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^{1}H-[^{13}C] NMR measurements of [4-^{13}C]glutamate turnover in human brain

(glucose metabolism/amino acid metabolism/*in vivo* NMR/tricarboxylic acid cycle)

D. L. ROTHMAN*, E. J. NOVOTNY†, G. I. SHULMAN*, A. M. HOWSEMAN‡, O. A. C. PETROFF§, G. MASON‡, T. NIXON‡, C. C. HANSTOCK¶, J. W. PRICHARD§, AND R. G. SHULMAN‡

Departments of *Internal Medicine, †Pediatrics, ‡Molecular Biophysics and Biochemistry, and §Neurology, Yale University School of Medicine, New Haven, CT 06510; and ¶Department of Chemistry and Biochemistry, James Cook University, Townsville, Australia

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ABSTRACT **A limitation of previous methods for studying human brain glucose metabolism, such as positron emission tomography, is that metabolic steps beyond glucose uptake cannot be studied. Nuclear magnetic resonance (NMR) has the advantage of allowing the nondestructive measurement of ^{13}C distribution in specific carbon positions of metabolites. In this study ^{1}H-[^{13}C] NMR spectroscopy in conjunction with volume localization was used to measure the rate of incorporation of ^{13}C isotope from infused enriched [1-^{13}C]glucose to human brain [4-^{13}C]glutamate. In three studies C4 glutamate turnover time constants of 25, 20, and 17 min were measured in a 21-cm^3 volume centered in the region of the visual cortex. Based on an analysis of spectrometer sensitivity the spatial resolution of the method can be improved to <4 cm^3. In conjunction with metabolic modeling and other NMR measurements this method can provide a measure of regional rates of the brain tricarboxylic acid cycle and other metabolic pathways.**

Glucose is the primary carbon source for brain energy production and amino acid synthesis (1). Methods such as positron emission tomography (PET) have been used to assess regional brain glucose uptake in humans from measurements of the distribution of radioactivity after infusion of isotopically labeled glucose or glucose analogues (2, 3). Limitations of methods using glucose analogues are that they may not reflect actual glucose metabolism and cannot provide specific information about fluxes through enzymes. Methods using radioactively labeled glucose cannot distinguish the signal from glucose and glucose metabolites. Nuclear magnetic resonance (NMR) potentially can overcome these limitations since it can noninvasively measure the distribution of ^{13}C, a stable isotope, in specific carbon positions of metabolites. Previous *in vivo* studies have used ^{13}C NMR to determine the incorporation of ^{13}C into metabolites during infusion of ^{13}C-enriched glucose in living animal brains (4) and in spectra of the human head (5). A limitation of the ^{13}C NMR method is poor spatial resolution due to low sensitivity. To improve the sensitivity of NMR detection of ^{13}C isotope, we have developed methods for the indirect detection of ^{13}C enrichment by ^{1}H-[^{13}C] NMR, thereby taking advantage of the high sensitivity of ^{1}H NMR. This method has been used to measure the rate of conversion of [1-^{13}C]glucose to the C4 and C3 carbons of glutamate (6, 7) in the rat brain. The sensitivity of this method for glutamate detection is theoretically 8-fold greater than direct ^{13}C NMR and this enhancement has been demonstrated in animal experiments (8). The glutamate ^{13}C enrichment data obtained with ^{1}H-[^{13}C] NMR from the rat brain have been analyzed by metabolic modeling to calculate the citrate synthase flux in the tricarboxylic acid (TCA) cycle and the rate of exchange between mitochondrial α-ketoglutarate and cytosolic glutamate (9). We present here the results of studies in which ^{1}H-[^{13}C] NMR was used in combination with spectroscopic localization to measure the turnover of [4-^{13}C]glutamate in normal human brain during a [1-^{13}C]glucose infusion. Preliminary results of this work have been presented elsewhere.‖**

METHODS

NMR Spectroscopy. NMR spectra were obtained with a 2.1-T, 1-m-bore magnet (Oxford Magnet Technologies, Oxford, U.K.) equipped with a Biospec I spectrometer (Bruker Instruments, Billerica, MA). Subjects were placed in the magnet supine. A 6-cm (diameter) distributed capacitance circular surface coil tuned to protons at 89.43 MHz was used for transmission and reception. A concentric circular 15-cm (diameter) ^{13}C surface coil tuned to 22.49 MHz was used for ^{13}C spin inversion and decoupling. The coils were positioned directly under the occiput with the subject laying supine in the magnet. In the spectroscopy signal was obtained from a $2 \times 3.5 \times 3$ cm^3 centered in the region of the visual cortex. Multislice sagittal and axial images of the brain were obtained by an inversion recovery gradient echo sequence. A 2-cm (diameter) sphere containing [^{13}C]formic acid was placed at coil center to calibrate the ^{13}C pulse angle. The performance of the pulse sequence was tested on a mold of a human head filled with 50 mM KCl and containing a 200-ml bottle of [2-^{13}C]acetate positioned directly above the coil. The spectrometer noise figure was measured by the two-temperature 50-ohm resistor method (10). Coil efficiency was determined by comparing the loaded and unloaded Q (10) as measured on a Hewlett Packard network analyzer.

Experimental Protocol. Studies involving glucose infusion were performed on two volunteers, both male, ages 42 and 55 years. One subject was studied three times. Both gave their consent according to procedures approved by the Yale Human Investigation Committee. After 30 min of baseline spectral acquisition, plasma glucose was rapidly raised from ≈5 mmol/liter to between 11 and 14 mmol/liter and maintained for 40–60 min at that level with an infusion of 40 g of 99% [1-^{13}C]glucose in a 20% (wt/vol) solution in the left

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Abbreviations: TCA, tricarboxylic acid; PET, positron emission tomography.

‖Rothman, D. L., Novotny, E. J., Howseman, A., Lantos, G., Petroff, O. A. C., Hanstock, C. C., Prichard, J. W. Shulman, G. I. & Shulman, R. G., Society Magnetic Resonance in Medicine 9th Annual Meeting, Aug. 12–18, 1989, Amsterdam, p. 1060 (abstr.).

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antecubital vein. Blood samples were obtained every 5 min during and after the infusion to determine plasma glucose concentration and ^{13}C fractional enrichment.

Analytical Measurements. Plasma glucose concentration was determined using a Beckman glucose analyzer. Glucose fractional enrichment was determined by gas chromatography/mass spectrometry of the pentacetate derivatives of plasma glucose after deproteinization and deionization (11) and by high-field (500 MHz) ^1H NMR in which the ^{13}C side bands determined the percentage enrichment.

Calculations. An estimate of the C4 glutamate exponential time constant for achieving ^{13}C isotopic equilibrium (turnover time) was obtained from the NMR data using a simplified version of the model of Mason *et al.* (9) that has been established in the rat brain. The model neglects the small glycolytic intermediate pools and models the flow of label from [1-^{13}C]glucose to C4 glutamate as a three-step reaction: C1 glucose, C3 lactate, C4 glutamate. Differential equations describing the inflow and outflow of mass and isotope were written for the indicated carbon positions. The differential equations were solved numerically to obtain a least squares fit to the [4-^{13}C]glutamate time course (9). Data input into the model was the enrichment of brain glucose, which was assumed to be the same as measured in plasma glucose, the concentration of brain lactate, and the measured C4 glutamate ^{13}C fractional enrichment. [4-^{13}C]Glutamate NMR signal intensity was converted to fractional enrichment by setting the intensity at the asymptote of the incorporation curve (see below) to one-half the [1-^{13}C]glucose fractional enrichment. A concentration of 0.6 mM was assumed for lactate based on previous NMR measurements (12).

Pulse Sequence. Volume selection was achieved by the 3d image-selected *in vivo* spectroscopy technique (13) using 8-msec phase swept hyperbolic secant pulses (14) (u = 5; bandwidth, 2000 Hz; truncation, 1%). Three-dimensional outer volume suppression was achieved by a noise pulse in z (15), a five-lobe sinc pulse applied to the sample surface in a y gradient (16), and a surface spoiler gradient during the spin echo delay in y (17), which was the axis perpendicular to the coil plane, as well as by the surface coil B_1 profile. The acquisition sequence consisted of a five-lobe sinc in an x gradient for excitation and a semiselective 22 pulse (18) followed by dephasing gradients for water suppression and refocusing. A 1-msec ^{13}C inversion pulse centered at 4 msec prior to acquisition $(2J_{^1H\text{-}^{13}C})^{-1}$ was applied to the [4-^{13}C]-glutamate resonance on alternate scans (19). Free induction decays (FIDs) acquired with the inversion pulse were subtracted from FIDs acquired with a 90_x90_{-x} ^{13}C pulse to obtain the edited spectrum, which contains signal from protons coupled to ^{13}C nuclei. Additional water suppression was obtained with a 30-msec three-lobe sinc pulse applied at the H_2O resonance frequency. ^{13}C decoupling at 15 W was applied during the 200-msec acquisition using either continuous wave (CW) or composite pulse (20) decoupling. The total echo time was 16 msec and repetition time was 2.8 sec. Localized shimming with the outer volume suppression sequence yielded *N*-acetylaspartate linewidths of 6–7 Hz.

RESULTS

To obtain a high signal-to-noise ratio ^1H NMR spectra from cerebral glutamate it was necessary to use short-spin echo times (TE < 20 msec) to prevent loss of intensity due to dephasing from homonuclear couplings (16). Fig. 1 shows an unedited ^1H-^{13}C NMR spectrum from a human subject obtained prior to [1-^{13}C]glucose infusion. In a previous study we have shown that the intensity of the 2.29-ppm resonance is primarily from C4 glutamate (16).

Fig. 2 shows 12-min ^1H-[^{13}C] edited spectra obtained before and during a [1-^{13}C]glucose infusion. No resonances were observed in the C4 and C3 glutamate region (2.5–2.0 ppm) prior to infusion as illustrated by the control spectrum. In some edited spectra during infusion there was contamination from lipid signals at 1.6 ppm due to subject motion and Bo field shifts during blood sampling. The resonance that appears during infusion centered at 2.29 ppm is assigned to [4-^{13}C]glutamate with possible contributions from [3-^{13}C]glutamate and [4-^{13}C]glutamine. The C4 glutamate resonance appears as a partial doublet due to incomplete collapse (Jr = 18 Hz) of the ^1H-^{13}C J coupling by the CW decoupling field.

Fig. 3 shows the time courses of three experiments. A fourth, which is not shown, was performed with a slower

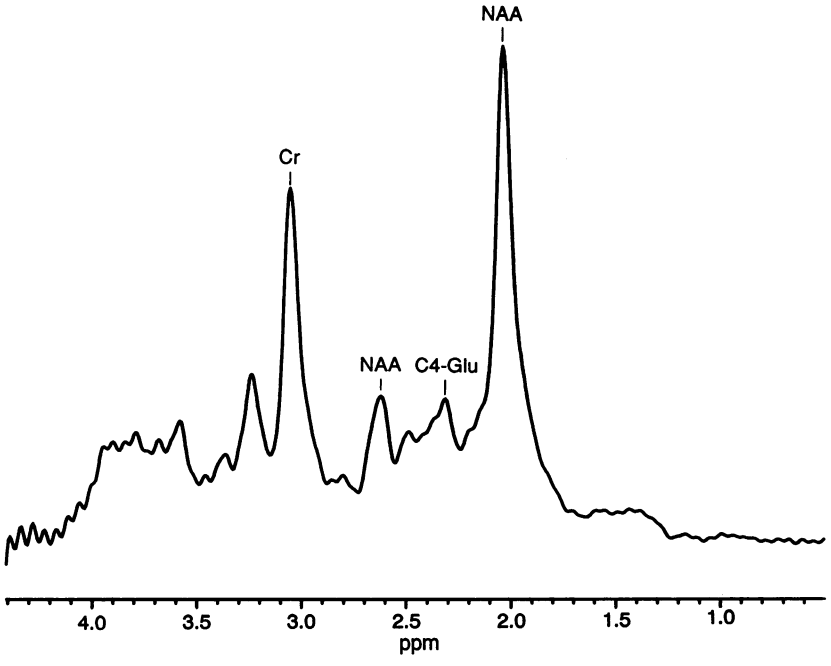

FIG. 1. Unedited ^1H-[^{13}C] NMR spectrum of human brain. Assigned resonances (16) are the acetyl and aspartyl group of *N*-acetylaspartate (NAA), the C4 resonance of glutamate (C4-Glu), and the methyl resonance of creatine (Cr). The spectrum was obtained from 3 min of data acquisition that were apodized with a 2-Hz exponential function prior to Fourier transformation.

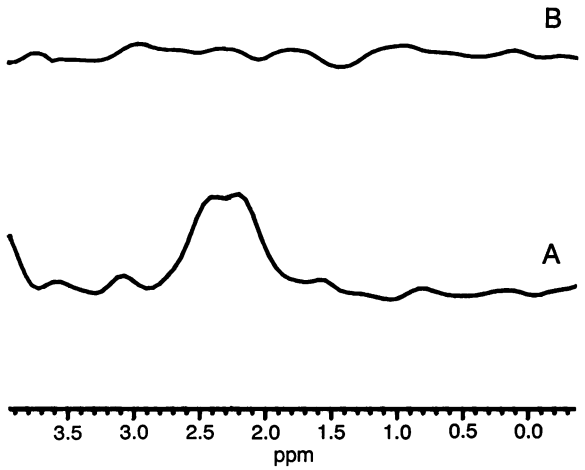

FIG. 2. *In vivo* ^{13}C edited glutamate spectra. Twelve-minute ^{1}H-[^{13}C] difference spectra obtained before (control, B) and after (time = 60 min, A) 1-^{13}C glucose infusion are shown. The time domain data were apodized with 10-Hz exponential broadening for optimal sensitivity. The resonance at 2.29 ppm in the 60-min spectrum corresponds to [4-^{13}C]glutamate. No resonances are observed in the control spectrum prior to infusion.

glucose infusion rate. The open circles show the time course of [1-^{13}C]glucose enrichment. The solid circles show the measured resonance amplitudes of [4-^{13}C]glutamate with the maximum amplitude set to 10 units. In two of the studies shown, the experiment lasted long enough for the [4-^{13}C]-glutamate concentration to reach an apparent steady-state value.

The NMR data from the three experiments with a rapid glucose infusion were analyzed to estimate the C4 glutamate turnover time constant. Two of these experiments were performed on the same subject, from which time constants of 20 and 17 minutes were obtained. On the second subject turnover time of 25 min was obtained, giving a three-measurement average of 21 min.

Potential sensitivity improvements (see ref. 8) were calculated from measurements of probe and spectrometer sensitivity relative to the ideal case, solution measurements of the ^{13}C inversion, and glutamate *J* modulation and the residual ^{1}H-^{13}C *J* coupling in the *in vivo* spectrum. As shown in Table 1, a 5-fold signal-to-noise improvement from the same brain volume as for the experiment in Fig. 2 is achievable from spectroscopic and electronic improvements, allowing the present sensitivity to be achieved with a 4-cm^3 spatial resolution. Improved localization, water suppression, and blood sampling should eliminate baseline stability problems. Use of either [1,6-^{13}C]glucose or [U-^{13}C]glucose precursor would provide an additional factor of 2 increase in sensitivity.

DISCUSSION

The results of the study indicate that ^{1}H-[^{13}C] NMR can be used to measure the incorporation of ^{13}C label into C4 glutamate in human brain. The results are consistent with an isotopic turnover time for the C4 glutamate pool of ≈21 min (range, 17–25 min). This time constant is a maximum estimate since the delay in precursor enrichment due to the brain glucose pool was not taken into account and the resonance in the edited ^{1}H-[^{13}C] spectrum contains contributions from C4 glutamine and C3 glutamate, which, based on animal data, have slower turnover times (9, 21).

The C4 glutamate turnover time can be used to calculate the rate of the TCA cycle if it is assumed that the observed glutamate pool is in fast equilibrium with α-ketoglutarate (9), as has been shown in the rat (9). If a glutamate concentration

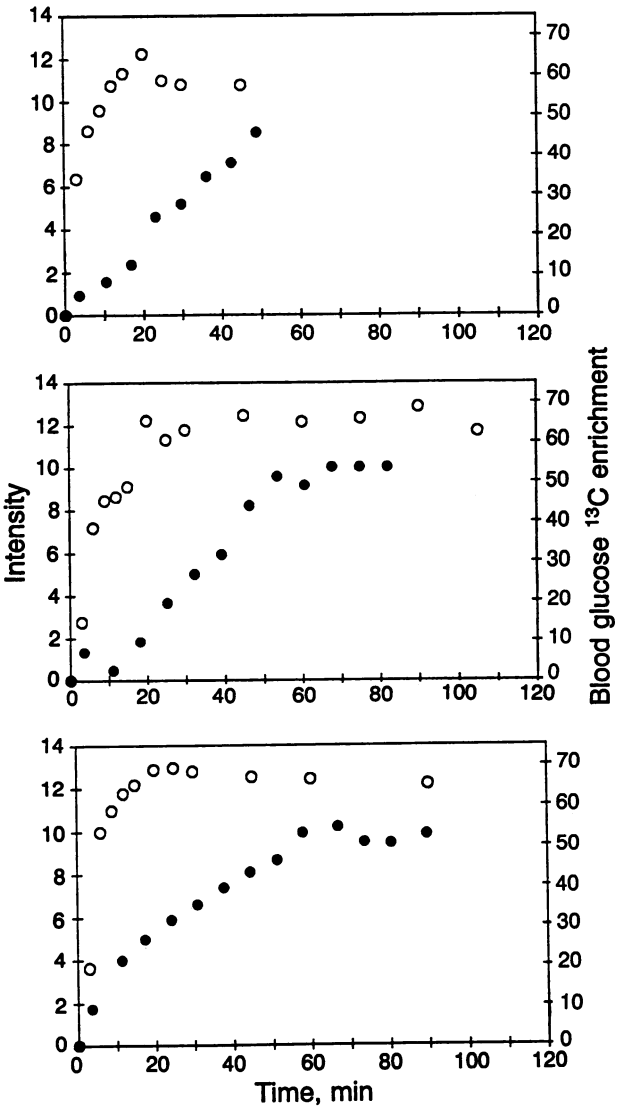

FIG. 3. [4-^{13}C]Glutamate amplitude vs. time. The [4-^{13}C]glutamate amplitude in arbitrary units is plotted for three of the glucose infusion experiments as solid circles. The plasma glucose 1-^{13}C fractional enrichment is plotted as open circles. The [4-^{13}C]glutamate and the plasma glucose enrichment time courses are seen to be highly reproducible. For the [4-^{13}C]glutamate measurement the time domain NMR data were apodized with a 5-Hz Lorentzian to Gaussian filter and linear baseline correction was performed between 3.3 and 2.0 ppm.

of 10 mM (22) is assumed, an average rate for the citrate synthase step of the TCA cycle (V_{tca}) of 0.5 μmol/g·min is calculated from the C4 glutamate turnover data. This V_{tca} rate can be compared with previous brain O_2 consumption measurements by assuming that glucose is the only carbon source for oxidation with a stoichiometry of $C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2$

Table 1. Potential sensitivity improvements

Source	Present	Theoretical	Improvement
Coil efficiency	0.8	1	1.3
Noise figure	3.5 decibels	<0.5 decibels	1.4
Echo time	16 msec	8 msec	1.3
Decoupling	Jr = 18 Hz	Jr < 3 Hz	1.4
^{13}C inversion	0.9	1	1.1
Pulse sequence	0.7	1	1.4
Isotope	[1-^{13}C]Glucose	[U-^{13}C]Glucose	2.0
Total			10

+ $6H_2O$. Using this stochiometry the calculated V_{tca} rate predicts an O_2 consumption rate of 1.5 μmol of O_2/g·min, which is in agreement with that measured in nonactivated visual cortex by ^{15}O PET in studies by Fox *et al.* (23) of 1.7 ± 0.2 μmol of O_2/g·min.

The TCA cycle rate obtained from the ^1H-[^{13}C] NMR data must be considered a preliminary estimate. However most of the assumptions made in the calculation can be tested by NMR experiments. As recently demonstrated, ^{13}C NMR can be used to determine brain glucose concentrations and transport kinetics, which can be used to calculate brain glucose ^{13}C enrichment (24). Implementation of broadband ^{13}C decoupling would allow measurement of [3-^{13}C]glutamate, which Mason *et al.* (9) used to test the assumption of rapid exchange between α-ketoglutarate and glutamate. Although substantially lower in sensitivity, direct ^{13}C NMR (4, 5) of metabolites that exhibit slower kinetic time courses such as glutamine, in combination with ^1H-[^{13}C] NMR measurements of glutamate, may allow investigations into compartmentation of metabolism. These measurements should be of value for studying normal brain metabolism and pathological conditions that alter amino acid metabolism, such as epilepsy and hepatic encephalopathies (25), both of which have been studied by ^1H NMR (26, 27).

In summary, our results demonstrate that ^1H-[^{13}C] NMR can be used to measure regional incorporation of ^{13}C isotope from blood glucose into human brain C4 glutamate. An estimate of 21 min was made for the C4 glutamate isotopic turnover time constant, which is consistent with PET O_2 consumption measurements. In combination with metabolic modeling and other recently developed NMR methods, ^1H-[^{13}C] NMR may potentially be used to determine regional rates of the TCA cycle and other metabolic pathways. With spectroscopic improvements and use of the optimum isotope these determinations can be performed on brain volumes of <4 cm^3 at 2.1 T.

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