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Localized ^{13}C NMR Spectroscopy of *myo*-Inositol in the Human Brain *in Vivo*

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Natural abundance ^{13}C NMR spectra obtained from 144-cm^3 volumes in the human brain contained well-resolved resonances of *myo*-inositol after 60 min of data accumulation. A mean concentration of 7.2 ± 0.5 μmol/g (±SE, $n = 7$) was calculated from the comparison with phantoms. ^{13}C NMR spectroscopy thus provides a complementary role in the quantitation of metabolites also observed in the crowded ^1H spectrum. © 1992 Academic Press, Inc.

INTRODUCTION

The large chemical-shift dispersion of the ^{13}C nucleus makes *in vivo* ^{13}C NMR potentially useful for quantitative measurements of metabolites that are difficult to resolve in the ^1H spectrum. However, due to the inherently low sensitivity of the ^{13}C nucleus and its low natural abundance of 1.1%, *in vivo* ^{13}C NMR of humans has been limited to observations of highly concentrated compounds, such as lipids in adipose tissue (*1, 2*), glycogen in liver (*3*) and muscle (*4*), and citrate in the prostate (*5*). The glycogen C1 peak is well resolved (*3, 4*) and has been the subject of many human studies performed in this and other laboratories.

Nonlocalized ^{13}C spectra from the human head are dominated by lipid signals that originate in the scalp (*6, 7*) as are ^{13}C spectra obtained from most other tissues (*8*). The application of ^{13}C NMR to the human head had been limited due to an estimated detection limit of 0.4 to 0.6 m*M* in ^{13}C concentration (*7*). Recently, ^{13}C NMR spectra of the human head obtained during intravenous infusions of enriched [1-^{13}C]glucose have been reported (*9, 10*). Signals from metabolites other than glucose were observed in unlocalized spectra only after the fractional enrichment of glucose in the infusate was raised to 99% (*9*). We have performed localized quantitative measurements of D-[1-^{13}C]glucose concentrations in the human brain at 2.1 T using adiabatic pulses and nuclear Overhauser enhancement (NOE) with enhanced sensitivity. In addition, shimming with all first and second order shim coils resulted in narrow lines and further sensitivity improvements (*10*). Results obtained during that study indicated an improved detection limit of approximately 0.1 m*M* in ^{13}C concentration after 60 min of data accumulation. This corresponds to 10 m*M* natural abundance which is approximately the concentrations reported for *N*-acetyl-aspartate, glutamate, creatine, and *myo*-inositol in the human brain (*11*). The present study demonstrates that the

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signals from *myo*-inositol can in fact be observed and quantified with localized natural abundance ^{13}C NMR in the human brain *in vivo*.

METHODS

Five healthy adults (aged 27–39 years) were studied after written informed consent was obtained from the subjects using forms and procedures approved by the Yale Human Investigations Committee.

The subjects lay supine in a 2.1-T whole-body magnet (ORS-Bruker, Billerica, MA) on a double surface coil consisting of a 7-cm diameter ^{13}C coil and a concentric 14-cm ^{1}H coil displaced by 1 cm relative to the carbon coil. A volume of 144 ml ($6 \times 4 \times 6$ cm^3) was localized within the occipitoparietal region of the brain that excluded major blood vessels and the ventricles. Localization was based on inversion-recovery ^{1}H magnetic resonance images obtained as gradient-recalled echoes (TR = 2500 ms, TI = 800 ms, TE = 14 ms) in three transverse and one sagittal plane.

The radiofrequency (RF) power was adjusted on a sealed sphere (2 cm diameter) at the coil center containing an aqueous solution of ^{13}C formic acid as follows: The power for the ^{13}C coil was adjusted with a 180° pulse of 150 μs duration by minimizing the upfield signal from the formate sphere. The ^{1}H decoupling power was set for a 900-μs 90° pulse by minimizing the ^{13}C NMR signal of the sphere by adjusting the θ pulse power in the sequence 90°(^{13}C)–1/2J–θ(^{1}H)–acquire(^{13}C) (*12*). The 90° pulse duration used for decoupling was then set to 1.2 ms. Adjusting RF power rather than pulse durations had the advantage that the decoupler and transmitter RF field strength distribution in space did not vary from study to study. Localized shimming of all first and second order shim coils was achieved with an adaptation of a previously reported procedure (*13*).

The spectral localization sequence which is based on the ISIS technique (*14*) is shown in Fig. 1. Hyperbolic secant pulses (*15*) of 8 ms duration were used for magnetization inversion with a 2-kHz bandwidth. A 5-ms adiabatic half passage sin/cos pulse with a numerically optimized modulation function was used for excitation. For a numerical simulation of the corresponding pulse profiles, see Ref. (*16*). In order to minimize the z magnetization and to ensure proper localization, the pulse used for excitation was applied again at the end of the acquisition (*17*). The spectrometer base frequency was set to 73 ppm and the excitation pulses were offset 100 Hz upfield to 69.5 ppm. During the signal acquisition time T_{aq} of 102 ms, WALTZ-16 proton broadband decoupling (*18*) was used with a power of at most 13 W. The signal intensity of *myo*-inositol was increased with an NOE generated by 1 W of continuous wave irradiation at 3.7 ppm in the ^{1}H spectrum during the recovery delay T_{rec} of 1.5 s. The maximum tissue RF power deposition was calculated to be below 2 W/kg using a magnetic vector potential model (*19*).

Spectra were zero-filled to 819 ms, multiplied with an exponential function corresponding to 5 Hz line broadening, Fourier transformed, and phase corrected using a zero-order phase correction term only.

RESULTS

A region from an unlocalized ^{13}C NMR spectrum of a human head is shown in Fig. 2. The spectrum is dominated by the glycerol backbone signals from scalp fat,

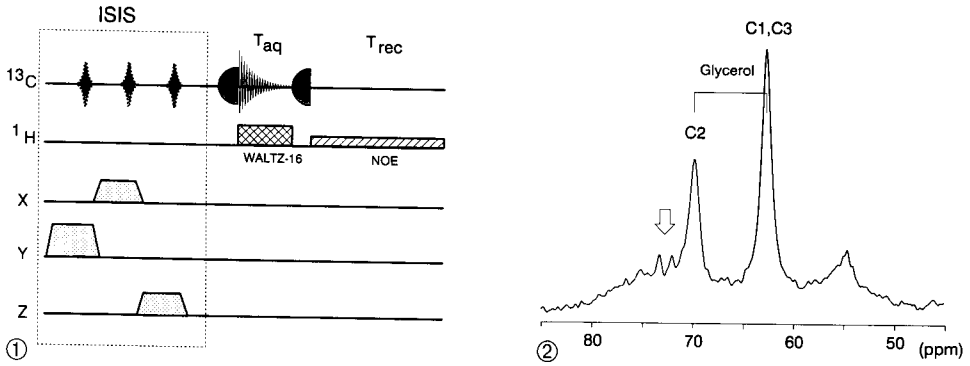

FIG. 1. Pulse sequence. The localization of a 6 × 4 × 6-cm^3 volume was achieved using ISIS (*14*). The inversion pulses are indicated by broken lines. The pulse used for excitation was applied again at the end of the acquisition to ensure minimal z magnetization. Chemical-shift displacement errors were minimized for the resonances of *myo*-inositol by setting the spectrometer base frequency at 73 ppm. WALTZ-16 proton decoupling was applied during the acquisition time T_{aq} and an NOE was generated by ^{1}H continuous wave irradiation during the recovery delay T_{rec}.

FIG. 2. *myo*-Inositol region of an unlocalized spectrum from the human head. The spectrum was acquired from a human head in 30 min (1024 averages) using the pulse sequence in Fig. 1 by omitting the ISIS part (repetition time TR = 1.7 s). Processing consisted of exponential multiplication corresponding to 5 Hz line broadening and zero-filling prior to Fourier transformation. The large resonances of glycerol at 69.7 ppm (C2) and at 62.5 ppm (C1, C3) dominate the unlocalized spectrum in this region. The arrow indicates the *myo*-inositol peaks at the downfield side of the glycerol C2 peak.

but the *myo*-inositol peaks are observable downfield from the glycerol C2 peak (arrow in Fig. 2). Figure 3A shows a localized spectrum obtained in 90 min from a 144-cm^3 volume using the sequence of Fig. 1. The sensitivity in this measurement was approximately twice that in the other six measurements due to an increase of approximately 20 dB in ^{13}C preamplifier gain, which improved the system noise figure to 2 dB. The improvement was also confirmed on the formate signal. The absence of the glycerol C2 peak at 69.7 ppm in this and the other *in vivo* spectra indicates localization of the *myo*-inositol signals to the brain. The chemical-shift error was minimized for this spectral region by placing the spectrometer base frequency at 73 ppm in the carbon spectrum. The *in vivo* signals of *myo*-inositol are superimposed on a weak background signal with an asymmetric shape covering the range from 10 to 80 ppm. The spectral region is otherwise free from detectable resonances of other compounds, in agreement with natural abundance ^{13}C spectra from excised brain (*20*).

The *in vivo* resonances were assigned by comparison with a localized ^{13}C spectrum of a bottle containing an aqueous solution of 50 m*M* *myo*-inositol (Sigma Chemical Co., St. Louis), shown in Fig. 3B. The spectrum of *myo*-inositol contains two degenerate carbon resonances, one at 73.3 ppm (C1 and C3) and the other at 72.0 ppm (C4 and C6), that were always visible *in vivo* after 30–60 min of data accumulation, as well as a single carbon resonance at 75.3 ppm (C5). The remaining carbon (C2) resonates at 73.1 ppm which is 0.2 ppm, i.e., 4 Hz upfield from the C1/C3 peak. The C2 peak could not be resolved despite an experimental linewidth of 2–3 Hz at half height, but broadened the C1/C3 resonance relative to that of C4/C6 (Fig. 3B). Spectral resolution

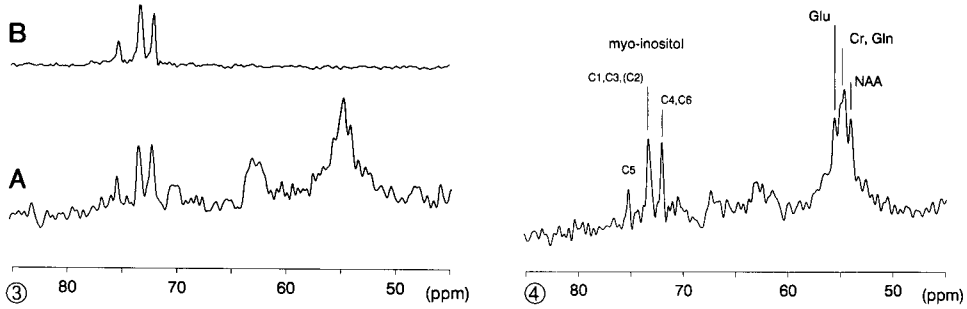

FIG. 3. Expansion from localized ^{13}C NMR spectra of *myo*-inositol. The spectra were obtained from 144-cm^3 volumes using the pulse sequence in Fig. 1. (A) In 90 min, 3072 averages were collected from a human brain *in vivo;* (B) 1024 averages were collected from a bottle containing an aqueous solution with 50 m*M* *myo*-inositol (45 m*M* phosphate buffer, pH = 7.1, 2 m*M* NaN_3, 295 K). Processing consisted of 5 Hz exponential multiplication, zero-filling, FT, and zero-order phase correction. No baseline correction was applied. The *in vivo* spectrum (A) was obtained after substantial improvements in spectrometer sensitivity were achieved by improving the carbon preamplifier gain.

FIG. 4. Sum of six localized *in vivo* measurements. This spectrum shows the glycerol region from the sum of six *in vivo* spectra (corresponding to 17,408 averages, excluding that in Fig. 3A, which was obtained after spectrometer sensitivity improvements) processed with 2 Hz line broadening prior to FT. Resonance assignments are according to previously reported chemical shifts and intensities (*20*): C5 of *myo*-inositol at 75.3 ppm, C1 and C3 at 73.3 ppm (plus C2 at 73.1 ppm broadening this peak slightly), and C4 and C6 at 72.0 ppm. In addition to *myo*-inositol we tentatively assigned C2 of glutamate at 55.6 ppm, C2 of glutamine and C4 of creatine at 55.0 and 54.7 ppm, respectively, as well as C2 from *N*-acetyl-aspartate at 54.0 ppm (*20*).

was limited by the acquisition time of 102 ms as confirmed by prolonging the acquisition time to 408 ms in phantom measurements which showed the C2 resonance at 73.1 ppm clearly resolved from the C1/C3 resonance at 73.3 ppm (data not shown).

In addition to the signals from *myo*-inositol, several resonances were observed in the region from 53 to 56 ppm (Fig. 4). On the basis of previously reported chemical shifts and contents of excised brain (*20*), we tentatively assign these resonances to the C2 of glutamate, glutamine, *N*-acetyl-aspartate, and the C4 of creatine. Solution measurements indicate that the spectral resolution can be potentially improved for this region by increasing the acquisition time.

In order to estimate the *myo*-inositol concentrations, *in vivo* spectra were baseline corrected with a second order polynomial and integrated from 71 to 74 ppm. The integral values were compared to those from a bottle containing a 50 m*M* solution of *myo*-inositol (Sigma Chemical Co., St. Louis) in 45 m*M* phosphate-buffered saline (30 m*M* Na_2HPO_4, 15 m*M* NaH_2PO_4, pH = 7.1, 2 m*M* NaN_3, 295 K) measured on the same day at the same position relative to the surface coil using identical procedures. The differential effect of loading on signal strength was determined according to previously described procedures (*15*), by integrating the upfield peak of the formate doublet in separate, fully relaxed spectra. Brain *myo*-inositol signals corresponded to a mean concentration of 7.2 ± 0.5 μmol/ml brain volume (mean ± standard error, $n = 7$) as calculated from seven such measurements in five healthy volunteers aged between 27 and 39 years. Since the intensity of *myo*-inositol in solution spectra did

not change when the repetition time was prolonged from 1.7 to 5.2 s, we considered effects of the carbon T_1 to be negligible. The effect of NOE buildup on the signal was assessed *in vivo* on different days in two volunteers by comparing unlocalized spectra obtained without NOE generation (TR = 3.2 s) with spectra acquired with the *in vivo* acquisition parameters including NOE (TR = 1.7 s). The results were consistent within 20% with the assumption of an identical NOE enhancement factor of 2.0 *in vivo* and in solution. Prolonging T_{rec} (Fig. 1) to 5 s resulted in the maximal enhancement factor of 3.0 in solution measurements.

DISCUSSION

This study reports the detection and quantitation of natural abundance ^{13}C NMR signals from *myo*-inositol in the human brain *in vivo*. *myo*-Inositol concentrations have been reported to change slowly upon autolysis and to be evenly distributed within the mammalian brain, with species-specific differences (*21*). The *in vivo* concentration in the occipital lobe can therefore be compared to extracts from the human brain. *In vitro* studies on brain biopsy (*11*) or postmortem tissue (*22*) report between 6 and 7 μmol/g for an age range comparable to this study. One study reports a concentration of 7 μmol/g in 27 brain samples that were a mixture of gray and white matter obtained from the temporal lobe during surgery (*11*). Assuming for brain tissue a specific gravity of 1 g/ml, there is good agreement between the reported chemical measurements and the *in vivo* ^{13}C NMR value of 7.2 μmol/ml, which supports the reliability of the ^{13}C NMR quantitation procedure. The same quantitation procedure has been previously used to determine [1-^{13}C]glucose concentrations in the human brain during infusion of enriched [1-^{13}C]glucose (*10*).

The single carbon *myo*-inositol resonance at 75.3 ppm represents the *in vivo* signal corresponding to a natural abundance concentration of 7 μmol/g detected from 144-cm^3 volumes with a signal-to-noise ratio of 7–8 in 90 min (Fig. 3A). This represents a signal-to-noise ratio of 0.8 $(\mathrm{m}M)^{-1}$ $(\mathrm{h})^{-1/2}$. This sensitivity was achieved in the seventh measurement, shown in Fig. 3A, after the carbon preamplifier gain was increased, resulting in a twofold signal-to-noise improvement. The sum of the six earlier measurements acquired prior to this improvement is shown in Fig. 4. With anticipated improvements in insertion loss, preamplifier gain, and, possibly, coil design, this represents a lower bound in the achievable sensitivity at 2.1 T using the present technique. Measurements might be improved at higher fields or with polarization transfer techniques.

The peaks from glutamate, glutamine, *N*-acetyl-aspartate, and creatine which are present at similar concentrations (*11*) should be observable with the similar signal-to-noise ratios as C5 at 75.3 ppm in Fig. 3A. In ^1H spectra of the human brain, peaks from these metabolites have been observed at a much higher spatial resolution which is not surprising considering the higher abundance and gyromagnetic ratio of the proton. However, the quantitation of metabolite signals in ^1H spectra of the human brain is complicated by several problems, including inadequate suppression of the intense water signal which can cause baseline distortions or even spurious signals in the spectral region of metabolite signals, localization methods involving T_2 and diffusion weighting, and extensive spectral overlap of strongly coupled resonances (*23*). For example, the *myo*-inositol peak at 3.55 ppm in the ^1H spectrum is close to the

large H_2O resonance at 4.73 ppm and overlaps at 2.1 T with that of glycine at 3.57 ppm, whose concentration is on the order of 1 μmol/g (*11, 21*). In contrast, there are no large resonances to suppress in the brain ^{13}C NMR spectrum, the localization can be performed with minimal T_2 and diffusion weighting, and several well-resolved singlet resonances can generally be observed for each metabolite in broadband decoupled ^{13}C spectra of brain (*20*) due to the large chemical-shift dispersion, even at *in vivo* linewidths as demonstrated here for *myo*-inositol.

Evidence that the method can be extended to other metabolites, e.g., creatine, glutamate, glutamine, and *N*-acetyl-aspartate, is provided by the observation of the respective resonances in the region from 53 to 56 ppm (Fig. 4). It should in particular be possible to resolve the resonances of glutamate and glutamine in the 30–35 ppm region where the resonances are separated by 3.1 ppm, if the chemical-shift displacement error is eliminated for that spectral region, e.g., by setting the spectrometer base frequency to that region. However, simultaneous quantitation of peaks is confined to 10 ppm wide regions of the spectrum when using 5 mT/m gradient strengths, due to the large chemical-shift displacement error of 0.42 mm/ppm. Nevertheless, the elimination of nearby resonating signals from scalp lipids, such as the C2 peak of glycerol backbone in this study can be used as an indicator of accurate localization of metabolite signals to the brain. For quantitation purposes, the comparison with the phantom measurement will then automatically correct for this shift in displacement, when using surface coils.

We conclude that natural abundance ^{13}C NMR has the potential to provide quantitative measurements of metabolites in the human brain. The high resolution of the ^{13}C NMR spectrum should aid in the interpretation of the highly sensitive but poorly resolved ^{1}H NMR spectrum. ^{13}C NMR may even become the method of choice for measuring metabolite concentrations in those diseases where regional differences are of minor importance. For instance, the polyol *myo*-inositol may have a role in regulation of intracellular water balance in the brain (*24, 25*) and is the precursor of inositol phosphates which have important regulatory functions. Alterations of *myo*-inositol levels may be important in the development of cerebral edema. Considering the good agreement between ^{13}C NMR and the few *myo*-inositol concentration measurements which have been reported so far for the human brain by chemical methods (*11, 22*), the *in vivo* measurement by ^{13}C NMR may potentially permit a better understanding of the functional role of *myo*-inositol in normal and pathological conditions of the human brain.

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