Localized $^{1}H[^{13}C]$ NMR Measurement of N-acetyl-aspartate Turnover in Rat Brain

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Introduction

Dynamic ^{13}C NMR spectroscopy combined with ^{13}C label administration has been used to study brain metabolism non-invasively. N-acetyl aspartate (NAA) metabolism has been considered to be slow [1-3] and to have a similar turnover time as glycogen [3]. Most studies employed direct ^{13}C detection to measure ^{13}C label in NAA, with an additional external reference to calculate the isotopic enrichment (IE) [4]. Compared to the direct ^{13}C measurement, ^{1}H $[^{13}C]$ spectroscopy can provide higher sensitivity and spatial resolution for detecting ^{13}C in the acetyl resonance of NAA at 2.01 ppm and allows to directly determine IE. However, the enrichment of the NAA C6 resonance is typically low and overlapped with the more intense Glx C3 resonance (2.04-2.13ppm). The aim of the present study was to measure the NAA C6 turnover rate in vivo in the rat brain using a recently described selective resonance suppression ^{1}H $[^{13}C]$ approach [5].

Materials and Methods

Animal preparation: Five Sprague-Dawley rats were fasted overnight before the experiment. The femoral artery and vein were catheterized for blood sampling (monitoring blood gases and plasma glucose level), as well as glucose and α-chloralose infusion. After giving a bolus of 99%-enriched $[U\text{-}^{13}C_6]$ glucose over 5 min, that doubled plasma glucose concentration, 67% enriched $[U\text{-}^{13}C_6]$ glucose was infused continuously at a stable rate for up to 19h.

NMR spectroscopy: All experiments were carried out on an actively-shielded 9.4T/31cm scanner (Varian/Magnex) using a homebuilt 10mm (^{13}C)/14mm (^{1}H quad) surface coil. Shimming was performed with FASTMAP. The ^{13}C labeling time course of the acetyl group of NAA was measured by ^{13}C-edited and -decoupled ^{1}H NMR spectroscopy with selective resonance suppression using asymmetric adiabatic RF pulses as described previously [5]. A voxel of 75µl was measured and each scan contained 64 averages (~ 8.5 min). At the end of the study the animals were sacrificed by focused microwave irradiation (1.4s) and the brains were prepared for perchloric acid extracts. The brain extracts measurements were performed on a 600 MHz vertical bore spectrometer (Bruker, Fallanden) to verify the ^{13}C IE of NAA C6 at the end of the infusion. The peak area was measured using built-in spectrometer software (VNMRJ) to calculate the ^{13}C IE for the time course.

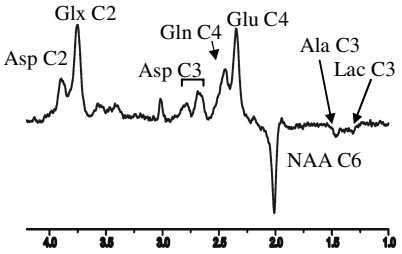

Figure 1. The difference spectrum of the spectra acquired using a resonance suppression ^{1}H $[^{13}C]$ approach during the last five hours of 19 hrs $[U\text{-}^{13}C_6]$ glucose infusion (gf=0.11).

Results and Discussion

In the difference spectrum (Fig. 1), the intensive labeling of the Glx C3 signal was completely suppressed and NAA C6 was directly detected as a resolved resonance. The ^{1}H resonances whose ^{13}C coupling partner is upfield from Glx C3, such as NAA C6, Lac C3 and Ala C3 inverted relative to the resonances whose ^{13}C chemical shift is downfield (Glx C2, C4 and Asp C2, C3). ^{13}C label of Glx C3 was completely suppressed allowing to *directly* observe label in NAA C6 within the first hour of glucose infusion (Fig.2A). NAA C6 labeling signal increased slowly but was observed already at a very low IE (Fig. 2). The NAA C6 ^{13}C IE of the last time point of each subject agreed well with that measured in the brain extract (slope=1.01, R^2=0.93).The turnover time courses of 5 rats show a high reproducibility of the measurements (Fig.2 B). The turnover time course of NAA C6 was fitted using $\frac{^{13}NAA_6(t)}{NAA} = 0.67 - 0.659e^{\left(\frac{-t}{\tau_{NAA}}\right)}$ [4] (Fig.2 C), since brain glucose enrichment was measured in extracts at ~ 67%, resulting in τ_{NAA6} = 34 ± 2.5 h (Mean ± SEM, n=5). Based on a total [NAA] of the rat brain of 8.5 µmol/g [6] the turnover rate of NAA C6 was 0.24 µmol/g/h which was in the range of previously published studies [1, 4].

We conclude that NAA turnover can be measured at very low levels of NAA enrichment by ^{1}H $[^{13}C]$ spectroscopy. The technique used in this study allows to study NAA metabolism in a specific region.

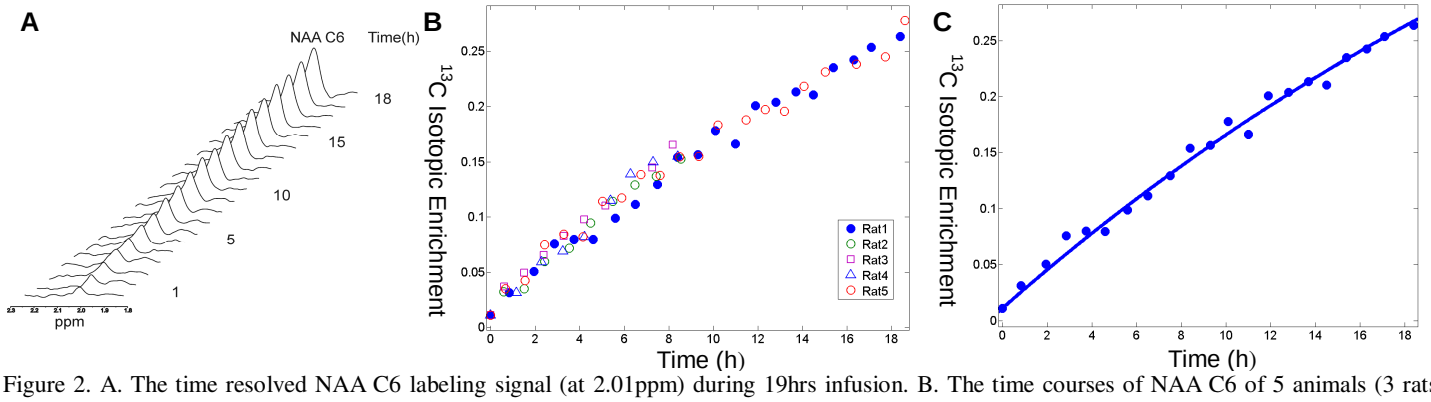

Figure 2. A. The time resolved NAA C6 labeling signal (at 2.01ppm) during 19hrs infusion. B. The time courses of NAA C6 of 5 animals (3 rats were infused for 9hrs and 2 rats were infused for 19hrs). C. The fitting of turnover time course of Rat 1.

References

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Acknowledgements

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