Vtca^{g}, while Vx^{g}= 5 Vtca^{g} resulted in substantial fit residuals (not shown).

2. In the case of [1-^{11}C]acetate infusion, the model was fitted successfully to the different tissue activity curves of 6 animals, by varying the glial mitochondrial fluxes and Vnt. A glial composite rate constant Kgt^{g} = Vgt^{g}/[Ace]$_{plasma}$ was extracted from the experimental data. Considering an average acetate concentration in plasma of 1 µmol/g [8] and the negligible additional amount injected, we found an average of Vgt^{g} = 0.077± 0.018 (n=6), which is in good agreement with the NMR measurements in the present study and the literature [8].

3. Another metabolite entering in consideration when using radioactive ^{11}C measurements is the production of radioactive $^{11}CO_2$. Based on the high diffusivity of CO_2 across the blood-brain barrier, the model predicts small amount of $^{11}CO_2$, 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 CO_2 through the TCA cycle (i.e. ^{11}C flowing directly from acetate to CO_2 without passing through the glutamate pools). The height of this initial peak is thus dependent on the value of the glial Vx^{g} relative to Vtca^{g}.

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. ^{11}C PET presents a faster alternative to the glial Krebs cycle measurement, potentially applicable to human PET imaging.

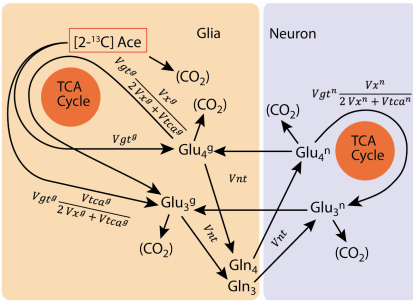

Fig.1: *Model used for [2-^{13}C]acetate infusion (NMR study). A similar model [2] was used for ^{11}C studies, with adapted label positions (i.e. glutamate and glutamine C5 followed by C1)*

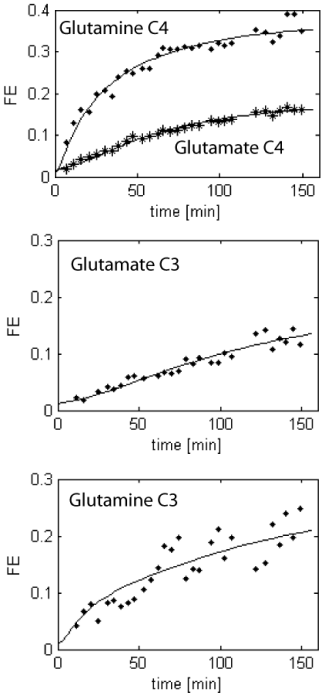

Fig.2: *Fits of the MRS data averaged on 5 animals, using the model shown in figure 1.*

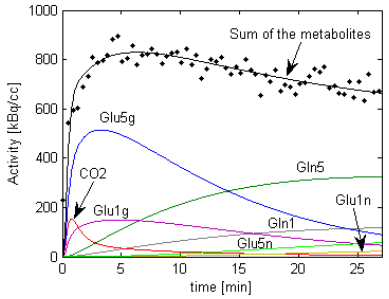

Fig.3: *Typical fit of beta-probe data, using a similar model than in figure 1, adapted to the different labeled carbon positions.*

References: **1.** RA de Graaf, et al. *NMR Biomed.* 16:339 (2003) **2.** B Lanz et al. *Proc. Intl. Soc. Mag. Reson. Med.* 16: 1643 (2008) **3.** R Gruetter, et al. *Am. J. Physiol. Endocrinol. Metab.* 281:100 (2001) ; **4.** K Uffmann, et al. J. Neurosci. Res. 85(13):3304 (2007) ; **5.** B Weber, et al. *J. Cereb. Blood Flow Metab.* 23:1455 (2003) ; **6.** MT Wyss, et al. *NeuroImage* 35:1086 (2007) ; **7.** B Weber, et al. *Eur. J. Nucl. Med.* 29:319 (2002) ; **8.** N Cetin, et al. *Neurochem. Int.* 5:359 (2003);

Acknowledgments: Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations; SNF grant No. 3100A0-116220.