Measuring NAA synthesis *in vivo* using proton MRS

S. Xu[1], J. Yang[1], and J. Shen[1]
[1]Molecular Imaging Branch, NIMH, Bethesda, MD, United States

Introduction

N-acetyl aspartate (NAA) is found in high concentrations (~10 mM) exclusively in the nervous system. It has been used as a neuronal marker in the study of cerebral disorders. Abnormalities in NAA synthesis, transport and/or breakdown may contribute to altered steady-state NAA level [1,2]. Canavan disease, for example, is an NAA metabolic disorder due to *N*-asparto-acylase deficiency [2]. The *in vivo* synthesis rate of NAA (V_{NAA}) has been directly measured using ^{13}C MRS in both rats [3] and humans [2]. Since the *N*-acetyl methyl group of NAA is the major resonance (2.02 ppm) in the ^{1}H MR spectroscopy, a potentially useful approach is to determine NAA synthesis by measuring the ^{13}C satellite signals of NAA in the ^{1}H NMR spectrum. The resonances of the ^{13}C satellite peaks are symmetrically located with respect to the central resonance of the methyl protons attached to ^{12}C. The ratio of the satellite peaks to the total NAA peaks reveals the ^{13}C isotopic enrichment of the acetyl moiety of NAA. In this study, we demonstrate that V_{NAA} can be measured *in vivo* using a localized long echo-time (TE) ^{1}H MRS method.

Methods

Male adult Sprague-Dawley rats (169-217 g, n = 12) were anaesthetized with isoflurane (1.5%) and infused with uniformly ^{13}C-labeled glucose ([U-^{13}C]glucose, 99% enriched, 20% wt/vol, i.v.). Plasma glucose level was maintained at 19.6 ± 1.6 mM during the experiment. All experiments were performed on a Bruker 11.7 T spectrometer interfaced to an 89 mm i.d. vertical-bore magnet. A 15-mm inner diameter ^{1}H surface coil was used for excitation and detection, and was positioned ~0-1 mm posterior to bregma. Adjustment of all first- and second-order shims was accomplished with a fully automatic procedure described previously [4]. The pulse sequence was similar to that used in a previously study [5] but with a longer echo-time (TE = 100ms). A ^{1}H MR spectrum was acquired from a 6 x 3 x 6 mm^3 voxel centered on the midline of the brain with 4096 data points, a spectral width of 4000 Hz , and TR of 3.2 s. For each data block, 440 acquisitions were accumulated over 24 minutes. After acquisition of each data block, a 6 minutes interval was used for re-shimming to maintain B_0 homogeneity over a total experimental duration of 10 hours. Satellite NAA methyl peaks were analyzed using the MATLAB curve-fitting toolbox (The mathWorks, Inc., Natick, MA). To reduce interference from the glutamate methylene group at 2.04 - 2.11 ppm region, the upfield NAA satellite peak at 1.89 ppm was used in the calculation. Because the observed kinetics of ^{13}C label incorporation into the acetyl moiety of NAA was found to be approximately linear within the experimental time frame (*vide infra*) the time course of ^{13}C isotopic enrichment of the NAA methyl group was modeled using a linear equation $^{13}NAA(t)/NAA_0 = 0.011 + V_{NAA} \times t$, where NAA_0 is the total concentration of NAA (10 µmol/g). The NAA synthesis rate V_{NAA} was determined by a least-squares fitting of the measured time course of the ^{13}C isotopic enrichment of NAA to the above equation.

Results

The time course of ^{13}C label incorporation into the *N*-acetyl methyl group of NAA in an individual rat brain is shown in Fig 1. The *in vivo* spectra show high sensitivity and spectral resolution at 11.7 T. The time course of the ^{13}C isotopic enrichments of the *N*-acetyl methyl group of NAA (Fig. 2) was fitted to the linear equation described in Methods and the estimated NAA synthesis rate was V_{NAA}= 0.19 ± 0.02 µmol/g h (mean ± SD, n = 12).

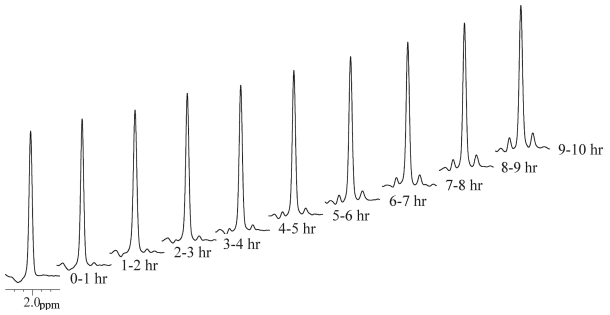

Fig. 1. Detection of dynamic ^{13}C labeling incorporation into the *N*-acetyl methyl group of NAA resonance.

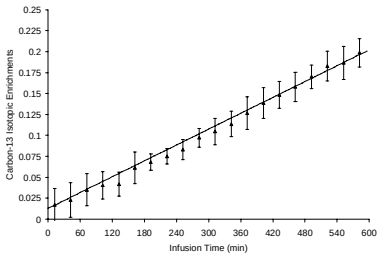

Fig. 2. The ^{13}C labeling time course of the *N*-acetyl methyl group of NAA (mean ± SD, n = 12) .

Discussion

We demonstrated the feasibility of using only the proton channel for *in vivo* detection of the rate of NAA synthesis in the brain, which takes advantage of the high sensitivity of proton detection and spectral resolution of ^{13}C satellite peaks when no heteronuclear decoupling is applied. In addition, since the technique only requires a single channel for excitation and detection, it simplifies the hardware requirement. Compared with a previous report based on *in vivo* direct ^{13}C MRS method (V_{NAA}= 0.7 ± 0.1 µmol/g h) [3], our V_{NAA} value is substantially lower. This may be due to the difference in anesthesia and in methods in quantification of ^{13}C-labeled signals. One advantage of using proton MRS without heteronuclear decoupling is that the total NAA signal remains unchanged and can be easily used as a reliable concentration reference. In contrast, direct ^{13}C MRS methods lack an internal concentration reference and may be prone to quantification errors. We note that our V_{NAA} is close to the low end value found in a study using brain extracts (V_{NAA}= 0.3 - 0.6 µmol/g h [6]. In conclusion, the high sensitivity and easy implementation of our method make it potentially applicable to future clinical studies of human brain disorders with manifestation of abnormality in total NAA concentration.

References

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