Sensitivity enhanced and localized 13C MRS to study glucose uptake and metabolism in mouse brain

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Introduction

In vivo ^{13}C MRS measurements provide a valuable technique for a better understanding of metabolism in neuroactivation and neuropathologic diseases. Feasibility of this technique in transgenic mice models would be of particular interest. Recent ^{13}C MR studies in rat brain *[Sibson, 2001]* already have contributed to a quantitative determination of fluxes through the glycolysis, tricarboxylic acid cycle and glutamate-glutamine cycle using a metabolic model. However, ^{13}C MRS in mice brain implicates small voxel sizes, which requires enhanced signal intensity. Thus far, only unlocalized ^{13}C MRS has been performed at non-steady state conditions in mice *[Rivenzon-Segal, 2001; Renema, 2003].* Here we present an experimental setup that enables localized ^{13}C MRS detection at near physiological steady state glucose conditions in mice. We show that high temporal and spectral ^{13}C MRS resolution can be achieved using polarization transfer techniques in combination with efficient RF coils. This enables monitoring of metabolic fluxes in mouse models.

Materials and Methods

Localized *in vivo* measurements were performed on C57BL6 mice. After four hours of fasting, the tail vain was canulated and the mouse was anesthetized with 1.5% isoflurane (35% O_2, 65% N_2O). A warm water blanket maintained body temperature. 50 μl 210 mU/ml insulin was administered prior to the [1-^{13}C] glucose injection. A first bolus of 100 μl 1M [1-^{13}C] glucose dissolved in water was followed by a decreasing infusion rate, based on the protocol for rats as presented by Fitzpatrick et al. *[JCBF&M,1990].* After the first 5 minutes the infusion rate was kept constant at 3.3 μl/min during the rest of the measurement. Glucose metabolism and neurotransmitter dynamics were monitored before and during infusion by ^{13}C MRS on a 7 T system (MR Research systems, UK). A birdcage coil was used for homogeneous ^{1}H excitation, combined with a 12 mm surface coil optimized for ^{13}C MRS of the mouse brain. Localisation was performed by ISIS at the ^{1}H frequency (bandwidth of 6.5 kHz). This was combined with a semi adiabatic version of Distortion enhanced polarization transfer (DEPT) using BIR4 pulse of 0.5 ms segments at the ^{13}C channel. Broadband ^{1}H decoupling was used (WALTZ 16, 60 ms) during ^{13}C MRS detection. The bandwidth was about 80ppm; sufficient for glucose metabolism detection. Other parameter values were: voxel size 6x6x4 mm^3 (fig. 1a), TR 1000ms, 256 averages. The temperature was monitored on the top of the head (close to the ^{13}C conductor) to check SAR conditions. Phantom measurements were performed to validate the SNR gain of the DEPT compared to direct ^{13}C detection. Spectra were analysed using jMRUI.

Results and discussion

The phantom measurements yielded a more than four fold increase in SNR when comparing the DEPT sequence to the unlocalized pulse acquiredmeasurements. This confirms the expected signal enhancement and the valuable effect of the adiabatic RF pulses. After the infusion rate started, a clear increase of α- and β-glucose was observed in the *in vivo* measurements (fig. 1c). Steady-state glucose signals can be reached within one hour. The acquired MR spectra showed the signals originating from several metabolites such as of Glutamate (Glu_x), Glutamine (Gln_x), γ-aminobutyric acid ($GABA_x$) and lactate (Lac_3) among others (fig. 1b). No contaminating residual lipids are detected, thereby validating the quality of localization and making subtraction methods redundant. The SNR, temporal and spectral ^{13}C MRS resolution demonstrated by these measurements are sufficient for the determination of glucose metabolism and neurotransmitter cycle rates in mouse models. For this purpose the neurometabolic model for rats *[Gruetter et al., 2001]* has to be adapted for mice.

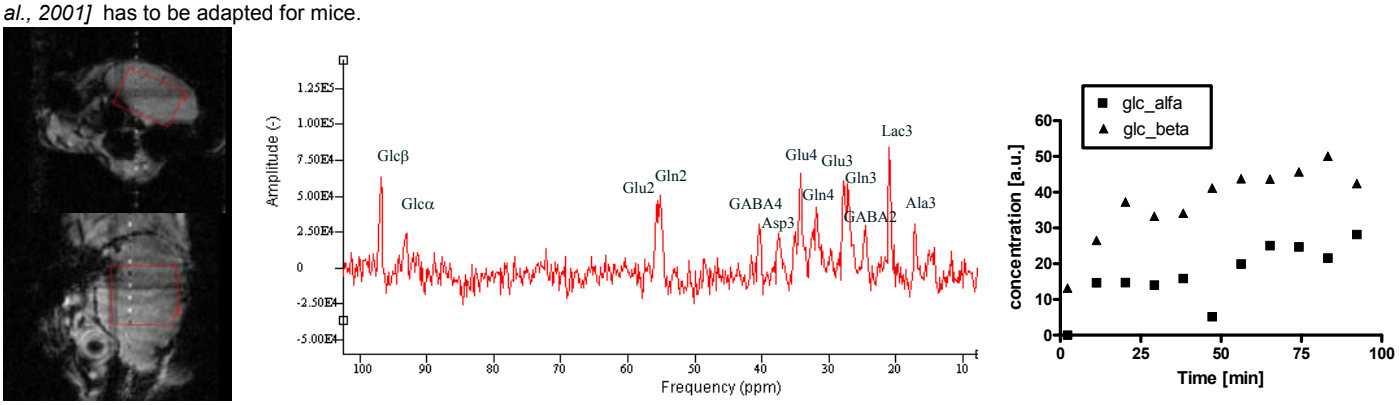

Fig. 1. ISIS localized ^{13}C MRS in mouse brain

- *a. Gradient echo MR images of the mouse brain (top: coronal, bottom horizontal) with the projection of the ISIS localized voxel (nominal voxel volume: 6x6x4 mm^3) from which the ^{13}C MR spectra were obtained*
- *b. Corresponding in vivo ^{13}C MR spectrum in mouse brain, DEPT with broadband ^{1}H decoupling, TR 1000ms, 2560 averages*
- c. *Dynamics of [1αβ-^{13}C] glucose signal as measured in the same voxel*

Conclusion

Here we demonstrated for the first time that ISIS ^{1}H-^{13}C localized polarization transfer provides sufficient sensitivity enhancement and accurate localization for ^{13}C MRS in mouse brain. Several key compounds of brain energy metabolites were detectable at a useful time resolution. This opens a window on studying *in vivo* neurometabolism in various neurologic mouse models.

References

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