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Localized ^{13}C NMR Spectroscopy in the Human Brain of Amino Acid Labeling from D-[1-^{13}C]Glucose

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Abstract: Cerebral metabolism of D[1-^{13}C]glucose was studied with localized ^{13}C NMR spectroscopy during intravenous infusion of enriched [1-^{13}C]glucose in four healthy subjects. The use of three-dimensional localization resulted in the complete elimination of triacylglycerol resonance that originated in scalp and subcutaneous fat. The sensitivity and resolution were sufficient to allow 4 min of time-resolved observation of label incorporation into the C3 and C4 resonances of glutamate and C4 of glutamine, as well as C3 of aspartate with lower time resolution. [4-^{13}C]Glutamate labeled rapidly reaching close to maximum labeling at 60 min. The label flow into [3-^{13}C]glutamate clearly lagged behind that of [4-^{13}C]-glutamate and peaked at t = 110–140 min. Multiplets due to homonuclear ^{13}C–^{13}C coupling between the C3 and C4 peaks of the glutamate molecule were observed in vivo. Isotopomer analysis of spectra acquired between 120 and 180 min yielded a ^{13}C isotopic fraction at C4 glutamate of 27 ± 2% (n = 4), which was slightly less than one-half the enrichment of the C1 position of plasma glucose (63 ± 1%), $p < 0.05$. By comparison with an external standard the total amount of [4-^{13}C]glutamate was directly quantified to be 2.4 ± 0.1 μmol/ml-brain. Together with the isotopomer data this gave a calculated brain glutamate concentration of 9.1 ± 0.7 μmol/ml, which agrees with previous estimates of total brain glutamate concentrations. The agreement suggests that essentially all of the brain glutamate is derived from glucose in healthy human brain. **Key Words:** ^{13}C NMR—Glutamate—Glutamine—GABA—Isotopomers—Human brain.
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NMR spectroscopy has been shown to be a powerful noninvasive technique that can be used to determine the concentration of many metabolites in living tissue (Prichard and Shulman, 1986; Williams and Gadian, 1986; Tofts and Wray, 1988; Radda et al., 1989; Bachelard and Badar-Goffer, 1993). Initially, most studies used the ^{31}P nucleus to monitor intracellular energy metabolism. Subsequently, ^{1}H NMR spectroscopy has gained interest due to its high sensitivity enabling a high spatial resolution in animals (Behar et al., 1983) and in humans (Bottomley et al., 1989; Kreis et al., 1992; Rothman et al., 1992*a,b*; Michaelis et al., 1993). To overcome the low sensitivity and natural abundance (1.1%) of ^{13}C it has been necessary to infuse ^{13}C-labeled glucose to follow brain metabolism. In a series of experiments it has been possible to follow the metabolism of [1-^{13}C]glucose in the rat (Rothman et al., 1984; Fitzpatrick et al., 1990) and human brain (Rothman et al., 1992*b*) by indirect ^{1}H NMR detection of ^{13}C concentration based on J coupling, thereby following the ^{13}C label with proton sensitivity.

Direct observation by ^{13}C NMR of brain metabolites requires larger volumes of interest due to the reduced sensitivity but provides more chemically specific information due to the large chemical shift range. Behar et al. (1986) showed in the rabbit that it was possible to observe [1-^{13}C]glucose being metabolized in vivo to brain glutamate. Subsequent rat studies were used to measure the steady-state ^{13}C NMR intensities of the α- and β-anomers of glucose (Mason et al., 1992*b*) at different plasma glucose concentrations. The values were used to evaluate the kinetic parameters of a Michaelis–Menten model of glucose transport.

Using gradient localized spectroscopy and localized shimming we have quantified the brain glucose signal and derived glucose transport kinetics (Gruetter et al., 1992*a*) in the human brain. We have recently also shown that even natural abundance peaks of metabolites, if concentrated like *myo*-inositol, can be quantified (Gruetter et al., 1992*b*), which indicated that the

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Abbreviations used: IF, plasma glucose isotopic fraction; MRI, magnetic resonance imaging; NAA, *N*-acetylaspartate; NOE, nuclear Overhauser enhancement; rf, radiofrequency; TCA, tricarboxylic acid; TE, echo time; TR, repetition time.

detection limit for ^{13}C NMR in the human brain is ~0.1 m*M* in ^{13}C concentration.

In a recent study by Beckmann and coworkers (1991), [1-^{13}C]glucose was infused and the label incorporation into the cerebral glutamate/glutamine peaks was observed using a surface coil for localization and a pulse-acquire sequence. The ^{13}C NMR difference spectra obtained in this study gave similar results to the early rabbit studies (Behar et al., 1986). The improvements in shimming, localization, and decoupling, demonstrated in our *myo*-inositol and glucose transport studies (Gruetter et al., 1992*a,b*), indicated the feasibility of quantitative ^{13}C NMR measurements of ^{13}C incorporation into pools of various amino acids during [1-^{13}C]glucose infusion. As shown below, the improved sensitivity has allowed a time resolution of 4–8 min for localized spectra of the glutamate and glutamine C4 peaks, measurement of glutamate isotopomers, and detection of label incorporation into aspartate and GABA. A preliminary report has appeared (Gruetter R., Novotny E. J., Boulware S. D., Rothman D. L., Tamborlane W. V., Shulman R. G., 11th Annual Meeting, Society of Magnetic Resonance in Medicine, Berlin, August 8–14, 1992, p. 1921).

MATERIALS AND METHODS

Subjects

Four healthy subjects (age, 18–22 years; three male, one female) were studied after written informed consent was obtained from the subjects using forms and procedures approved by the Yale Human Investigations Committee. Sensory stimulation was minimized by having the subjects wear earplugs and an eye mask.

Infusion protocol

Plasma glucose concentration and fractional enrichment were controlled by using the glucose clamp technique (DeFronzo et al., 1979; Shulman et al., 1990) modified as follows (Gruetter et al., 1992*a*): The fractional enrichment of [1-^{13}C]glucose (percentage of total plasma glucose) was raised by infusing initially 30 g of 99% enriched D-[1-^{13}C]-glucose in a 20% weight/volume solution into the antecubital vein. After 5–10 min, the infusion was switched to an enriched dextrose infusate containing ≈66% enriched D-[1-^{13}C]glucose to maintain a constant plasma isotopic fraction during the study. Plasma glucose concentration was controlled by adjusting the infusion rate of glucose based on blood samples (DeFronzo et al., 1979) obtained from the opposite arm every 5 min during the study and measured immediately in a Beckman glucose analyzer (Beckman, Fullerton, CA, U.S.A.). Plasma glucose was maintained at a hyperglycemic plateau (~15 m*M*) for 5–10 min. Subsequently, plasma glucose was allowed to drop to 7.5 m*M* where it was maintained.

The [1-^{13}C]glucose plasma isotopic fraction was measured in samples obtained every 10 min by gas chromatography–mass spectrometry of the pentacetate derivatives of plasma glucose after deproteinization and deionization (Shulman et al., 1990). Additional deproteinized samples were analyzed by ^{1}H NMR at 360 MHz in D_2O to determine the degree of enrichment at the C1 position.

In vivo NMR spectroscopy protocol

The subjects laid supine in a 2.1-T whole body magnet (ORS-Bruker, Billerica, MA, U.S.A.) equipped with active-shielded gradients (Oxford Magnet Technology, Oxford, U.K.) on a double surface coil consisting of a 7-cm-diameter ^{13}C coil and a concentric 14-cm ^{1}H coil. A volume of 144 ml (6 × 4 × 6 cm^3) was localized within the occipital–parietal region of the brain that excluded major blood vessels and ventricles, based on inversion recovery ^{1}H magnetic resonance images (MRI) obtained just before the start of the infusion [repetition time (*TR*) = 2,500 ms, inversion time (T_1) = 800 ms, echo time (*TE*) = 10 ms]. Before infusion a baseline spectrum was acquired that verified the absence of lipid signal.

Spectrometer parameters

Spectrometer adjustments were performed before the start of the infusion as described previously (Gruetter et al., 1992*a,b*); i.e., the radiofrequency (rf) power was adjusted on a sphere at the coil center containing an aqueous solution of ^{13}C formic acid to give a 100-μs 180° pulse. ^{1}H decoupling power was set for a 900-μs 90° pulse by minimizing the ^{13}C NMR signal of the sphere with the sequence 90°(^{13}C)-$\frac{1}{2}J$-θ(^{1}H)- acquire(^{13}C) (Bax, 1983). The 90° pulse duration used for decoupling was set to 1.2 ms. Localized shimming of the H_2O resonance using all first- and second-order shim coils was achieved with an automated sequence (Gruetter and Boesch, 1992; Gruetter, 1993).

The localization was based on the ISIS technique (Ordidge et al., 1986) using a previously described sequence (Gruetter et al., 1992*b*) with modifications; i.e., the chemical shift displacement error was minimized by placing the spectrometer frequency at 31 ppm in the spectrum and by using 5.5-ms-long hyperbolic secant pulses (Luyten et al., 1989) to produce a magnetization inversion bandwidth of 4 kHz (μ = 8, β = 2,000). A 3-ms adiabatic half passage sin/cos pulse with a numerically optimized modulation function was used for excitation (Gruetter et al., 1992*b*). Bilevel WALTZ-16 proton broadband decoupling (Shaka et al., 1985) was applied at 2.5 ppm in the ^{1}H spectrum with a power of at most 13 W during the signal acquisition time of 204 ms and 1 W during the recovery delay of 3 s to generate a nuclear Overhauser enhancement (NOE) of 1.3 in vivo as assessed from the C2 resonances of glutamate and glutamine in unlocalized spectra. The maximum tissue rf power disposition was calculated to be <2 W/kg using a magnetic vector potential model (Bottomley et al., 1989).

Data analysis

The spectra were zero-filled to 819 ms, multiplied with an exponential function corresponding to 2 Hz line broadening, Fourier transformed, phase corrected, and the baseline was corrected between 45 and 15 ppm using a fourth-order polynomial. To reduce the effect of noise in individual spectra, the baseline correction was performed on the sum of 1 h of measurements and applied to each individual spectrum. It was assumed that label incorporation into the broad underlying signal was negligible and this was verified by visual inspection. Time courses were obtained from peak amplitudes with a 4-min time resolution for the glutamate C4 resonance, 8 min for the glutamine C4 resonance, and 16 min for the glutamate C4 and C3 homonuclear coupling sidebands and the peaks from aspartate C3 and GABA C4. The measured amplitudes were converted to integrals by multiplication by conversion factors determined from inte-

gration of these resonances in 1 h of summed spectra. Intensity distortion due to chemical shift displacement error was determined to be negligible within the spectral bandwidth studied based on measurements of glutamate in solution.

Quantification of [4-^{13}C]glutamate concentration

Brain [4-^{13}C]glutamate concentrations were determined according to a procedure used previously for the quantification of glucose and natural abundance metabolites (Gruetter et al., 1992*a,b*). The integrals of the localized in vivo signals were compared with those obtained under identical experimental conditions from a phantom solution containing 50 m*M* glutamate and equimolar taurine at natural abundance (equivalent to 0.55 m*M* [4-^{13}C]glutamate) plus ~150 m*M* phosphate buffer (pH 7.1), 2 m*M* NaN_3, and 10 m*M* acetate. The effect of loading was measured by integrating the upfield peak of the formate doublet in separate, fully relaxed spectra obtained from the small sphere placed at the coil center. The combined effect of NOE and T_1 was assessed in vivo in unlocalized spectra by determining the ratio of the C2 resonances of glutamate and glutamine at 55.6 and 55.1 ppm, respectively, obtained with NOE generation using the parameters of the localization experiment (*TR* 3.3 s) to that of the signal obtained under fully relaxed conditions (*TR* 5.3 s) in the absence of NOE generation. The C2 resonances were chosen because they are not overlapped by lipid signals in the unlocalized spectrum. Based on the similarity of the correction factors measured in solution for the glutamate C2, C3, and C4 resonances, the measured in vivo glutamate C2 correction factor was applied to the other glutamate resonance positions.

Isotopomer analysis

Within the glutamate molecule peaks due to homonuclear coupling were seen in all four studies (J_{CC} 35 Hz for J_{23} and J_{43}). The isotopic fraction of the glutamate molecule was calculated as follows: The glutamate C3 isotopic fraction was calculated from the sum of the homonuclear peaks centered at glutamate C4 representing the sum of the indistinguishable [3,4-^{13}C]glutamate and [2,3,4-^{13}C]glutamate isotopomers divided by the total glutamate C4 intensity, i.e., the sum of the doublet plus center singlet intensity. Conversely, fractional enrichment of glutamate C4 was calculated by dividing the intensity of the C4 glutamate doublet by the total intensity of glutamate C3. The latter was determined by summing the glutamate C3 center peak (27.9 ppm) with two times the intensity of the downfield peak of the associated doublet. The upfield peak of the doublet was omitted in the calculation due to overlap with glutamine C3. To obtain higher sensitivity and to eliminate complications from the glutamate C2–C3 coupling, the amount of [3,4-^{13}C]-glutamate was set to the sum of the glutamate C4 doublet peaks.

RESULTS

Figure 1A shows the amino acid and methylene carbon region from an unlocalized natural abundance spectrum from the head of a 32-year-old female volunteer. The triacylglycerol resonances typically had a line width of ~22 Hz. Upon localization, the peaks completely vanished as shown in the bottom spectrum (Fig. 1B), indicating that these signals arise from noncerebral tissue (e.g., scalp and bone marrow of skull).

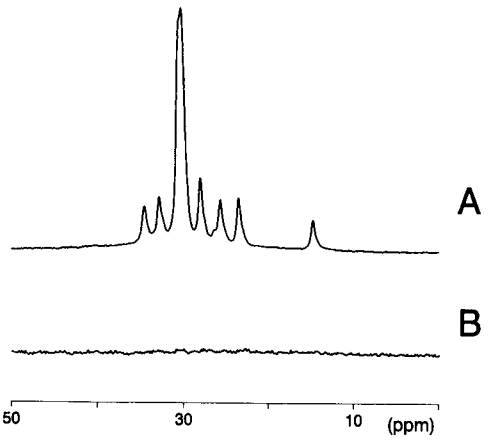

FIG. 1. Expansion of natural abundance ^{13}C NMR spectra of the human head. **A:** An unlocalized spectrum acquired in 4 min. **B:** The localized spectrum acquired in 30 min from 144 ml of occipital cortex from the same subject, a 32-year-old woman. The lack of triacylglycerol resonances in B indicates that the entire chemical shift range studied was localized to the brain. Acquisition parameters were as described in Materials and Methods.

Figure 2 shows the appearance of ^{13}C label with a 4-min time resolution in the localized spectra during the first hour of a study. Incorporation of label into the C4 resonance of glutamate was observed 4 min after the start of the infusion (arrow). Four consecutive 4-min spectra summed at the beginning and at the end of the 60-min period are shown to the right, demonstrating subsequent incorporation of label into aspartate C3 and glutamate C3 near the end of the 60-min period.

Figure 3 shows a typical broadband decoupled spectrum that is the sum of the spectra obtained between 120 and 180 min after the infusion started. The resonances from the glutamine C4 position (31.7 ppm), aspartate C3 (37.3 ppm), and the overlapping resonances of GABA C4 and *N*-acetylaspartate (NAA) C3 (40.4 ppm) are clearly discernible. The peak of [3-^{13}C]glutamine (27.9 ppm) overlapped and broadened the upfield peak of the homonuclear glutamate C3 doublet, causing the latter to be broader than its downfield counterpart. In addition to the resonances shown in Fig. 3, label incorporation was observed at the C2 position of glutamate (55.6 ppm), glutamine (55.1 ppm), and aspartate (53.1 ppm) in localized as well as unlocalized spectra that were not quantified (data not shown). Resonance line widths in the localized spectra were typically 2 Hz for glutamate C4. Note the absence of the intense lipid CH_2 peak at 30.5 ppm (Fig. 1) in the spectrum of Fig. 3.

Table 1 shows the measured concentration of [4-^{13}C]glutamate, the corresponding ^{13}C isotopic fraction at glutamate C4 and C3, and plasma glucose C1 of the four studies measured at specific time points between 90 and 130 min. It is noteworthy that the average isotopic enrichment (isotopic fraction minus 1.1% natural abundance ^{13}C) of glutamate C4 was only slightly less than one-half that of plasma glucose, 26 ± 2% versus

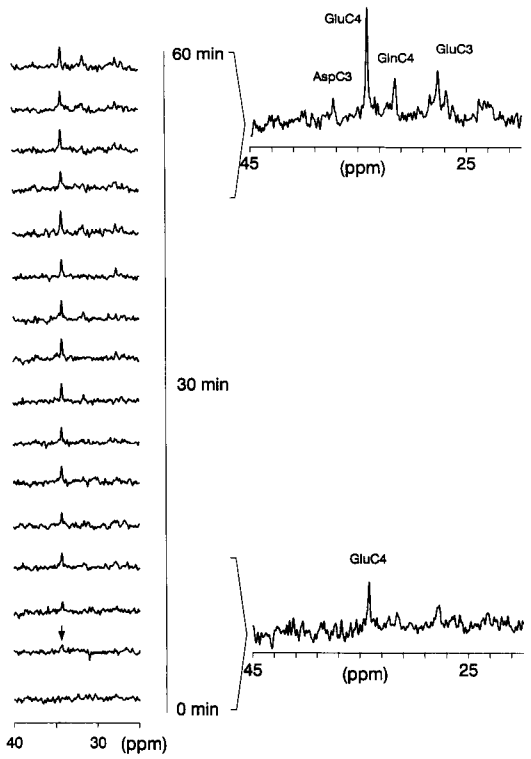

FIG. 2. Time course of localized ^{13}C NMR spectra at a 4-min time resolution (**left**) showing the rapid incorporation of label into glutamate C4 (arrow). The sum of the first and last 15 min is shown (**right**), indicating subsequent label incorporation into glutamine C4 (Gln C4), glutamate C3 (Glu C3), and aspartate C3 (Asp C3) at the end of the 60-min period. For peak assignments see Fig. 3.

31 ± 1%, with the average difference being 5 ± 2% (mean ± SEM, $p < 0.05$) and similar to that of glutamate C3.

Figure 4 illustrates the close relationship between the time course of plasma glucose isotopic fraction (open squares) and the time course of both the isotopic fraction and concentration of the glutamate C4 (filled circles). As described above, the NMR data allowed not only the quantification of ^{13}C label but also the estimation of the isotopic fraction of the glutamate molecule in vivo. This is reflected in Fig. 4 in that both axes apply to the [4-^{13}C]glutamate time course, whereas the plasma glucose isotopic fraction was divided by two (IF/2) before plotting. The similarity of plasma glucose IF/2 and the maximum enrichment of glutamate C4 illustrates the close to complete turnover of the C4 position of glutamate from glucose.

Table 2 gives the average concentration of other labeled metabolites determined relative to that of [4-^{13}C]glutamate, yielding for [4-^{13}C]glutamine 1.1 ± 0.1 μmol/ml, for [3-^{13}C]aspartate 0.26 ± 0.03 μmol/ml, and for [4-^{13}C]GABA plus natural abundance NAA 0.41 ± 0.05 μmol/ml at these time points assuming for all resonances similar correction factors in vivo as measured in solution.

Figure 5 shows the time course of the concentrations of [4-^{13}C]glutamate, [3-^{13}C]glutamate, and [4-^{13}C]-glutamine in an experiment in which plasma glucose isotopic fraction was maintained at a constant level throughout. The concentration of [4-^{13}C]glutamate was the most rapid approaching a maximum level at 60 min. The appearance of ^{13}C label at glutamate C3 clearly lagged behind that at C4. In all studies, similar levels of glutamate C3 and C4 labeling were reached ~2 h after the start of the infusion. The incorporation of label into glutamine C4 closely paralleled that of glutamate C4 with a slight lag.

DISCUSSION

The present study indicates that localized ^{13}C NMR spectroscopy can be used to measure quantitatively the time course of ^{13}C label incorporation from glucose into selected positions of aspartate, GABA, glutamate, and glutamine. Spectral resolution and sensitivity were sufficient to resolve the C3–C4 isotopomers of glutamate. Due to chemical shift offset of the selected volume, the spectral region completely within the brain was restricted and we confined quantitation to a 20 ppm bandwidth. Previous studies (Gruetter et al., 1992*a,b*) indicated that the presence of resonances from mobile triacylglycerides was a sensitive indicator for the spectroscopic volume containing extracerebral tissues. The absence of methylene and methyl resonances in the spectral region from 15 to 40 ppm in the present study (Figs. 1 and 3) verifies that the chemical shift range studied (25–40 ppm) was completely localized to the brain.

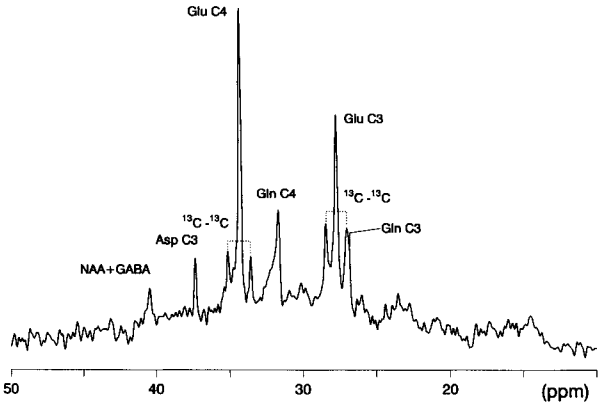

FIG. 3. Localized ^{13}C NMR spectrum from the human brain. Shown is the accumulation during 120–180 min after the infusion started. Processing consisted of 2-Hz exponential line broadening and no baseline correction and zero-order phase correction. Homonuclear peaks (^{13}C–^{13}C) with a splitting of 35 Hz were observed in the glutamate molecule. Peak assignments are based on chemical shift measurements reported for excised brain (Barany et al., 1985), which we found to be in agreement with solution measurements at 2.1 T, i.e., glutamine C3 (Gln C3), 27.9 ppm; glutamate C3 (Glu C3), 28.7 ppm; glutamine (Gln C4), 31.6 ppm; glutamate C4 (Glu C4), 34.2 ppm; aspartate C3 (Asp C3), 37.3 ppm; NAA + GABA, 40.4 ppm.

TABLE 1. *Isotopic fraction of [1-^{13}C]glucose in plasma, [4-^{13}C]- and [3-C^{13}]glutamate, and total brain [4-^{13}C]glutamate concentration (μmol/g wet weight)*

Study	Time (min)	IF (%)			Concentrations (m*M*)	
		Plasma glucose	Glu C4	Glu C3	[Glu C4]	$[Glu]_{total}$
1	97–116	65	27	24	2.3	8.5
2	85–100	64	33	23	2.4	7.3
3	100–115	60	24	21	2.4	10.0
4	134–149	64	24	25	2.5	10.4
Mean ± SEM		63 ± 1	27 ± 2	23 ± 1	2.4 ± 0.1	9.1 ± 0.7

Label incorporation into glutamate C4 was rapid approaching maximum levels within 60 min. The time course of label incorporation was similar to the rate of label incorporation into glutamate measured using $^1H\{^{13}C\}$ NMR in human brain (Rothman et al., 1992*b*). In contrast to the ^1H NMR measurement, the glutamate C3 resonance was well resolved and lagged behind the glutamate C4 labeling reaching a maximum at t = 110–140 min. A lag in glutamate C3 relative to C4 labeling had been reported in the rat brain using $^1H\{^{13}C\}$ NMR (Fitzpatrick et al., 1990) and was also observed in sequential analysis of animal brain extracts (Brainard et al., 1989; Shank et al., 1993) and guinea pig brain slices (Badar-Goffer et al., 1990*b*, 1992). As described in Mason et al. (1992*a*) a delay in glutamate C3 labeling indicates rapid exchange between glutamate and α-ketoglutarate. This exchange, which is believed to be catalyzed primarily by aspartate aminotransferase, results in ^{13}C label having to pass through the large glutamate pool before completing a turn of the tricarboxylic acid (TCA) cycle, which delays the movement of the label to the interior positions of α-ketoglutarate and resultant glutamate C3 labeling.

The [4-^{13}C]glutamate concentration when divided by the measured isotopic fraction gave a glutamate concentration of 9.1 ± 0.7 μmol/ml in the human brain (Table 1). This concentration represents a minimum estimate, because unlabeled glutamate pools and NMR-invisible pools (Kauppinen and Williams, 1991) would not be observed. Our values, however, are similar to the concentrations reported from recent ^1H NMR measurements of human brain (Rothman et al., 1992*a*; Michaelis et al., 1993) and the average concentration of 8.4 μmol/g from biopsy data (tabulated in Rothman et al., 1992*a*, and see Petroff et al., 1989) suggesting that the majority of brain glutamate is synthesized rapidly from plasma glucose. Furthermore, the average fractional enrichment of 26 ± 2% of the C4 of glutamate (Table 1) is close to one-half of the [1-^{13}C]-glucose fractional enrichment (31 ± 1%), which is also consistent with this conclusion. The small reduction in the glutamate enrichment relative to the plasma glucose precursor may be explained by label loss in the pentose shunt to CO_2 or label dilution due to unlabeled acetyl-CoA from plasma lactate and ketone bodies (Mason et al., 1992*a*). Alternatively, the glutamate

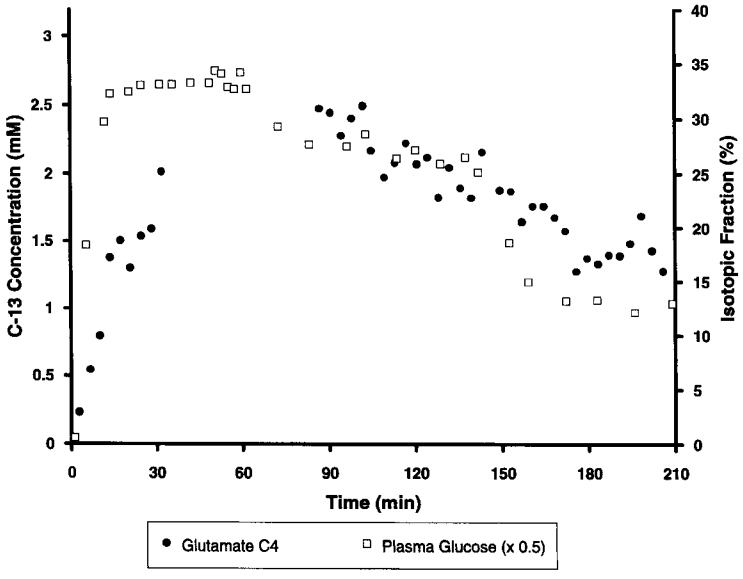

FIG. 4. Time course of the isotopic fraction and of the ^{13}C concentration of glutamate C4 (filled circles) determined as described in Materials and Methods is compared with the isotopic fraction of plasma glucose divided by 2 (open squares), which represents the theoretical maximum enrichment of glutamate. Shown is study 2. The time gap in glutamate C4 measurements was caused by the requirement to remove the subject from the magnet for urination. A firm head holder enabled accurate repositioning, as verified by coil loading factors, constant shimming, and MRI.

TABLE 2. *Average ^{13}C concentration of metabolites measured at the chemical shift indicated and corresponding minimum estimate of brain pool size, calculated by dividing ^{13}C concentration with isotopic fraction of glutamate C4 (Table 1)*

	Glutamate (34.2 ppm)	Glutamine (31.7 ppm)	GABA (40.4 ppm)	Aspartate (37.4 ppm)
^{13}C concentration	2.4 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	0.27 ± 0.03
Minimum total brain pool[a]	9.1 ± 0.7	4.3 ± 0.7	1.2 ± 0.3[b]	1.0 ± 0.2

Results are expressed as mean ± SEM values in m*M* (n = 4).
[a] Estimated by dividing the individual ^{13}C concentrations by the isotopic fraction of glutamate C4 (Table 1).
[b] Estimate for GABA pool size was calculated from ^{13}C concentration at the chemical shift indicated minus 0.1 m*M* contribution from natural abundance NAA.

pool may not have completely reached isotopomeric equilibrium, which would result in an underestimate of ^{13}C isotopic fraction using the ^{13}C sideband method. However, the isotopic fraction measurement was performed at the end of each infusion when both the glutamate C4 and C3 had reached an apparent plateau in enrichment that suggests near steady state was achieved.

Kauppinen and Williams (1991) reported for brain slices a change in glutamate peak height in ^{1}H NMR spin echo spectra (*TE* 120 ms) during anoxia. From this evidence they concluded that 20% of the glutamate is invisible to the NMR experiment under well-oxygenated conditions and probably reflects a vesicular glutamate pool. Although based on the good agreement between the ^{13}C NMR quantitation of glutamate and previous reports of human brain glutamate concentration (see above), we have no reason to propose an invisible pool; but the possibility cannot be ruled out because previous measurements of human brain biopsy material reported concentrations varying by >20% (see Rothman et al., 1992*a*). Compared with the present ^{13}C NMR method, the long echo ^{1}H NMR method used by Kauppinen and Williams (1991) is highly sensitive to changes in T_2 and susceptibility with anoxia, which may also explain their results.

The finding of near complete glutamate labeling is in contrast with recent ^{13}C isotopomer analyses of brain slices (Badar-Goffer et al., 1990*b*, 1992), which have found less than one-half the glutamate to incorporate ^{13}C label from glucose. The difference between the in vivo measurement and brain slice data may be explained by the existence of (a) glutamate pool(s) that require normal physiological activity to be metabolically active. Alternatively, the difference may be due to an artifact of the slice preparation where the pools represent different populations of cells that are metabolically compromised and have lost the ability to oxidize glucose. The rate of oxygen consumption in the brain slice is ~40% of its value in situ (Lipton and Whittingham, 1984), and this has been attributed to cell damage during slice preparation and hypoxia in cells deep in the core of the slice.

Based on earlier labeling kinetic studies (van den Berg, 1973) of animal brain and brain slices as well as direct immunocytochemical staining studies (Ottersen et al., 1992), it is believed that glutamine is synthesized entirely in the glia. It has also been suggested that the anapleurotic pathway exists primarily in astrocytes (Shank et al., 1985; Hertz and Schousboe, 1988). These data in combination with studies of isolated glial culture substrate transport have led to the concept of a glutamate/GABA/glutamine cycle in which glutamate or GABA released by the neurons

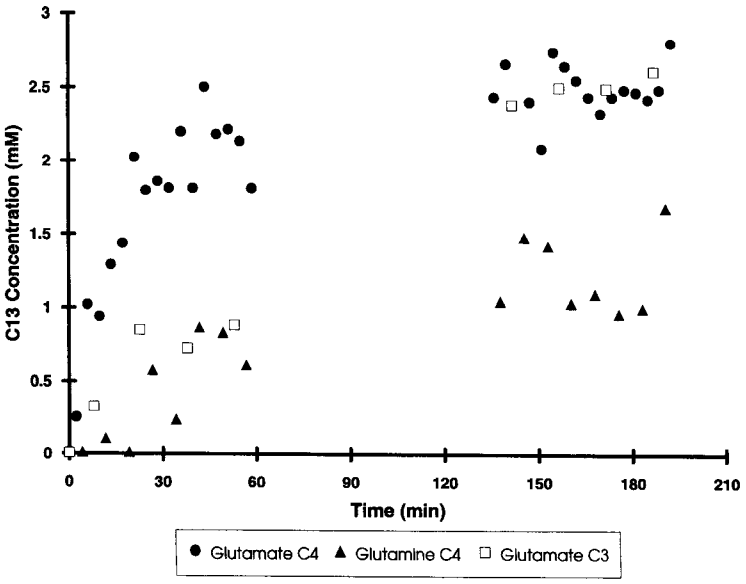

FIG. 5. Time course of ^{13}C metabolite concentrations (m*M*) in human brain in vivo. Time courses are given for [4-^{13}C]glutamate (filled circles), [3-^{13}C]-glutamate (open squares), and [4-^{13}C]glutamine (filled triangles). The data are from study 4, where isotopic fraction of plasma glucose was constant throughout the entire time period shown. Quantification of peaks is described in Materials and Methods. Time gaps in the measurements were caused by the requirement to remove the subject from the magnet for urination.

is taken up by glial cells, converted to glutamine by glutamine synthetase, and exported as glutamine or a TCA cycle intermediate to the extracellular fluid where it can be taken up again by the neurons (Hertz and Schousboe, 1988; Sonnewald et al., 1991). The observation of the time course of label entry into glutamine C4 paralleling that of glutamate C4 in human brain suggests that this cycle is highly active in the occipital region of the human brain under physiological conditions. A similar parallel incorporation of label into glutamate and glutamine C4 was observed in ^{13}C NMR study of brain slices given [1-^{13}C]glucose as a substrate when slice metabolic activity was increased by KCl depolarization (Badar-Goffer et al., 1992). In nonstimulated brain slice preparations either no or very little incorporation of label from glucose into glutamine was measured (Badar-Goffer et al., 1990*b*, 1992), suggesting that much of the label incorporation into glutamine observed in the human brain is related to normal physiological activity.

An estimate of the size of the glutamine pool that incorporates glucose was obtained by dividing the measured [4-^{13}C]glutamine concentration by the glutamate C4 fractional enrichment in the later time periods of the infusion (see Table 1). The glutamine pool size determined this way will be a minimum estimate because the glutamate pool was close to one-half the plasma glucose fractional enrichment. This calculation yielded a glutamine pool size of 4.3 ± 0.7 μmol/ml (Table 2), which is similar to the average values measured in surgically removed human tissue by conventional chemical assay tabulated in Rothman et al. (1992*a*) of 4.6 ± 1.2 μmol/ml. The presence of a large glutamine pool that is metabolically derived from glucose is in contrast to even the depolarized brain slice data (Badar-Goffer et al., 1992) in which approximately one-fourth of the glutamine pool was derived from glucose.

Glutamate is the precursor of GABA synthesis via the glutamate decarboxylase reaction, which predicts that the C2 enrichment of GABA follows that of glutamate C4 and that C3 and C4 of GABA follow glutamate C2 and C3 enrichments, respectively. A significant signal increase was observed at 40.4 ppm, which is the chemical shift of GABA C4 and NAA C3 in excised brain tissue (Barany et al., 1985). If it is assumed that the underlying NAA C3 resonance was not labeled, then its contribution to the GABA C4 resonance would represent ~0.06–0.1 μmol/ml of ^{13}C depending on whether NAA concentrations determined from extracts of surgically removed brain tissue (Petroff et al., 1989) or in vivo ^{1}H NMR (Michaelis et al., 1993) are used. Assuming the GABA C4 enrichment was the same as glutamate C3 in the 90–120-min period and using 0.1 μmol/ml for the NAA ^{13}C concentration yields a GABA pool size of 1.2 ± 0.6 μmol/ml. This concentration is similar to the value of 1.1 μmol/ml reported in a recent ^{1}H NMR study and is within the range of values reported for surgically removed human brain tissue that was rapidly frozen (0.5–1.4 μmol/ml, see Rothman et al., 1993), which suggests that most of the brain GABA pool is derived from glucose under physiological conditions. An alternate explanation of the increase in the 40.5 ppm resonance is due to label incorporation in the NAA C3 position, although this process has been demonstrated to be only 1–2% of the rate of label incorporation into aspartate (Margolis et al., 1960). However, if there were a contribution from NAA synthesis, the incorporation of label would be from aspartate labeled at the C3 position only because we did not observe any signal changes at the C2 of NAA in unlocalized spectra (54.1 ppm, not shown).

Synthesis of GABA or glutamine from a subpool of glutamate with a higher activity in pyruvate carboxylase relative to the TCA cycle rate will result in a higher fractional enrichment of GABA C2 over GABA C4 and glutamine C3 over glutamine C2 (Shank et al., 1993). However, at present, we do not have the accuracy to assess small differences in the relative labeling due to overlap of GABA C2 and glutamine C3 with the glutamate isotopomer resonances and the glutamine C2 resonance being outside the quantitated chemical shift range. However, the relatively slow increase in the GABA C4 resonance, which did not reach maximum levels until the 90–130-min period is in agreement with most previous work in animal brain (Badar-Goffer et al., 1992; Shank et al., 1993). It disagrees, however, with the report from Brainard et al. (1989) of rapid GABA C4 labeling during [1-^{13}C]glucose infusion determined from ^{13}C NMR analysis of sequential brain extracts, which led to their proposal of GABA being synthesized in a compartment with highly active pyruvate carboxylase and inactive pyruvate dehydrogenase. Additional information on metabolic compartmentation could be obtained with further improvements in the spectroscopic method and data analysis or by the use of C2-labeled glucose, which is only incorporated into noncarboxyl glutamate positions by anapleurotic reactions.

Labeling of lactate C3 was not observed even when all in vivo spectra were summed. This result is in contrast to a report of highly elevated lactate in nonlocalized human brain ^{13}C NMR spectra during [1-^{13}C]-glucose infusion (Beckmann et al., 1991). The sensitivity of the lactate C3 measurement may be reduced due to chemical shift offset and T_1 saturation of the methyl peak. However, if it is assumed that the lactate C3 position has one-half the glucose fractional enrichment, the result suggests a brain lactate concentration of <0.3–0.5 μmol/ml. The observation of low brain lactate levels is comparable with previous ^{1}H NMR estimates of 0.5–0.6 μmol/g (Hanstock et al., 1988; Prichard et al., 1991), considering the possibility of overlap with threonine and macromolecule resonances in these studies (Behar and Ogino, 1991, 1993).

Conclusions

The present study demonstrates that localized ^{13}C NMR spectroscopy permits the investigation of brain

amino acid metabolism in humans noninvasively. In contrast to previous ^{13}C NMR studies of brain slices, the human brain glutamate pool incorporated label at close to the theoretical value expected from the plasma glucose fractional enrichment. Estimates of the total GABA, glutamine, and glutamate pool sizes gave values similar to those reported by biopsy from the literature, which suggests that (1) almost the entire pools of these amino acids are derived from glucose in the normal human brain and (2) there are no large "NMR invisible" pools of these compounds. The present time course data should be sufficient to derive the rates of the brain TCA cycle, α-ketoglutarate/glutamate exchange, and the flow of label from glutamate to glutamine by using metabolic models similar to Mason et al. (1992*a*).

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