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Detection and Assignment of the Glucose Signal in ^{1}H NMR Difference Spectra of the Human Brain

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The difference between ^{1}H NMR spectra obtained during eu- and hyperglycemia exhibited well-resolved glucose peaks between 3 and 4 ppm as demonstrated by comparison with solution spectra. Estimated increases were consistent with recent ^{13}C NMR quantitations of intracerebral glucose. Difference spectra were measured in 36-ml volumes from the human brain every 3 min. © 1992 Academic Press, Inc.

INTRODUCTION

The normal human brain depends on adequate glucose and oxygen supply from blood. Whereas oxygen can diffuse freely into the brain, glucose has to be transported across the endothelial cell membranes comprising the blood–brain barrier. If energy demand is increased or transport altered, the rate of metabolism may be limited by transport such that energy production is insufficient to maintain normal brain function. The intracerebral glucose concentration is a function of metabolism and bidirectional transport rates across the blood–brain barrier (*1*).

In a recent study we have quantified brain glucose concentrations in humans using ^{13}C NMR and intravenous infusions of D-[1-^{13}C]glucose. These measurements showed that the brain glucose concentration was approximately 1 m*M* at basal plasma glucose levels (euglycemia) and increased with plasma glucose concentration (*2*). While the use of ^{13}C-enriched glucose has the advantage of allowing a direct observation in a region of the carbon spectrum that is free from overlap with other resonances, the method is limited by inherently low sensitivity and requires sophisticated infusion protocols.

^{1}H NMR offers an alternative method of measuring the brain glucose signal with a much higher sensitivity. However, its direct observation is hampered by the close proximity of the water signal and overlap with coupled resonances from taurine, inositol, glutamate, and glutamine, all of which are more concentrated in the brain (*3*). The presence of glucose signals has been reported in ^{1}H NMR spectra obtained from hyperglycemic diabetic patients (*4*). However, the resonances of glucose were not resolved in these spectra.

In order to remove the complications introduced by extensive overlap we have obtained ^{1}H NMR spectra of glucose from the brain of healthy volunteers by taking

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the difference between spectra obtained at euglycemia and during glucose infusions at hyperglycemia. As such difference spectra contain only signals from glucose, the high sensitivity of ^{1}H NMR allowed measurement of changes in glucose levels with a 3-min time resolution.

METHODS

All experiments were carried out on a 2.1-T whole-body magnet (Bruker-ORS, Billerica, MA). The subjects were positioned on a 6-cm-diameter surface coil in the supine position. Positioning was based on magnetic resonance images obtained in sagittal and transverse planes. Localized automatic shimming was performed on all first- and second-order shim coils according to a recently suggested procedure (*5*). A volume of 36 ml was localized in the occipital lobe of the brain using the pulse sequence depicted in Fig. 1, which is a variant of a previously used sequence (*6*): Three-dimensional localization was obtained with ISIS (*7*) in conjunction with 8-ms hyperbolic secant pulses ((*8*) μ = 5, 2 kHz bandwidth). Outer volume suppression using 10-ms noise pulses (*9*) was applied in the two planes perpendicular to the coil. The signal was detected as a spin echo (TE = 48 ms) generated by a sinc pulse defined over $\pm 5\pi$ exciting a slice parallel to the surface coil plane in conjunction with a semiselective binomial refocusing θ_x–τ–θ_{-x} pulse, where τ was set to 5 ms. The use of the semiselective refocusing pulse separates the spatial and frequency selectivity and generates a positive amplitude throughout the spectrum (*10*). A $\theta/3$ pulse prior to the localization sequence was used to reduce signals from high B_1 flux regions (*11*). Unwanted transverse coherences were dephased

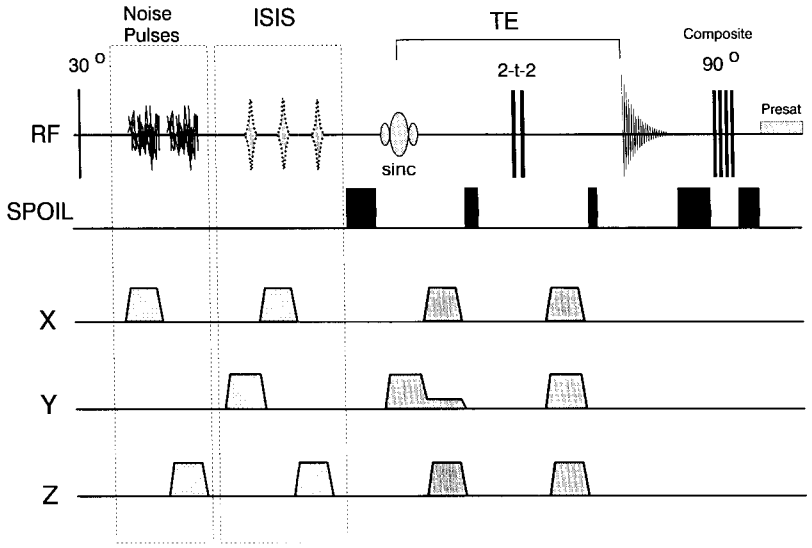

FIG. 1. Pulse sequence. The sequence uses the full sensitivity of the spin echo whose total echo time (TE) was set to 48 ms. Water suppression was achieved with the semiselective refocusing pulse and with low-power presaturation during the recovery delay of 2 s. The choice of τ = 5 ms led to a maximum signal at 3.55 ppm. The reduced signal intensities between 2 and 3 ppm are due to the second null of the excitation profile at 2.4 ppm (Fig. 2).

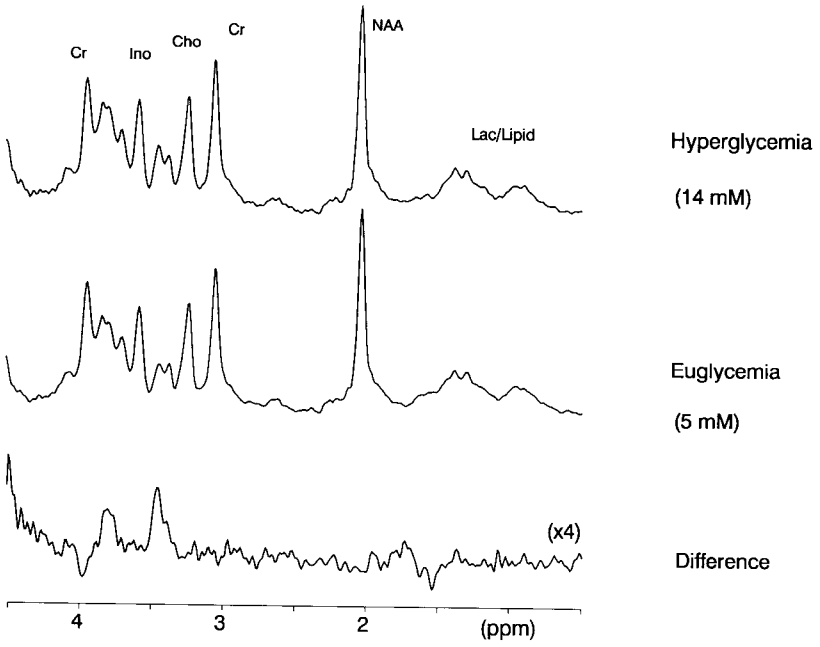

FIG. 2. ^{1}H NMR spectra acquired before and during glucose infusion. The spectra represent 9 min summations prior to glucose infusion (middle) where the plasma glucose concentration was 5 mM and 9 min during the infusion (top), at 14 mM plasma glucose. The difference spectrum was obtained by correcting for field drift only, i.e., by shifting the zero-filled spectra digitally. The FIDs were multiplied prior to Fourier transformation by an exponential corresponding to 1 Hz line broadening. Cr, (Phospho)creatine; Cho, choline; Ino, inositol; NAA, *N*-acetyl-aspartate which was used as chemical-shift reference (2.02 ppm).

by placing asymmetric surface gradient spoiler pulses (*12*) throughout the sequence. Longitudinal magnetization was minimized after the data acquisition period of 256 ms by applying a Levitt–Ernst 90° composite pulse (*13*), i.e., $(\theta/2)_x(\theta/2)_y(\theta/2)_x(\theta/2)_{-y}$. Water suppression was achieved by the semiselective refocusing pulse and by low-power presaturation (<0.2 Watts total) during the recovery delay, which was applied with a second low-power amplifier through a directional coupler. In order to correct for field drift during blood sampling, spectra were collected in 3-min blocks (64 scans, 2.5 s repetition time). Difference spectra were obtained by extensive zero filling and corrected for frequency shifts only by digital shifting.

Catheters were placed into the left antecubital vein for the glucose infusion and the right antecubital vein was catheterized to obtain the blood samples. Plasma glucose concentrations were measured every 5 min with the glucose oxidase technique in a Beckman glucose analyzer (Beckman, Fullerton, CA). A variable priming infusion of glucose (dextrose, 20% weight/volume) was begun at $t = 0$ min so that the plasma glucose concentration was raised acutely and maintained approximately 10 mM above baseline for 60 min according to previously described procedures (*14*). Two subjects, a 24-year-old male and a 27-year-old female, gave full written informed consent according to procedures and forms approved by the Yale Human Investigations Committee.

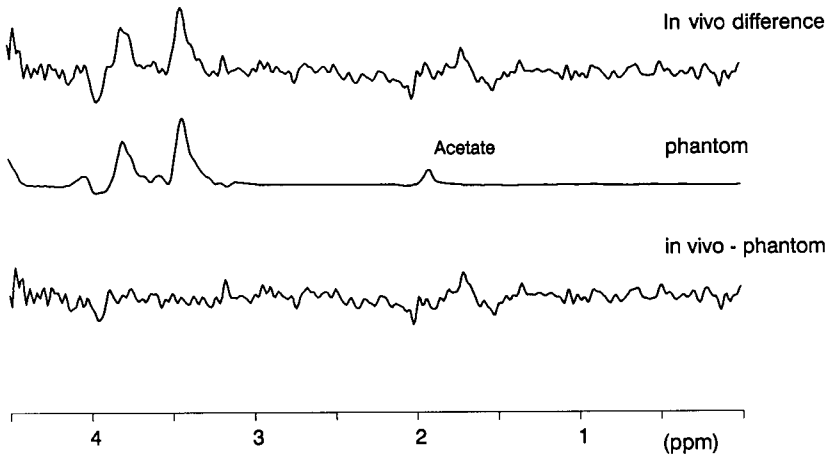

FIG. 3. Comparison of *in vivo* difference spectra to that of glucose in solution. The *in vivo* difference spectrum (top) obtained from the same subject as in Fig. 2 represents here the difference of 45 min of data accumulation during hyperglycemia (13 m*M* plasma) and 9 min during euglycemia (5 m*M*). The solution spectrum (middle) was obtained under identical experimental conditions from a preheated 2-liter bottle (37°C) placed on the surface coil containing 50 m*M* glucose, 45 m*M* phosphate buffer (pH = 7.1), and 10 m*M* acetate, and was line broadened until the linewidth of acetate matched that of singlets (Cr, NAA) in the *in vivo* spectra. The amplitude of the solution spectrum was adjusted to match that of the *in vivo* difference spectrum at 3.44 ppm. The bottom spectrum is the difference of the top two spectra.

RESULTS AND DISCUSSION

Figure 2 shows localized brain ^{1}H NMR spectra obtained before and during glucose infusion. A linewidth of 4 Hz was achieved on the *N*-acetyl-aspartate (NAA) and creatine (Cr) peaks and a stable baseline was present up to 4.3 ppm.

We observed in both subjects after the start of the infusion a distinct rise in the spectra at 3.4 ppm accompanied by an increase at 3.8 ppm which is shown by the fourfold-enlarged difference spectrum at the bottom of Fig. 2. The changes are, however, small relative to background resonances so that the overall spectral appearance is only slightly altered. Figure 3 compares an *in vivo* difference spectrum with a spectrum obtained under identical experimental conditions from a phantom solution (50 m*M* glucose, 45 m*M* phosphate buffer, pH = 7.1, 10 m*M* acetate, 37°C). The resonances at 3.44 and at 3.81 ppm show close correspondence to the glucose resonances in the solution spectrum. By subtracting the solution spectrum (middle trace in Fig. 3) from the *in vivo* difference spectrum (top) the increase in resonance intensity can be cancelled to the level of spectral noise (bottom). This is consistent with the difference spectrum being entirely due to glucose. The stability of the spectra was high, which allowed difference spectra to be obtained every 3–4 min during the infusion as shown in Fig. 4A. A plot of the area of the peak at 3.44 ppm against time is compared with the steep rise in plasma glucose in Fig. 4B. A lag in the increase in brain glucose signal relative to the time course of plasma glucose is observed.

In order to estimate the increase in glucose concentration the increment in glucose signal at 3.44 ppm was integrated from 3.3 to 3.6 ppm and compared to the area of creatine at 3.03 ppm integrated from 2.96 to 3.15 ppm in spectra baseline corrected

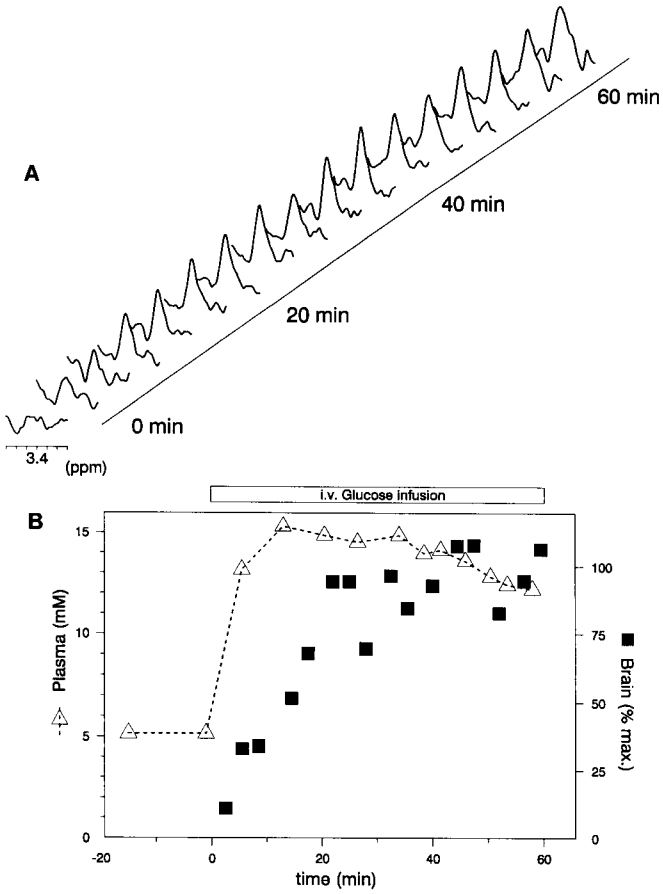

FIG. 4. Time course of the change in the glucose signal. (A) Stacked plot of the 3.44-ppm resonance of glucose in the *in vivo* spectra. A time resolution of 3 to 4 min was achieved by subtracting an averaged 9-min spectrum obtained at euglycemia from each 3-min spectrum obtained during infusion (same subject as in Fig. 2). Processing consisted of 4 Hz exponential multiplication and baseline correction from 2.5 to 4.5 ppm. (B) Time course of the peaks in (A) integrated from 3.3 to 3.6 ppm (solid squares), expressed in percentage of the maximum increase. Plasma glucose concentrations (triangles) were measured in a Beckman glucose analyzer.

from −1 to 8 ppm. To correct for the frequency selective excitation profile of the semiselective refocusing pulse and for J modulation, the pulse sequence was applied to a phantom containing equimolar glucose and creatine and an integral ratio of approximately 1.5 was obtained. Assuming the integral of the total creatine signal (including contributions from other metabolites, e.g., GABA) to represent *in vivo* approximately 10 mM (*3*), a 1.3 and a 1.5 mM increment was estimated in the two subjects, where plasma glucose concentration rose from euglycemia (5 mM) to 14 and 17 mM, respectively. These changes are consistent with ^{13}C NMR measurements (*2*), where a brain glucose concentration of 2.5 mM was measured at 12 mM plasma glucose and 1.0 mM at 5 mM in plasma glucose. However, the influence of relaxation

times and diffusion losses due to the localization sequence need to be determined *in vivo,* before changes in glucose concentration can be accurately quantified.

These results demonstrate the presence of glucose signals in localized ^{1}H NMR difference spectra from the human brain. A limitation of ^{1}H NMR is that signals from glucose are obscured in the ^{1}H NMR spectrum by several other resonances (*4*), whereas after infusion of D-[1-^{13}C]glucose, the quantitation can be performed directly in a region of the carbon spectrum that is free from other resonances (*2*). We estimate that at euglycemia glucose contributes at most a third to the amplitude of the peak at 3.44 ppm since an estimated 1.5 m*M* increase in brain glucose produced a 40–55% increase in the signal amplitude at 3.44 ppm in the baseline spectrum. Accurate direct quantitation of total glucose concentration in the 3–4 ppm region is therefore unlikely. However, with further improvements in technology it may be possible to observe and quantitate the ^{1}H peak of α-glucose at 5.24 ppm, which should be free from overlap with other resonances provided the intense water peak can be adequately suppressed.

As demonstrated in Fig. 4 the high sensitivity of the ^{1}H measurement allows dynamic changes in brain glucose to be measured. Potential applications range from a regional assessment of glucose transport kinetics to dynamic studies of glucose metabolism during functional stimulation.

ACKNOWLEDGMENTS

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REFERENCES

1. H. LUND-ANDERSEN, *Physiol. Rev.* **59,** 305 (1979).
2. R. GRUETTER, E. J. NOVOTNY, S. D. BOULWARE, D. L. ROTHMAN, G. I. SHULMAN, G. F. MASON, R. G. SHULMAN, AND W. V. TAMBORLANE, *Proc. Natl. Acad. Sci. USA* **89,** 1109 (1992).
3. O. A. C. PETROFF, D. D. SPENCER, J. R. ALGER, AND J. W. PRICHARD, *Neurology* **39,** 1197 (1989).
4. T. MICHAELIS, K. D. MERBOLDT, W. HÄNICKE, M. L. GYNGELL, AND J. FRAHM, *NMR Biomed.* **5,** 90 (1991); R. Kreis and B. D. Ross, "10th Annual Meeting, Society of Magnetic Resonance in Medicine, San Francisco, August 10–16, 1991," p. 401.
5. R. GRUETTER AND CH. BOESCH, *J. Magn. Reson.* **96,** 323 (1992).
6. J. W. PRICHARD, D. L. ROTHMAN, E. J. NOVOTNY, O. A. C. PETROFF, T. KUWABARA, M. J. AVISON, A. M. HOWSEMAN, C. C. HANSTOCK, AND R. G. SHULMAN, *Proc. Natl. Acad. USA* **88,** 5829 (1991).
7. R. J. ORDIDGE, A. CONNELLY, AND J. A. B. LOHMAN, *J. Magn. Reson.* **66,** 283 (1986).
8. M. S. SILVER, R. I. JOSEPH, AND D. I. HOULT, *J. Magn. Reson.* **59,** 347 (1984); J. Baum, R. Tycko, and A. Pines, *Phys. Rev. A* **32,** 3435 (1985).
9. A. CONNELLY, C. COUNSELL, J. A. B. LOHMAN, AND R. J. ORDIDGE, *J. Magn. Reson.* **78,** 519 (1988).
10. P. BLONDET, M. DECORPS, S. CONFORT, AND J. P. ALBRAND, *J. Magn. Reson.* **75,** 434 (1987).
11. A. J. SHAKA AND R. FREEMAN, *J. Magn. Reson.* **62,** 340 (1985).
12. W. CHEN AND J. J. H. ACKERMAN, *NMR Biomed.* **1,** 205 (1989).
13. M. H. LEVITT AND R. R. ERNST, *J. Magn. Reson.* **55,** 247 (1983).
14. G. I. SHULMAN, D. L. ROTHMAN, T. JUE, P. STEIN, R. A. DEFRONZO, AND R. G. SHULMAN, *N. Engl. J. Med.* **322,** 223 (1990).