Quantitative Measurement of Neurotransmitters in Rat Brain Tissue Using HR MAS ^{13}C NMR Spectroscopy and the ERETIC Method

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Introduction

Tracing labeled metabolic compounds based on ^{13}C labeled precursors by ^{13}C nuclear magnetic resonance (NMR) spectroscopy is used extensively to study metabolic interactions between neurons and glia in the brain [1]. Established extraction procedures of brain tissue are time consuming and may result in degradation of certain substances. High resolution magic angle spinning (HR MAS) has showed great potential in studying the metabolism *ex vivo* of intact biological samples [2, 3]. Absolute quantification in combination with HR MAS has so far been challenging. However, recently a new quantification method based on a synthesized reference signal has been introduced called ERETIC (Electronic REference To access *In vivo* Concentrations) [4]. This method in combination with microwave fixation of the brain tissue make it possible to do quantitative HR MAS ^{13}C NMR spectroscopy of intact brain tissue. To investigate this possibility we have compared ^{13}C NMR spectra of PCA extracted brain tissue with HR MAS ^{13}C NMR spectra of intact brain tissue both quantitatively and qualitatively.

Experimental

Six Sprague-Dawley rats were given an i.p. injection of sodium [1,6-^{13}C]glucose (3 mmol/kg; 99% ^{13}C enriched) and fifteen minutes later the brains of the animals were subjected to micro-wave fixation, 4kW, 2.2s (Model GA5013, Gerling Applied Engineering, California, USA). The thalamus was dissected out and ^{13}C NMR spectroscopy on perchloric acid (PCA) extracts were performed on one of the thalamic hemispheres and HR MAS spectroscopy of intact tissue on the other hemisphere. The sample weight of the hemispheres was between 21 and 34 mg. Both the extracted samples and the intact tissue were run on a Bruker Avance DRX600 spectrometer (14.1T). The tissue extracts were run on a Bruker BioSpin CryoProbe and the intact tissue on a Bruker HR-MAS dual ^{1}H/^{13}C probe. For both protocols ethylene glycol dissolved in D_2O were used as internal standard and total acquisition time for the ^{13}C NMR spectroscopy was 750 min at 4°C. Proton decupled carbon spectra with NOE were acquired with the same parameters both for PCA extracts and intact tissue. Spectra without NOE and long relaxation delay (20s) were also run both for PCA extracts and intact tissue to calculate correction factors for NOE and T_1 effects. For the intact tissue a 50 µl HR MAS rotor were used and spin rate was 5000Hz. Quantitative proton spectra were also obtained for all the samples. The ^{13}C signal from ethylene glycol in the HR MAS rotor was calibrated via proton signals towards a pseudo-FID ERETIC signal that was transmitted through the carbon channel of the HR MAS probe. A standard curve of five different concentrations of ethylene glycol in D_2O (0.05, 0.1, 0.2, 0.25, and 0.5Vol% respectively) were obtained both for proton and carbon spectra. Statistical differences of concentrations between the two groups were analyzed using unpaired two-tailed Student's t-test.

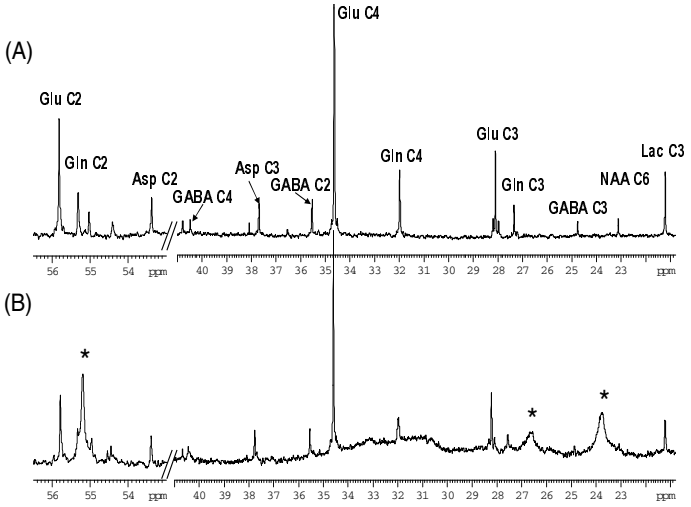

Fig. 1 Typical ^{13}C NMR spectrum of PCA extract (A) and HR MAS ^{13}C NMR spectrum of intact tissue (B). * Residual lipid signals

Results and discussion

The HR MAS ^{13}C NMR spectra obtained from intact brain tissue were of high resolution quality comparable to the spectra of the PCA extracted tissue (Fig 1). Some broad lipid signals are still visual in the HR MAS spectra (asterix in Fig 1b) and the baseline is also affected by signals from macromolecules that have not been cancelled out by spinning the sample (Fig 1b). Amounts of ^{13}C labeling at different positions (C2. C3 and C4) in glutamate, glutamine, GABA and aspartate measured either in intact tissue or in PCA extracts were not statistically significantly different (Fig 2). Quantification of total metabolite concentrations from the proton NMR spectra showed the same tendency as the ^{13}C experiments.

Conclusions

To our knowledge this is the first demonstration of absolute quantification of ^{13}C NMR experiments on intact brain tissue *ex vivo*. Based on the quantitative comparison between the HR MAS spectra and the PCA spectra presented in this study we believe that the HR MAS spectroscopy in combination with the ERETIC method might be fruitful in quantitative in ^{13}C NMR studies of intact tissue *ex vivo*.

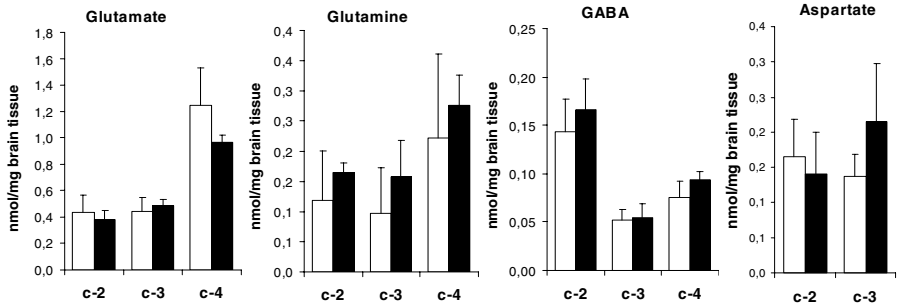

Fig. 2 Concentrations of ^{13}C labeled metabolites derived from [1,6-^{13}C]glucose metabolism in the rat thalamus. White bars represent PCA extracts (n=4) and black bars intact tissue (n=4). C2, C3 and C4 indicate labeling in different carbons within the molecule Data are presented as mean ± S.D and level of significance was set at $p<0.05$

References

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