

^1H -Observe/ ^{13}C -decouple spectroscopic measurements of lactate and glutamate in the rat brain *in vivo*

(brain metabolism/ ^1H NMR/ ^{13}C NMR/fractional ^{13}C isotope enrichment)

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ABSTRACT We have used $\{^{13}\text{C}\}$ - ^1H NMR spectroscopy at 360.13 MHz to resolve the ^{13}C coupled proton resonance of glutamate and lactate in the rat brain *in vivo*. The time required for the ^{13}C fractional enrichment of the 4- CH_2 position of brain glutamate to reach isotopic steady state was determined during a continuous infusion of D-[1- ^{13}C]glucose. Under conditions of ischemia, measurements made of the 3- CH_3 of lactate in $\{^{13}\text{C}\}$ - ^1H NMR spectra revealed the relative contribution of brain glucose and glycogen to lactate formation. $\{^{13}\text{C}\}$ - ^1H NMR was 11 times more sensitive than ^{13}C NMR for the detection of ^{13}C in the 3- CH_3 position of lactate and 6 times more sensitive for the detection of ^{13}C in the 4- CH_2 of glutamate under similar *in vivo* conditions.

The low NMR sensitivity of the ^{13}C nucleus has limited *in vivo* ^{13}C NMR studies (1–3) to the measurements of enriched compounds whose concentrations are above 1 mM. An alternate method of measuring ^{13}C enrichment by NMR, adopted for use in cell suspensions, is to measure the intensity of the ^{13}C side bands of protons directly bonded to ^{13}C nuclei in the ^1H spectrum (4–7). The advantages of this technique over conventional applications of ^{13}C NMR are 2-fold: the greater sensitivity of the ^1H nucleus and the ability to resolve resonances from protons bound to both ^{12}C and ^{13}C nuclei, the latter allowing direct measurement of ^{13}C fractional enrichments (4).

We report here the use of a $\{^{13}\text{C}\}$ - ^1H NMR spin-echo difference sequence, as suggested by Bendall and Gordon (8), to observe selectively the ^{13}C coupled ^1H resonances of metabolites in rat brain *in vivo* during a continuous infusion of D-[1- ^{13}C]glucose. This method has enabled us to follow the ^{13}C enrichment of glutamate and lactate in the rat brain with 1-min time resolution.

METHODS

***In Vivo* NMR Measurements.** ^1H NMR spectra were obtained with a WH-360 wide-bore spectrometer (Bruker Instruments) operating at 360.13 MHz for ^1H . A probe with a Plexiglas insert was used to restrain a rat vertically within the magnet. The surface coil (12 × 16 mm) and probe circuit, which was double-tuned to the ^1H and ^{13}C resonant frequencies and used for ^1H transceiving and ^{13}C decoupling, has been described elsewhere (9).

In vivo ^1H NMR spectra were acquired by using a ^1H spin-echo sequence with gated ^{13}C decoupling (see Fig. 1), which will be referred to as the $\{^{13}\text{C}\}$ - ^1H sequence. A τ delay of 7.8 msec was used, which is approximately $1/J$ for the ^1H - ^{13}C J coupling of [3- $^{13}\text{CH}_3$]lactate and [4- $^{13}\text{CH}_2$]glutamate ($J \approx 127$ Hz). Scans acquired with continuous and gated ^{13}C decou-

pling were accumulated alternately in blocks of 8 scans and stored in separate computer memory locations. ^{13}C difference spectra (see Fig. 1) were obtained by computer subtraction of the ($^{12}\text{C} + ^{13}\text{C}$) and ($^{12}\text{C} - ^{13}\text{C}$) subspectra. The phase of the π pulse was changed by 180° relative to the $\pi/2$ pulse during alternate scans to eliminate phase artifacts from surface coil H_1 inhomogeneity. The full four-phase cycling scheme suggested by Bendall and Gordon (8) was not necessary because the relevant artifact signals were sufficiently dephased by H_0 inhomogeneity (14) ($\Delta\nu_{1/2} \approx 25$ –35 Hz).

The pulse duration that gave the maximal H_2O signal (25–30 μsec) was used for the $\pi/2$ pulse. H_0 inhomogeneity was minimized by shimming on the tissue H_2O signal using this pulse duration. All *in vivo* spectra were acquired with 1024 data points and a width of 7000 Hz (73-msec acquisition time) centered at the H_2O resonance. Free induction decays were zero filled to 4096 data points prior to Fourier transformation. Single-frequency (non-phase cycled) ^1H irradiation (≈ 200 mW) was used to saturate the H_2O signal (15) for 912 msec prior to the spin-echo pulse sequence. All *in vivo* ^1H chemical shift values are referenced to the methyl proton resonance of endogenous *N*-acetyl aspartate at 2.023 ppm (16).

Animal Preparation for *In Vivo* ^1H NMR Experiments. Rats of the Charles River strain (150–160 g) were starved for 24 hr with free access to water prior to the NMR experiment. They were tracheotomized under enflurane anesthesia, mechanically ventilated with 25% O_2 in nitrous oxide, and paralyzed with *d*-tubocurarine chloride (1.2 mg/kg, s.c.) and pancuronium bromide (0.6–2.7 mg/kg, s.c.). Propranolol was administered (1.9–5.7 mg/kg, i.p.) prior to tracheotomy to counter stress-induced hyperglycemia. A catheter was placed in the left femoral vein for infusion of [1- ^{13}C]glucose. Electrocardiogram leads were placed on the left extremities. To ensure that only the contents of the skull were observed, the skin and temporalis muscles were retracted and fixed in a position away from the skull. The rat was then mounted and restrained vertically within the probe and a surface coil was centered 6 mm posteriorly to the bregma. A more detailed description of the animal preparation will appear elsewhere (17).

[1- ^{13}C]Glucose Infusion. D-[1- ^{13}C (92 atom %)]Glucose (0.25 g/ml in 0.9% saline) was delivered i.v.; an initial bolus of 0.021 g (0.75 mmol/kg of body weight) was followed by an exponentially decreasing infusion rate, adjusted manually, once every minute. After 15 min the rate was adjusted to supply a steady flow of 0.007 g/min (0.25 mmol/kg of body weight per min) for the remainder of the experiment. The infusion schedule was designed to produce a high fraction of [1- ^{13}C]glucose in the blood rapidly and to maintain the concentration at that level for the remainder of the infusion, similar to a protocol established for deoxy[^{14}C]glucose (18).

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Abbreviation: S/N, signal-to-noise.

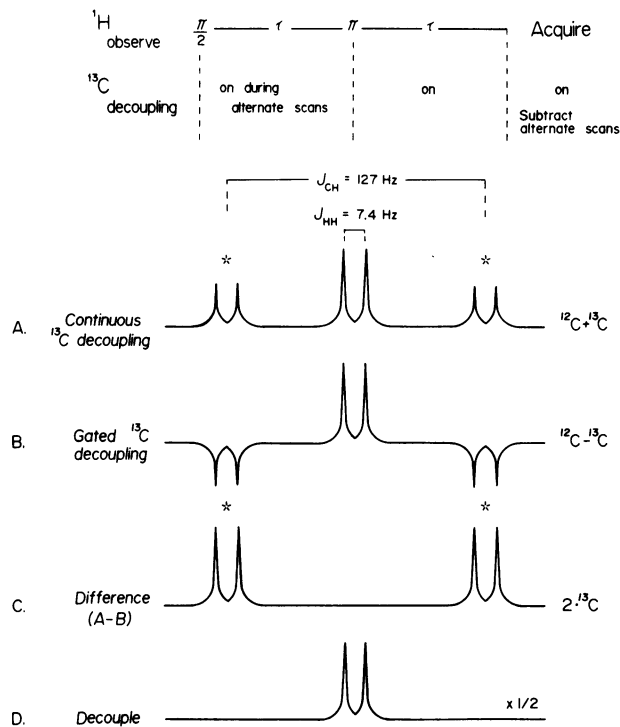


FIG. 1. Fig. 1 (top) shows the $\{^{13}\text{C}\}$ - ^1H NMR pulse sequence (8). A τ delay of $1/J_{\text{CH}}$ was used, where J_{CH} is the ^1H - ^{13}C coupling in Hz. Single-frequency ^{13}C irradiation was used to decouple selectively the ^1H - ^{13}C coupling of the proton resonance. This sequence is analogous to published sequences that use gated ^1H decoupling to edit the ^{13}C NMR spectrum (10, 11) where a detailed theoretical explanation may be found. Similar $\{^{13}\text{C}\}$ - ^1H sequences that use a π radian ^{13}C pulse instead of ^{13}C decoupling have also been published (12, 13). (A and B) The differential phase relationship at acquisition of the proton resonances of $[3\text{-}^{13}\text{CH}_3]$ - and $[3\text{-}^{12}\text{CH}_3]$ lactate depending on whether continuous or gated ^{13}C decoupling is applied during the ^1H spin-echo sequence. When continuous ^{13}C decoupling is applied (A), the resonances of protons bound to ^{12}C (central doublet) and ^{13}C nuclei (starred doublets) are indistinguishable during the spin echo and are acquired with the same phase ($^{12}\text{C} + ^{13}\text{C}$). The two resonances of $[3\text{-}^{13}\text{CH}_3]$ lactate (starred doublets) are separated by ≈ 127 Hz, which is the value of J_{CH} . The smaller couplings present (≈ 7.4 Hz) are the ^1H - ^1H homonuclear couplings between the lactate 2-CH and 3- CH_3 protons. In the text A is referred to as the ($^{12}\text{C} + ^{13}\text{C}$) subspectrum. When ^{13}C decoupling is gated off during the first τ delay (B), the two resonances of $[3\text{-}^{13}\text{CH}_3]$ lactate precess 180° in phase and are acquired inverted relative to the resonance of $[3\text{-}^{12}\text{CH}_3]$ lactate ($^{12}\text{C} - ^{13}\text{C}$). Subtraction of A and B results in a spectrum containing only the two resonances of $[3\text{-}^{13}\text{CH}_3]$ lactate at twice their respective intensity in A as shown in C ($2 \times ^{13}\text{C}$). ^{13}C decoupling during acquisition collapses the ^1H - ^{13}C coupling and the two resonances in C are acquired as the single resonance shown in D, referred to in the text as the ^{13}C difference spectrum. The fractional ^{13}C enrichment of the 3- CH_3 position of lactate is calculated by dividing half the sum of the intensity of the $[3\text{-}^{13}\text{CH}_3]$ lactate resonance in the decoupled ^{13}C difference spectrum (D) by the sum of the intensities of $[3\text{-}^{12}\text{CH}_3]$ - and $[3\text{-}^{13}\text{CH}_3]$ lactate in the ($^{12}\text{C} + ^{13}\text{C}$) subspectrum (A). When ^{13}C decoupling is used during acquisition, the resonances of $[3\text{-}^{13}\text{CH}_3]$ - and $[3\text{-}^{12}\text{CH}_3]$ lactate are acquired as a single resonance in the ($^{12}\text{C} + ^{13}\text{C}$) subspectrum, which in the text is referred to as $[3\text{-}^{12+13}\text{CH}_3]$ lactate. The description presented above holds for any ^1H resonance coupled to a single ^{13}C nucleus. For the proton resonances of $[3\text{-}\text{CH}_3]$ lactate and $[4\text{-}\text{CH}_2]$ glutamate, homonuclear J modulation during the $\{^{13}\text{C}\}$ - ^1H sequence can be ignored to a first approximation because the ^{13}C - ^1H couplings (≈ 127 Hz) are much larger than the ^1H - ^1H couplings present (≈ 7 Hz). The resonance intensities of protons coupled to ^{13}C nuclei will not be distorted in the $\{^{13}\text{C}\}$ - ^1H decoupling sequence provided that good ^{13}C decoupling is achieved across the entire ^1H sensitive volume. Based upon decoupling efficiency measurements made *in vitro* and *in vivo*, the intensity reduction due to incomplete ^{13}C decoupling was 5%, at most.

When strictly applied, this schedule is valid only for the infusion of "tracer" amounts of substrate; the large quantities used in this study may lead to a marked deviation from the desired constancy of ^{13}C fractional enrichment of blood glucose over the NMR measurement period.

RESULTS

Fractional Labeling of $[4\text{-}\text{CH}_2]$ Glutamate. Two rats were given a continuous infusion of $[1\text{-}^{13}\text{C}]$ glucose, as described in *Methods*, while glutamate was monitored in the $\{^{13}\text{C}\}$ - ^1H spectra. Provided that the fractional ^{13}C enrichment of brain glucose is maintained at a steady state over the measurement period, metabolic fluxes into and out of the brain glutamate pool(s) can be inferred by the time required for the $[4\text{-}\text{CH}_2]$ glutamate ^{13}C fractional enrichment to reach a steady-state value (i.e., turnover time).

Fig. 2B shows a $\{^{13}\text{C}\}$ - ^1H sequence ^{13}C difference spectrum of the resonance of $[4\text{-}^{13}\text{CH}_2]$ glutamate in a rat brain acquired 22 min after the start of a $[1\text{-}^{13}\text{C}]$ glucose infusion. The resonance of $[4\text{-}^{12+13}\text{CH}_2]$ glutamate (2.35 ppm) is resolved in the ($^{12}\text{C} + ^{13}\text{C}$) subspectrum (Fig. 2C).

Fig. 2A shows a plot of the resonance amplitude of $[4\text{-}^{13}\text{CH}_2]$ glutamate vs. time during an i.v. infusion of $[1\text{-}^{13}\text{C}]$ glucose. Because no changes in the resonance amplitude of $[4\text{-}^{12+13}\text{CH}_2]$ glutamate were observed during this period, as assessed in the ($^{12}\text{C} + ^{13}\text{C}$) subspectrum, the increase detected in $[4\text{-}^{13}\text{CH}_2]$ glutamate during the infusion was entirely due to an increase in isotopic enrichment. Isotopic steady state was observed for $[4\text{-}^{13}\text{CH}_2]$ glutamate about 15 min after the infusion began (Fig. 2A); in a second rat, isotopic steady state was reached within 20 min.

Fractional Labeling of $[3\text{-}\text{CH}_3]$ Lactate During Ischemia. During complete ischemia, lactate cannot enter or leave the brain because of the cessation of blood flow, and the oxidative metabolism of pyruvate is reduced to zero as tissue oxygen levels fall. When brain glucose is derived from blood glucose that is ^{13}C enriched following a $[1\text{-}^{13}\text{C}]$ glucose infusion, the rates of formation of total brain lactate ($d/dt [3\text{-}^{12+13}\text{CH}_3]$ lactate) and $[3\text{-}^{13}\text{CH}_3]$ -enriched brain lactate ($d/dt [3\text{-}^{13}\text{CH}_3]$ lactate) during ischemia are described by the following two equations:

$$d/dt [3\text{-}^{12+13}\text{CH}_3]\text{lactate} = V_{\text{glucose}} + V_{\text{glycogen}} \quad [1]$$

$$d/dt [3\text{-}^{13}\text{CH}_3]\text{lactate} = 1/2(A)V_{\text{glucose}} \quad [2]$$

V_{glucose} is the contribution to the rate of glycolysis from brain glucose that was derived directly from blood glucose and V_{glycogen} is the contribution to the rate of glycolysis from brain glycogen breakdown. Rates are given in units of mol per unit time. A factor of 2 is implicit in V_{glucose} and V_{glycogen} because one molecule of glucose will produce two molecules of lactate. It is assumed in the above equations that pentose shunt activity is negligible [$\approx 3\%$ of glycolytic flux (21)] and that lactate is in isotopic and chemical equilibrium with pyruvate so that the net rate of lactate formation ($d/dt [3\text{-}^{13+12}\text{CH}_3]$ lactate) will equal the net rate of brain glycolysis ($V_{\text{glucose}} + V_{\text{glycogen}}$). We also assumed that brain glycogen will not be enriched significantly by ^{13}C after a $[1\text{-}^{13}\text{C}]$ glucose infusion of short duration (22), so that $[3\text{-}^{13}\text{CH}_3]$ lactate will only be produced from intracellular $[1\text{-}^{13}\text{C}]$ glucose whose fractional enrichment mirrors that existing in blood, and also that glucose contained in other extracellular fluid compartments (e.g., cerebrospinal fluid) possessed the same isotopic enrichment as whole blood. The term "A" is the fractional enrichment of brain $[1\text{-}^{13}\text{C}]$ glucose derived from blood glucose; this quantity will be constant during ischemia because no new blood-derived glucose reaches the brain. A value of $1/2(A)$ is used in Eq. 2 because $[3\text{-}\text{CH}_3]$ lactate is produced equally from the enriched C-1 and unenriched C-6

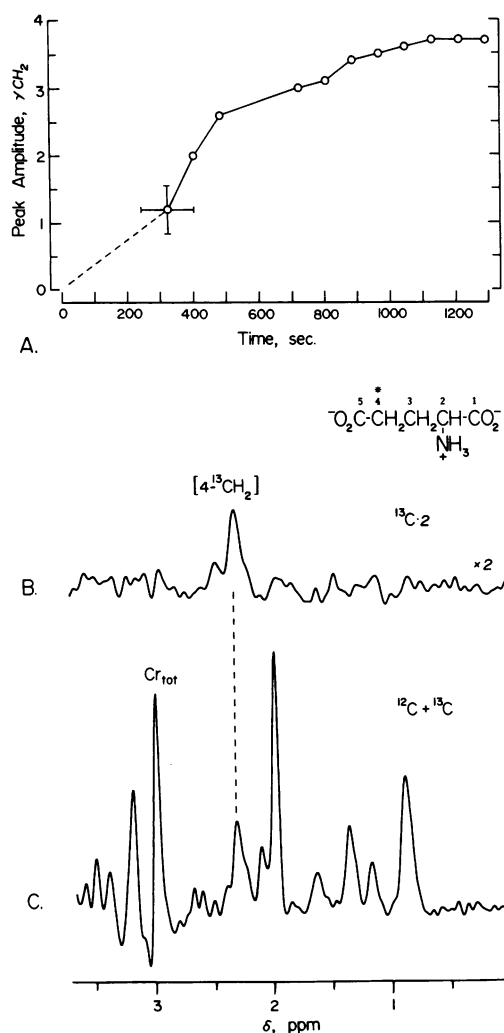


FIG. 2. (A) The amplitude (arbitrary units) of the proton resonance of [4-¹³CH₂]glutamate is plotted vs. time after the start of a [1-¹³C]glucose infusion. The infusion began at $t = 0$ on the plot and was continued throughout the experiment. ¹³C-¹H spectra were acquired with 2 W of single-frequency ¹³C decoupling centered at the ¹³C resonance of [4-¹³CH₂]glutamate at 34.2 ppm in the ¹³C NMR spectrum. Each datum point represents the sum of two 64-scan (1 sec per scan) ¹³C difference spectra accumulated over a 162-sec time interval (horizontal bar). Vertical bars represent the amplitude uncertainty due to rms noise. The total concentration of glutamate, determined from the [4-¹²⁺¹³CH₂]glutamate resonance amplitude in the (¹²C + ¹³C) subspectrum, remained constant during the infusion. Linewidths did not change during the experiment, so that the plotted amplitudes are directly proportional to resonance intensities. (B) A ¹³C-¹H sequence ¹³C difference spectrum of [4-¹³CH₂]glutamate. The spectrum is the sum of two 64-scan difference spectra (1 sec per scan) centered at 22 min after the start of a [1-¹³C]glucose infusion. The spectrum was processed with a mild Gaussian filter (19) and a convolution difference (20) to eliminate baseline tilt from incomplete subtraction of the tissue H₂O signal. Assuming that the resonance intensity of creatine + phosphocreatine (Cr_{tot}) at 3.03 ppm in the (¹²C + ¹³C) subspectrum (C) is 10.5 μmol/g of wet weight, then the concentration of [4-¹²⁺¹³CH₂]glutamate was calculated to be ≈1.5 μmol/g of wet weight. (C) Sum of the two (¹²C + ¹³C) subspectra, which are components of the two ¹³C difference spectra, whose sum is plotted in B. The resonance of [4-¹²⁺¹³CH₂]glutamate (indicated by dashed line) is resolved at 2.35 ppm. Resonance intensities in the spectrum in C can only be compared approximately with the [4-¹³CH₂]glutamate resonance intensity in the spectrum in B because more resolution enhancement was used in C.

glucose carbons. It can be shown from Eq. 2 that the total amount of brain lactate formed from brain glucose (blood glucose derived) during ischemia can be calculated by divid-

ing the amount of [3-¹³CH₃]lactate formed during the ischemic period by 1/2(A). The total amount of lactate formed from brain glycogen during ischemia is the difference between the total amount of lactate formed and the amount of lactate formed from brain glucose.

After 29 min of a continuous infusion of [1-¹³C]glucose, the O₂ fraction of a rat's breathing mixture was lowered to 10%. Following 6 min of hypoxia at this level, the rat was then made completely anoxic (0% O₂, 100% N₂O). The ischemia, which subsequently followed (≈80 sec after the reduction of the inspired oxygen, as monitored by the electrocardiogram), led to rapid increases in the resonances of both [3-¹³CH₃]- and [3-¹²⁺¹³CH₃]lactate (Fig. 3 Lower).

The ¹³C fractional enrichment in the 3-CH₃ position of brain lactate, measured from the sum of three ¹³C-¹H spectra acquired during the last 4 min of hypoxia, was found to be 38% ± 8%. Because there was no efflux of lactate from the brain during ischemia, the ¹³C fractional enrichment of newly formed lactate during ischemia can be calculated by dividing the change in the amplitude of the resonance of [3-¹³CH₃]lactate between successive ¹³C-¹H spectra ($\Delta[3-^{13}\text{CH}_3]\text{lactate}$) by the change in the amplitude of [3-¹²⁺¹³CH₃]lactate ($\Delta[3-^{12+13}\text{CH}_3]\text{lactate}$) during the same time period. The fractional enrichment of the lactate formed between 45 and 131 sec after ischemia began fell to 20% ± 4% (Fig. 3 Upper)—an indication that lactate was formed from unenriched carbon sources, the most likely source being brain glycogen (23). After 5 min of ischemia, the fractional enrichment of the 3-CH₃ position of brain lactate was 25% ± 0.7%. Assuming that the lactate fractional enrichment—measured at the end of hypoxia (38% ± 8%)—equaled 1/2 the brain glucose 1-¹³C fractional enrichment [1/2(A)] (i.e., lactate was in an isotopic steady state with brain glucose during hypoxia), it can be calculated from Eq. 2 that 34% ± 13% of the lactate formed during the first 5 min of ischemia was derived from unenriched carbon sources. This amount corresponds to 4.5 μmol/g of wet weight of lactate if total creatine (≈10.5 μmol/g of wet weight) at 3.03 ppm is used as a concentration standard (16). A similar dilution of the fractional enrichment of [3-CH₃]lactate was observed in a second rat exposed briefly to hypoxia followed by complete anoxia.

Sensitivity Comparison Between ¹³C-¹H NMR and ¹³C NMR. In principle, the ¹H nucleus should be 63 times more NMR sensitive than the ¹³C nucleus—21 times if the ¹³C nucleus develops a full ¹H nuclear Overhauser effect during decoupling. Signal-to-noise (S/N) measurements were made on the double-tuned probe with a solution of 20 mM [2-¹³CH₃]acetate in 150 mM KCl/²H₂O. For equal numbers of nuclei, and resonances of equal linewidth, the probe's ¹H channel was 8.4 times more sensitive than its ¹³C channel, the latter complete with ¹H decoupling and a ¹H nuclear Overhauser effect of 3. The reason(s) why the ¹H channel is less sensitive by a factor of 2.5 than expected has not yet been determined.

¹³C NMR sensitivity (S/N per unit time) was measured on the brains of two intact rats after death, each having been infused previously with [1-¹³C]glucose (Fig. 4). The ¹³C NMR sensitivity to detection of cerebral lactate and glutamate was compared to that obtained by ¹H NMR using the ¹³C-¹H pulse sequence *in vivo*. When corrections were made for concentration differences, ¹H NMR gave a S/N enhancement over ¹³C NMR of 11-fold for [3-¹³CH₃]lactate detection (3.6 per proton) and a 6-fold S/N enhancement for [4-¹³CH₂]glutamate (3.0 per proton). A factor of 2 in potential ¹H sensitivity enhancement was lost *in vivo* (relative to an analytical sample) because the ¹H linewidths ($\Delta\nu_{1/2} \approx 30\text{--}40$ Hz) were ≈4 times broader than ¹³C linewidths ($\Delta\nu_{1/2} \approx 7\text{--}10$ Hz). A small additional loss of ¹H sensitivity was due to low-frequency baseline oscillations in the ¹³C difference spectrum caused by the method of water suppression.

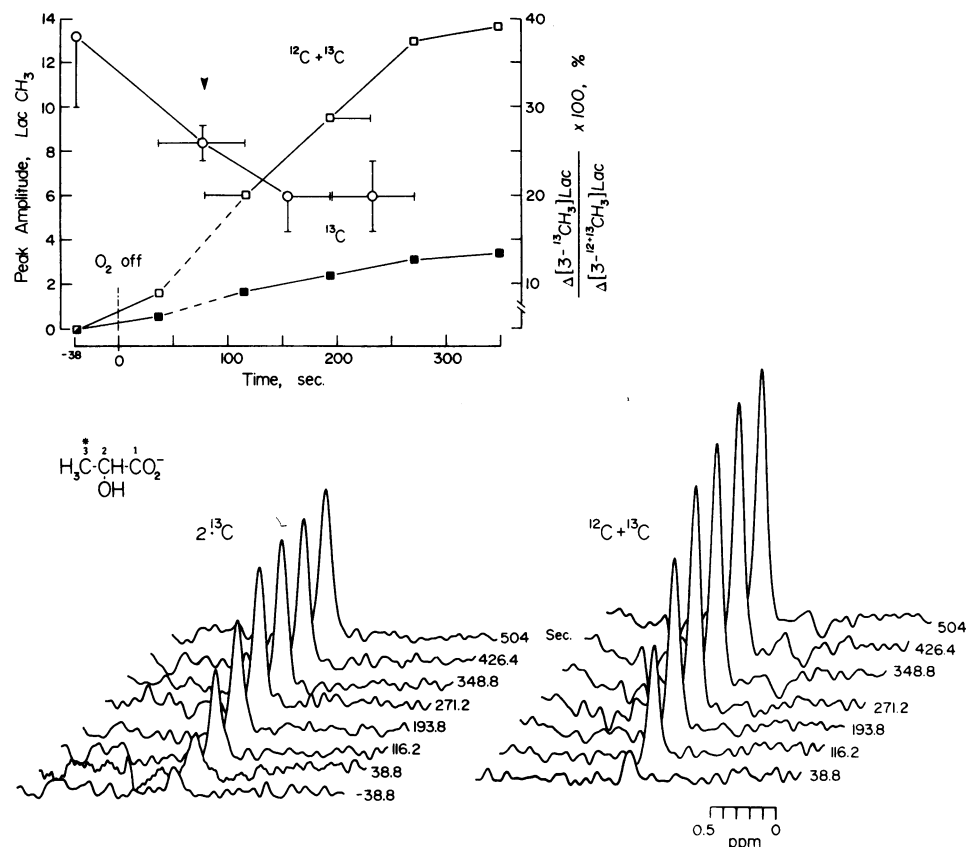


FIG. 3. (Upper) Amplitudes (arbitrary units) of the proton resonances of [3-¹³CH₃]lactate (■) and [3-¹²⁺¹³CH₃]lactate (□) are plotted as a function of time after the start of terminal anoxia ($t = 0$). Spectra were acquired by using the {¹³C}-¹H sequence with 2 W of single-frequency ¹³C decoupling centered at the resonance of [3-¹³CH₃]lactate at 20.9 ppm in the ¹³C NMR spectrum. Each spectrum required 83 sec to acquire (horizontal bar), of which 64 sec was used for acquisition (64 scans; 1 sec per scan). The amplitude uncertainty, derived from rms noise, is ± 0.09 amplitude units (vertical scale). Because linewidths did not change during the measurement period, the plotted resonance amplitudes are directly proportional to resonance intensities. Amplitudes of both [3-¹³CH₃]- and [3-¹²⁺¹³CH₃]lactate are set equal to zero at $t = -38$ sec, which represents the midpoint time of the last measurement made during hypoxia (10% O₂). Cessation of brain perfusion (estimated from the electrocardiogram) is indicated on the plot by an arrowhead at 80 sec. In addition, the fractional ¹³C enrichment of newly formed lactate measured over 83-sec time intervals (horizontal bar) during ischemia is plotted as $\{(\Delta[3-^{13}\text{CH}_3]\text{lactate})/(\Delta[3-^{12+13}\text{CH}_3]\text{lactate})\} \times 100$ (○). The fractional enrichment value at $t = -38$ sec was measured from the sum of three spectra acquired during the last 4 min of hypoxia (6 min at 10% O₂). Vertical bars are the fractional enrichment uncertainty calculated from rms noise. Fractional enrichment values are all overestimated by the same factor ($\approx 15\%$) because they were not corrected for the greater T₁-related saturation of [3-¹²CH₃]lactate than [3-¹³CH₃]lactate and the small increase in linewidth of [3-¹³CH₃]lactate ($\Delta\nu_{1/2} = 35$ Hz) relative to [3-¹²CH₃]lactate ($\Delta\nu_{1/2} = 30$ Hz) due to incomplete decoupling. (Lower) Stacked plots of the resonances of [3-¹³CH₃]- and [3-¹²⁺¹³CH₃]lactate, as plotted in Upper. The midpoint time (in seconds) to the right of each spectrum refers to the corresponding plot time in Upper. Resonances of [3-¹³CH₃]lactate were obtained from ¹³C difference spectra while resonances of [3-¹²⁺¹³CH₃]lactate were obtained by subtracting from each (¹²C + ¹³C) subspectrum acquired after anoxia, the last (¹²C + ¹³C) subspectrum acquired during hypoxia ($t = -38$ sec). The resonance of [3-¹³CH₃]lactate at $t = -38$ sec (hypoxia) represents a concentration of ≈ 0.6 $\mu\text{mol/g}$ of wet weight when normalized to the intensity of total creatine and corrected for T₁-related differential saturation. Spectra were processed as in Fig. 2B.

DISCUSSION

The results obtained in this study show that ¹H NMR and the {¹³C}-¹H sequence have several advantages over ¹³C NMR for measuring *in vivo* the ¹³C fractional enrichment of brain metabolites. Application of the technique led to a 6-fold S/N enhancement over ¹³C NMR for detection of [4-¹³CH₂]glutamate and an 11-fold enhancement for [3-¹³CH₃]lactate; presumably, these enhancements can be brought closer to the theoretical limit in the future. The ability to monitor both ¹³C-labeled and unlabeled metabolites allows the increase in the ¹³C fractional enrichment of [4-CH₂]glutamate to be distinguished from an increase in glutamate concentration, while the contribution of unlabeled substrates to brain glycolysis during ischemia is readily measured. When {¹³C}-¹H NMR is used in conjunction with ¹³C NMR, the concentrations of ¹³C-enriched metabolite resonances in the ¹³C spectrum can be determined: this is accomplished by comparison of resonance intensities in the ¹³C difference spectrum with

the creatine resonance in the (¹²C + ¹³C) subspectrum (16). This is a particularly useful approach for ¹³C NMR-based studies as no suitably stable natural abundance signal(s) exists at concentrations high enough to detect by ¹³C NMR. However, concentrations can be assigned to resonances in the ¹³C NMR spectrum by interleaving {¹³C}-¹H spectra with normal ¹³C NMR spectra (see Fig. 4).

Because the primary goal of this study was to show the uses of this spectroscopic method, the fractional ¹³C enrichment of blood glucose was not measured. In combination with measurements of blood glucose fractional enrichment, as shown in a recent ¹³C NMR study of rabbit brain (24), the present sensitivity of the {¹³C}-¹H technique should allow accurate measurement of turnover times for brain metabolites with concentrations above 1 mM; these include aspartate, γ -aminobutyric acid, glutamate, glutamine, and, under certain conditions, lactate. For those resonances that are partially overlapped in the (¹²C + ¹³C) subspectrum, a recently introduced technique based on ¹H-¹H J couplings (25) has been

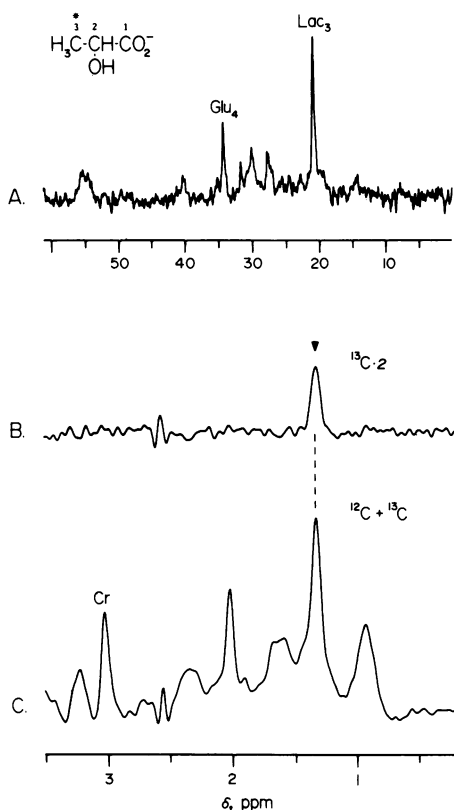


FIG. 4. (A) A ^1H decoupled ^{13}C NMR spectrum acquired post-mortem (≈ 1 hr after death) from the brain of an intact rat infused i.v. with 360–370 mg of $[1-^{13}\text{C}]$ glucose prior to death. The spectrum was acquired through the ^{13}C channel of the double-tuned $^1\text{H}/^{13}\text{C}$ probe. The spectrum is the sum of 2048 scans with a 1.1-sec recycle time (4096 data points, 15-KHz sweep width centered at 50 ppm). Broad-band (6 ppm) ^1H decoupling at 2 W was used during acquisition (137 msec) and this was maintained at 0.2 W to allow nuclear Overhauser effect development. Labeled resonances are $[3-^{13}\text{CH}_3]$ lactate at 20.9 ppm (Lac_3) and $[4-\text{CH}_2]$ glutamate at 34.2 ppm (Glu_4). The spectrum was processed with an exponential filter (10 Hz) to give optimal S/N. (B) A $\{^{13}\text{C}\}$ - ^1H sequence ^{13}C difference spectrum (32 scans; 6 sec per scan) of $[3-^{13}\text{CH}_3]$ lactate (arrowhead) acquired immediately prior to the spectrum in A. By comparing the intensity of the resonance of $[3-^{13}\text{CH}_3]$ lactate in the spectrum in B with the intensity of the creatine methyl resonance (Cr_{tot}) in the $(^{12}\text{C} + ^{13}\text{C})$ subspectrum (C), a concentration of $\approx 5.4 \mu\text{mol/g}$ of wet weight was estimated for the $[3-^{13}\text{CH}_3]$ lactate resonance in the ^{13}C NMR spectrum (A).

shown to resolve cleanly several unlabeled ^1H resonances in the rat brain (26) and, in conjunction with the $\{^{13}\text{C}\}$ - ^1H sequence, should allow the accurate measurement of absolute fractional enrichments. It should be possible to increase the S/N by a factor of 2 in studies of this type by using $[1,6-^{13}\text{C}]$ - or $[U-^{13}\text{C}]$ glucose. Although the use of $[1,6-^{13}\text{C}]$ glucose would be advantageous, as information about labeling patterns is not lost, it is not currently practical due to its high cost and limited availability. We expect that no loss in sensitivity should occur with $[U-^{13}\text{C}]$ glucose, as a result of two and three bond ^1H - ^{13}C couplings, because these couplings

are small ($J_{\text{CH}} \approx 5$ Hz) and will be collapsed by off-resonance ^{13}C decoupling during acquisition.

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