Detecting Natural Abundance Carbon Signal of NAA Metabolite Within 12-cm³ Localized Volume of Human Brain Using ¹H-¹³C NMR Spectroscopy

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NMR spectroscopy has been applied extensively to study metabolism noninvasively in the human brain and other tissues. However, it usually suffers from poor signal-to-noise ratio due to low NMR sensitivity and low metabolite concentrations. In this study, the technique of proton-observe-carbon-edited (POCE) NMR spectroscopy combined with a single-shot localization sequence was used to detect the natural abundance carbon signal of the amino acid N-acetyl aspartate from a 12-cm³ localized volume in the occipital lobe of humans at 4 T. The results suggest that NMR spectroscopy is sensitive enough to detect signals from low concentration metabolites (<60 nmol/g) from small volumes in the human brain within several minutes of data acquisition. This reveals that *in vivo* NMR spectroscopy is a promising technique for detecting small metabolite changes and low traces of ¹³C isotopic labeling for dynamic metabolism studies aimed at investigating physiological and pathological questions.

Key words: NMR spectroscopy; spectral editing; NMR sensitivity; N-acetyl aspartate.

INTRODUCTION

Spatially localized multinuclear MRS has been developed and applied to detect metabolites noninvasively in regions of the human body. In view of the predominance of hydrogen and carbon atoms in biological molecules, a significant fraction of such studies have naturally used ¹H and ¹³C nuclei. Studies based on ¹³C MRS take advantage of the ~1% natural abundance and the large chemical shift dispersion to monitor label accumulation in intermediary metabolites after infusion of ¹³C-enriched exogenous substrates (e.g., 1–4). This approach was used, for example, in measuring glutamate C4 turnover during infusion of [1-¹³C] glucose (see reviews in refs. 5 and 6), and the turnover information can be related to the tricarboxylic acid (TCA) cycle rate and thus cerebral oxygen metabolic rate (CMR_{O₂}) (7, 8). This is important for a better understanding of the regulation of energy

metabolism during functional activation in local regions of the human brain, which has been shown to exhibit transient changes of metabolites such as lactate (9), glucose (10, 11), and creatine kinase activity (12) during visual stimulation. The utility and reliability of such an approach in tissues with regional metabolic heterogeneity necessarily depends on the sensitivity for detecting signals from low-concentration metabolites in a small tissue volume. This goal, however, is impeded by the lower sensitivity of the ¹³C nucleus and often low ¹³C fractional enrichment of metabolites. To alleviate this problem, the use of indirect detection of protons bonded to carbon atoms has been used (e.g., refs. 13–17). A 14-fold improvement in sensitivity to detect the CH₃ group signal in the rabbit brain by indirect detection through the ¹H nucleus compared to direct carbon spectroscopy has been reported (18). Therefore, it is conceivable that such sensitivity gains can be combined with increased signal-to-noise ratio (SNR) provided by high magnetic fields to detect low metabolites and investigate regional metabolism in the human brain. In this study, we demonstrated the feasibility to detect the *natural abundance* carbon signals of N-acetyl aspartate (NAA) from a small localized region in the occipital lobe of the human brain using a heteronuclear editing technique.

METHODS

All NMR experiments were performed on a Varian (Palo Alto, CA) console interfaced to a Siemens (Erlangen, Germany) 4T whole-body system equipped with a head-gradient insert. Subjects were placed supine in the magnet. The studies were approved by the institutional review board of the University of Minnesota Medical School. A multinuclear surface-coil probe was designed, consisting of a 10-cm single-loop surface coil with distributed capacitance for ¹H excitation and reception and a 15-cm dual-loop quadrature surface coil for ¹³C spin inversion and decoupling. Electrical decoupling among the coils was achieved by adjustment of the geometrical overlap between the coils. The probe was mounted on an acrylic glass former and was positioned under the occipital lobe of the subjects used in this study. Each coil loop was connected to balanced tune/match circuitry. A similar coil configuration for ¹H-decoupled ¹³C spectroscopy was described previously (19). A 1-cm-diameter sphere containing [¹³C]-formic acid was placed near the center of the probe to calibrate the power needed for ¹³C inversion and decoupling.

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Indirect detection and spatial localization were achieved using a modified POCE pulse sequence (15, 16) combined with a single-shot PRESS technique (20) (Fig. 1). An optimized pulse with improved profile for slice selection (21) was used for 180° refocusing pulses. BISTRO (B_1 -insensitive selective train to obliterate signal) pulse train was used for outer volume suppression (OVS), taking advantage of its high suppression efficiency, low RF power requirement, B_1 insensitivity, and sharper saturation profile (22, 23). It was generated using a series of hyperbolic secant pulses (24) (2-ms pulse width) combined with slice selection gradients. The peak B_1 values for each pulse were varied according to a transposed hyperbolic secant function (22). Spoiling gradients were inserted after each hyperbolic secant pulse to dephase the transverse magnetization. The OVS pulses were applied in three dimensions, except the side of the localized volume perpendicular to the coil axis and distant from the coil. The OVS pulses were optimized to maximize the outer volume signal suppression and minimize the signal loss from inside the localized volume. Water suppression was performed by using three Gaussian-shaped chemical shift selective imaging pulses (25, 26) applied on the water resonance before the PRESS localization. A ^{13}C inversion hard pulse (0.56 ms) centered at $1/(2J_{\text{CH}}) \approx 4$ ms before data acquisition was applied to invert the ^{13}C resonance of the carbon bonded to the edited protons on alternate spectra acquired every 16 scans. Subtractions between the FIDs acquired in the presence and absence of the inversion pulse gives edited spectra that contain signals from protons coupled to ^{13}C nuclei (15). Broadband ^{13}C decoupling was achieved using a WALTZ-16 pulse (27). The ^{13}C inversion and decoupling pulses were optimized for those CH_n groups that resonate between 20 and 30 ppm in the ^{13}C spectrum. The average specific absorption ratio for the entire pulse sequence was below the Food & Drug Administra-

FIG. 1. Schematic presentation of the localized POCE pulse sequence. Outer-volume signal and lipid contamination are eliminated by using the BISTRO outer-volume suppression sequence. The water resonance is suppressed by a chemical shift selective imaging sequence. The PRESS sequence is used for single-shot localization using 90° – 180° – 180° frequency-selective pulses combined with slice selection and dephasing gradients in three dimensions. A ^{13}C hard inversion (IV) pulse centered at $1/(2J)$ before data acquisition is used for heteronuclear editing. A WALTZ-16 sequence is applied for ^{13}C decoupling (Dec.) during the data acquisition (Acq.) period.

tion guideline (<3 W/Kg). A 7- to 9-Hz linewidth of NAA resonance was routinely obtained within a localized volume using localized manual shimming.

The POCE spectra were obtained from a $2.0 \times 2.0 \times 3.0$ cm^3 localized volume centered in the primary visual cortex. Position of the localized volume was guided by multislice T_1 -weighted gradient-echo images that covered the main part of the calcarine fissure as demonstrated in Fig. 2a. A 23-ms TE was used. The total TR was 3.2 s. FIDs were acquired with 2048 data points and a spectral width of 4000 Hz. Spectra were processed with zero-filling and exponential line broadening (LB) before Fourier transformation. A 3-Hz LB was used for conventional localized PRESS spectra and a 5-Hz LB was used for POCE spectra and all SNR comparisons.

RESULTS

In Fig. 2b, a single-scan PRESS spectrum is shown, acquired from a 12-cm^3 localized volume located in the primary visual cortex of a representative subject, as shown in Fig. 2a. This single-scan spectrum shows the excellent sensitivity and superior lipid signal suppression achievable with the pulse sequence shown in Fig. 1. All ^1H visible metabolites were detected in the spectrum. The SNR of the methyl proton peak of NAA at 2 ppm was 125:1 for a single-scan acquisition. Based on this SNR value, it is possible to predict the SNR for the NAA methyl protons attached to natural abundance ^{13}C nuclei that are split into two satellites due to heteronuclear J -modulation. Considering that 448 scans (24-min acquisition time) were acquired and a 1.1% ^{13}C natural abundance, we predict an SNR of 14.7 for either one of the two J -modulated NAA methyl proton satellites. This indicates that the natural abundance ^{13}C signals from NAA should be detectable through indirect ^1H detection using the present pulse sequence (Fig. 1). In Fig. 3, a series of PRESS spectra (16 scans and 0.9-min acquisition time) acquired from an individual subject during the same NMR session are shown. The proton peaks from glutamate C4 (2.36 ppm) and glutamine C4 (2.46 ppm) were well resolved, and the 8-Hz homonuclear J -modulation splitting of the glutamate peak at 2.4 ppm was indicated. The series shows excellent stability with negligible variation among different spectra. This is critical for minimizing subtraction errors in the POCE spectra to detect natural abundance carbon signals of low-concentration metabolites.

In Fig. 4, the comparison between the conventional PRESS spectrum and the POCE spectrum is illustrated, without ^{13}C decoupling acquired from a 12-cm^3 localized volume (448 scans, 24-min acquisition time). Two satellite peaks were assigned to arise from the NAA protons attached to natural abundance ^{13}C carbon (CH_3), based on their positions being centered around the NAA proton peak attached to ^{12}C carbon (2 ppm), and splitting by the frequency difference of 126 Hz, which is typical of heteronuclear J -coupling constants of similar molecular groups (28). The SNR for either one of the two NAA satellites was 13, in excellent agreement with the predicted value of 14.7. The ^{13}C natural abundance enrichment calculated from the signal ratio between ^1H NAA

FIG. 2. (a) Sagittal scout image and the localized volume (covered by $2 \times 2 \text{ cm}^3$ in the box) for acquiring spectra. The localized volume is chosen to cover most of the calcarine fissure in the occipital lobe. (b) Localized ^1H spectrum (LB = 3 Hz) obtained from a single-scan and 12-cm^3 localized volume as shown in (a) without ^{13}C editing. It shows ^1H detectable signals from different metabolites such as NAA, glutamate (Glu), glutamine (Gln), creatine (Cr), and choline (Ch).

peak and the sum of two ^{13}C NAA satellites is 1%, which is close to the theoretical enrichment value of 1.1%. The assignment of these satellite peaks to NAA protons attached to natural abundance carbon is further corroborated in Fig. 5, in which the spectral comparison between the ^{13}C decoupled and nondecoupled POCE spectra is shown (each 224 scans, 12-min acquisition time). When ^{13}C decoupling was applied during the acquisition, the two resonances collapsed to a single peak with double signal amplitude at the normal NAA chemical shift.

DISCUSSION

By taking advantage of the high sensitivity contributed by (a) full NMR sensitivity of PRESS sequence compared to 50% NMR sensitivity of STEAM sequence (29), (b) relatively short TE, (c) approximately linear sensitivity gain for MRS as a function of magnetic field strength (30, 31), (d) excellent stability of our 4-T NMR system and thus minimal subtraction errors, and (e) optimization of sequence parameters, we have successfully used the localized POCE technique to detect the ^{13}C satellite signals of natural abundance NAA metabolite from a 12-cm^3 volume in the visual cortex of the human brain with a hitherto unachieved sensitivity. Assuming an average $10\text{-}\mu\text{mol/g}$ NAA concentration in the human brain (30, 32, 33), the concentration of each ^{13}C satellite from natural abundance NAA is only 56 nmol/g, which is equivalent to $0.67 \mu\text{mol}$ of ^{13}C natural abundance NAA metabolite within a 12-cm^3 localized volume in the occipital lobe. This low metabolite concentration, which is approximately 200 times lower than the concentration of the NAA signal detected by most ^1H MRS, can be reliably measured using the POCE sequence. Furthermore, when considering that ^{13}C decoupling collapses two NAA satellites to a single peak with double intensity, as demonstrated in Fig. 5, the same SNR (SNR = 13:1) of the ^{13}C NAA signal, as shown in Fig. 4, can be obtained

within a 6-min acquisition time when ^{13}C decoupling is applied. These results demonstrate the promising capability of $^1\text{H}\text{-}(^{13}\text{C})$ NMR spectroscopy for detecting signals from significantly low concentrations of ^{13}C -labeled metabolites and/or small metabolic changes in a local brain region. The spectral simplification achieved with heteronuclear editing and sensitivity gains achieved by a combination of the aforementioned factors is critical in resolving and detecting these resonances.

One exciting application of this approach is to measure the regional rate of C4 glutamate isotopic turnover in the visual cortex during a $[1\text{-}^{13}\text{C}]$ glucose infusion and compare the rate differences between resting condition and photic stimulation. The turnover information can be used to determine the changes of TCA rate and CMR_{O_2} during the stimulation for addressing the debated issue about how brains demand and consume energy through oxidative metabolic pathway during neuronal firing (34). The regional CMR_{O_2} changes within a small region of interest can be robustly detected based on the superior sensitivity demonstrated in this work. This makes it possible to differentiate metabolic rates between the gray and white matters, activated and nonactivated

FIG. 3. Stacked plot of localized ^1H spectra (LB = 3 Hz, 16 scans, 51-s acquisition time, and 12-cm^3 localized volume) acquired during the same NMR session showing excellent reproducibility and stability.

areas in the human brain. Compared to other heteronuclear editing techniques (13, 14), the POCE technique may provide a better choice to achieve a short TE and a better sensitivity to detect carbon signals. In addition, the POCE sequence also provides a direct measurement of ^{13}C fractional enrichment by comparing the intensities of ^{12}C and ^{13}C peaks, as the example shown in Fig. 4. All other metabolite signals preserved in the original ^1H spectra can be recovered by summing the spectra in the presence and absence of ^{13}C inversion. This makes it possible to use an internal metabolite signal with known concentration such as creatine for quantifying the concentrations of ^{13}C -labeled compounds. All of the information about the fractional enrichment and quantification of ^{13}C -labeled metabolite is important for modeling and calculating TCA rate and CMR_{O_2} .

CONCLUSIONS

The technique of POCE NMR spectroscopy combined with a single-shot localization has been used successfully to detect the natural abundance carbon signal of the amino acid NAA in the occipital lobe of the human brain. It has been demonstrated experimentally that ^1H MRS with ^{13}C -based editing at 4 T is sufficiently sensitive to detect metabolites with low intracellular concentrations (56 nmol/g) from a small localized volume (12 cm^3) in the human brain. These results reveal the promising capability of MRS for detecting and differentiating regional metabolism and metabolic alterations, which should increase our understanding of the regulation of brain energy metabolism during focal activation.

FIG. 4. (a) Unedited localized ^1H - $\{^{13}\text{C}\}$ spectrum showing ^1H signals from NAA, glutamate (Glu) and other metabolites. (b) Edited localized ^1H - $\{^{13}\text{C}\}$ spectrum showing two ^{13}C satellites from natural abundance NAA that are split by 126 Hz. The spectra were obtained from a 12-cm^3 localized volume in the visual cortex and 24 min of data acquisition (448 scans). A 5-Hz exponential LB was used before Fourier transformation.

FIG. 5. (a) Edited localized ^1H - $\{^{13}\text{C}\}$ spectra in the absence of ^{13}C decoupling showing two ^{13}C satellites from natural abundance NAA that are split by a 126-Hz J -coupling constant. (b) Edited localized ^1H - $\{^{13}\text{C}\}$ spectra in the presence of ^{13}C decoupling showing a single peak of the collapsed carbon signal from natural abundance NAA. The spectra in both (a) and (b) were obtained from a 12-cm^3 localized volume in the visual cortex and 12 min of data acquisition (224 scans). A 5-Hz exponential LB was used before Fourier transformation.

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