Selective Detection of [4-13C]-Glutamate and [4-13C]-Glutamine Turnover by 1H-[13C] NMR Spectroscopy in the Human Brain at 4 Tesla

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Introduction

Carbon-13 NMR spectroscopy in combination with ^{13}C-labeled substrate infusion (e.g. [1-^{13}C]-glucose) is a powerful technique to study important metabolic pathways in the animal and human brain *in vivo*. While ^{13}C NMR can resolve many important metabolites due to the large spectral dispersion, it is also characterized by low NMR sensitivity and hence acquisitions from large volumes (30–140 mL in human brain). ^{1}H-[^{13}C]-NMR spectroscopy can, in principle, provide the same information as ^{13}C NMR at a much higher sensitivity, allowing signal detection from volumes down to 6 mL (1,2). Unfortunately, strong spectral overlap due to the much smaller spectral range of ^{1}H-[^{13}C]-NMR has limited the detection to [4-^{13}C]-glutamate (1,2). Here we present a ^{1}H-[^{13}C]-NMR detection scheme based on the semi-selective POCE sequence of Henry et al (3) that allows the separate detection of [4-^{13}C]-glutamate, as well as [4-^{13}C]-glutamine and [3-^{13}C]-Glx, in the human brain *in vivo*.

Methods

All experiments were performed on a Bruker spectrometer interfaced to a actively-shielded Magnex 4 Tesla magnet and a Bruker BGK-38 head gradient insert (150 µs risetime, 30 mT/m). A 8 cm diameter proton surface coil was used for transception, while two orthogonal 13 cm diameter ^{13}C coils driven in quadrature were used for WALTZ-16 decoupling (B_{2max} ~ 500 Hz over 140 ms acquisition time). All semi-selective ^{1}H-[^{13}C] NMR spectra were acquired with the sequence shown in Fig. 1. To minimize signal loss due to RF inhomogeneities, the complete sequence is executed with adiabatic RF pulses, using 3D LASER for single-shot spatial localization. The proton semi-selective SSAP water suppression pulses gave optimal excitation/refocusing at the glutamate-H4 position. The two ^{13}C RF pulses were adiabatic BIR-4 pulses with nutation angles (α_1, α_2) of (0°, 0°), (0°, 180°), (+90°, +90°) and (+90°, -90°) in four separate experiments. The ^{1}H-[^{13}C]-difference spectra were calculated as a linear combination of the four sub-spectra. *In vivo* experiments were performed on the occipital cortex (2.5 × 1.5 × 2.5 cm = 9.4 mL) of a healthy volunteer before and after the oral dosing of 75 grams of [1-^{13}C]-glucose dissolved in 150 mL water.

Results

Figure 2 shows ^{1}H and ^{1}H-[^{13}C]-NMR spectra acquired between 1.5 and 2 hours following the oral dosing of [1-^{13}C]-glucose. Despite the long echo-time required for LASER (TE = 35 ms), the glutamate/glutamine H3 and H4 resonances do not exhibit much signal loss due to the CPMG character of LASER which largely refocusses scalar coupling evolution. The first ^{1}H-[^{13}C]-NMR difference spectrum selectively edits the [4-^{13}C]-glutamate resonance, while the second ^{1}H-[^{13}C]-NMR difference spectrum edits the [4-^{13}C]-glutamine resonance, as well as a significant part of the [3-^{13}C]-glutamate+glutamine resonances.

Discussion

Using a semi-selective ^{1}H-[^{13}C]-NMR method it is possible to detect [4-^{13}C]-glutamate and [4-^{13}C]-glutamine turnover with the high sensitivity of protons. The method is similar to that described by Henry et al (3), who used it to discriminate between [4-^{13}C]-Glx and [3-^{13}C]-Glx at 3 Tesla, with several noticeable modifications. The method is completely adiabatic thereby minimizing user-interaction and maximizing sensitivity. The localization is a single-shot method, allowing the detection and correction of motion related artifacts and the water suppression is near-complete with the use of semi-selective excitation/refocusing pulses. The separate detection of glutamate and glutamine turnover in relatively small volumes now offers the possibility to study energy and neurotransmitter metabolism in different cerebral tissue types (1), as well as in functional activated areas (2). Further improvements in sensitivity can be obtained by replacing [1-^{13}C]-glucose with [U-$^{13}C_6$]-glucose.

Acknowledgements

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References

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[3] Henry et al, Magn. Reson. Med. 2000; 44: 395-400

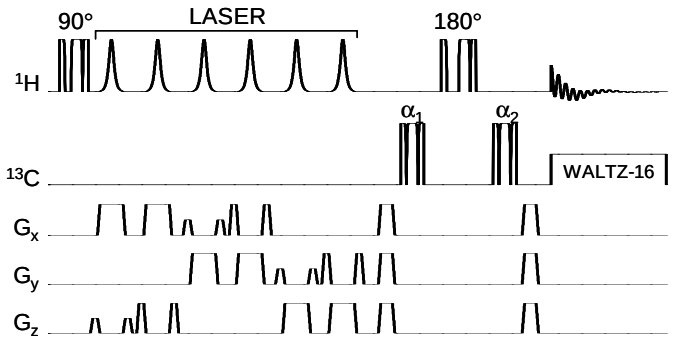

Figure 1: Semi-selective ^{1}H-[^{13}C]-NMR sequence.

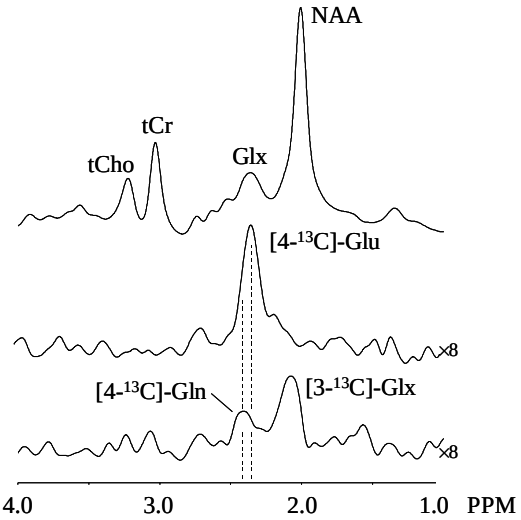

Figure 2 : Selective detection of [4-^{13}C]-glutamate (middle) and [4-13C]-glutamine (bottom) in the human brain at 4 Tesla.