Measurement of brain oxidative metabolism in monkeys using ^{1}H-NMR spectroscopy without ^{13}C editing and decoupling

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Introduction

NMR measurements of the brain TCA cycle flux (V_{TCA}) using ^{13}C-labeled glucose have greatly developed over the last decade. The sensitivity of ^{13}C spectroscopy being intrinsically low, the first *in vivo* brain studies were performed using indirect {^{13}C}-^{1}H detection of amino-acid labeling [1,2]. Due to the overwhelming signal of ^{12}C-bonded protons on ^{1}H spectra, the detection of ^{13}C-coupled protons of glutamate (Glu) has been performed using editing techniques such as POCE [1]. However heteronuclear editing techniques require both ^{1}H and ^{13}C radiofrequency transmitters and coils, as well as appropriate pulse sequences which are not implemented on most clinical MR systems. RF decoupling associated with heteronuclear editing is also problematical in human studies, due to power deposition to the tissue. Since the early POCE measurements in the mid-eighties, hardware improvement has led to higher spectrum quality and stability, so that the requirement for editing techniques is now questionable. In this context, our objectives were (i) to demonstrate that ^{13}C incorporation into brain glutamate could be detected during a ^{13}C-glucose infusion using a simple ^{1}H PRESS sequence without ^{13}C editing and decoupling, (ii) to develop a ^{13}C quantitation procedure based on LCModel analysis [3], taking advantage of the simultaneous detection of the decrease in ^{12}C-bonded protons and the increase in ^{13}C-coupled satellites of glutamate, and (iii) to measure V_{TCA} by fitting glutamate ^{13}C4 and ^{13}C3 enrichments time-courses.

Materials and Methods

Data acquisition. MR studies were conducted on 2 healthy macaque monkeys (2 studies for each monkey). Animals were anaesthetized using i.v. infusion of propofol (~200 µg/kg/min), intubated and ventilated. Experiments were conducted on a 3 Tesla whole-body NMR system (Bruker, Ettlingen, Germany) equipped with a home-made surface ^{1}H probe. After scout imaging, voxel positioning in the striatum (3.9 cm^3 voxel) and shimming down to 7 Hz, a baseline ^{1}H PRESS spectrum was acquired (TE/TR = 8/1000 ms, 512 transients, BISTRO-type OVS, VAPOR water suppression). Then ^{1}H PRESS spectra were collected during glucose infusion (3-min bolus of [U-$^{13}C_6$]glucose followed by 120-min continuous infusion). Blood samples were collected in order to measure glucose fractional enrichment using high resolution NMR spectroscopy.

Spectra processing. PRESS spectra collected under infusion of ^{13}C-labeled glucose were subtracted from the baseline spectrum. As a result of the subtraction, the uprising ^{13}C-coupled satellites of glutamate exhibit negative amplitudes during glucose infusion. Similarly, the vanishing resonances of ^{12}C-bonded protons exhibit increasing amplitudes during glucose infusion (Fig. 1a). In order to take full advantage of the simultaneous detection of ^{13}C-coupled satellites and ^{12}C-bonded protons, an original procedure was developed to quantify glutamate C4 and C3 fractional enrichments from the difference PRESS spectra (baseline - dynamic spectra). Automatic analysis was performed by LCModel using the following simulated lineshapes:

- a lineshape accounting for GluC4 enrichment, made of ^{12}C4-bonded protons (2.35 ppm) surrounded by two anti-phase ^{13}C4 satellites (±65 Hz away from the ^{12}C4 resonance), the peak area of ^{12}C4-bonded protons being the opposite of the total area of ^{13}C4 satellites (Fig 1d),
- a similar lineshape accounting for GluC3 enrichment, centered around 2.11 ppm (Fig 1e).

Both lineshapes were simulated using NMR-Sim 2.8 (Bruker, Ettlingen, Germany). Following automatic processing by LCModel, fractional enrichment time-courses were fitted by a single compartment mathematical model describing ^{13}C incorporation from glucose into brain glutamate [4], leading to V_{TCA}.

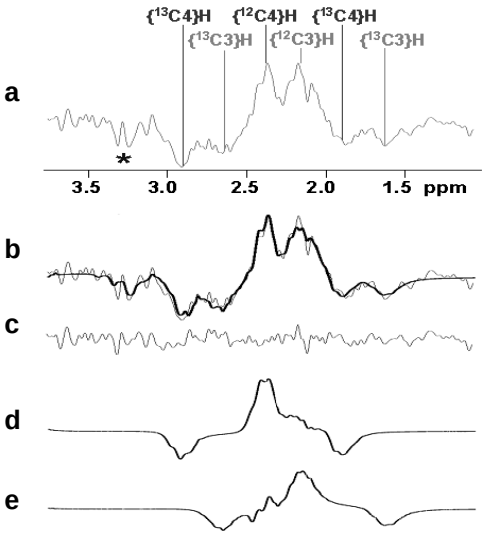

Fig. 1. *Difference PRESS spectrum analysis.* ***a:*** *Raw difference spectrum acquired at the end of glucose infusion.* ***b:*** *best fit by LCModel.* ***c:*** *residuals* ***d:*** *lineshape accounting for glutamate C4 enrichment.* ***e:*** *lineshape accounting for glutamate C3 enrichment. (*) points out ^{13}C glucose contribution.*

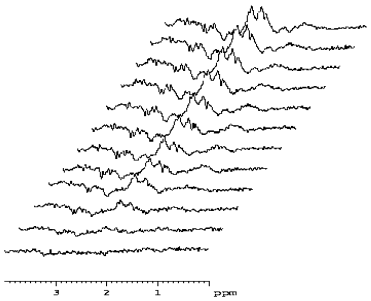

Fig. 2. *Stacked plot of difference PRESS spectra (9-min time resolution)*

Results and Discussion

The excellent signal stability over the 2-hour infusion allowed for the detection of ^{13}C incorporation into the C3 and C4 positions of glutamate (Fig. 2). LCModel analysis of a difference spectrum acquired at the end of glucose infusion is presented on figures 1b-e, showing a good agreement between experimental and modeled spectra. Figure 3 shows the best fit to the enrichment time-courses (averaged over the 4 studies), leading to a mean $V_{TCA} = 0.55 \pm 0.04$ µmol.g^{-1}.min^{-1} (*n=4, mean±s.d.*). This number is rather low in comparison with literature values of primate and human brain glucose metabolism [5,6]. This might be explained by the high contribution of white matter in our voxel of interest (46% of the tissue content as determined by T1-MRI segmentation).

Compared with other techniques such as direct ^{13}C or POCE, the PRESS approach allows for simultaneous detection of changes in ^{12}C-bonded and ^{13}C-coupled resonances. As a consequence, the measurement accuracy is increased by information redundancy, making it possible to measure V_{TCA} from a 3.9 cm^3 volume centered in the brain at 3 Tesla.

This work demonstrates the ability to accurately measure brain oxidative metabolism in a deep structure of the primate brain on a whole-body 3 Tesla system without ^{13}C editing or decoupling. The decreasing cost of ^{13}C labeled glucose combined with the increasing use of 3 T systems for clinical investigations could make [U-$^{13}C_6$]glucose a competitive substrate for metabolic studies in patients.

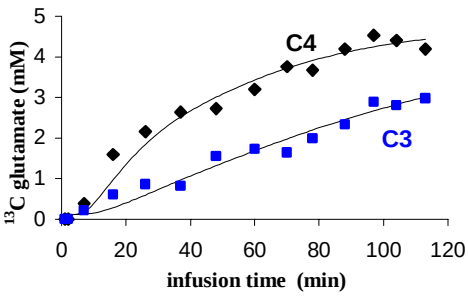

Fig. 3. *Averaged glutamate ^{13}C4 and ^{13}C3 enrichment time-courses (n=4) and best fits*

References

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