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Cerebral Glucose Is Detectable by Localized Proton NMR Spectroscopy in Normal Rat Brain *in Vivo*

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This contribution reports the first direct and noninvasive observation of cerebral glucose in normal anesthetized rats (n = 16) using short-echo-time localized proton NMR spectroscopy (2.35 T, STEAM, TR = 6000 ms, TE = 20 ms, 125 μl). In addition to resonances from *N*-acetyl aspartate (NAA), glutamate, total creatine, cholines, taurine, and *myo*-inositol, all spectra exhibit strongly coupled resonances from glucose (3.43, 3.80 ppm) that are readily identifiable using model solutions. The observed level of cerebral glucose in fasted rats covered a range of 15–40% of that of NAA giving absolute concentrations of 1.1–2.8 mM when NAA is taken to be 7 mM. The arterial blood glucose concentration was 7.7 ± 0.8 mM in the same group of animals.

INTRODUCTION

Glucose plays a central role in the energy metabolism of the brain providing more than 90% of its fuel (*1, 2*). Deviations of the cerebral glucose concentration under hypoglycemic or hyperglycemic (e.g., diabetes mellitus) conditions have pronounced consequences of foremost importance to clinical medicine. Analytical determinations of glucose in brain parenchyma (*3, 4*) require tissue destruction and are therefore only applicable to animals. Freeze-clamp techniques may also be subject to ischemic artifacts that develop in the time span between sacrifice of the animal and destruction of enzymatic functions, e.g., glucose delivery by glycogenolysis and glucose consumption by anaerobic glycolysis.

More recently, a noninvasive NMR approach has been reported (*5, 6*) for measuring brain glucose levels by means of an indirect proton-observe carbon-edited NMR technique in live animals. Unfortunately, the investigation requires the infusion of carbon-13-enriched glucose so that brain glucose levels are investigated in the presence of high plasma concentrations beyond physiological conditions. Here we report on the first direct observation of cerebral glucose levels in normal anesthetized rats using image-guided, localized proton NMR spectroscopy. Since proton NMR considerably simplifies the experimental setup as well as the absolute quantification of metabolite concentrations, this method is expected to facilitate further studies of glucose transport in the brain by correlating plasma levels with *in vivo* cerebral levels.

MATERIALS AND METHODS

All studies were carried out at 100 MHz using a Biospec system (Bruker, Karlsruhe, FRG) equipped with a 2.35-T 40-cm-bore horizontal magnet and an actively shielded

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gradient system (Oxford Instruments, Oxford, UK). Homogeneous radiofrequency (rf) excitation was performed using a homebuilt 12-cm Helmholz coil with signal reception by a 2-cm-diameter loop coaxial transmission line resonator. All modifications of the standard Bruker hardware configuration have been previously described (*7*). Localized proton NMR spectra were obtained with the use of a STEAM sequence employing three chemical-shift-selective (CHESS) rf pulses for water suppression:

$$[\text{CHESS–Spoil}]_3\text{–}90°(x)\text{–TE/2–}90°(y)\text{–TM–}90°(z)\text{–TE/2–Acq.}$$

In order to minimize T_2 relaxation losses and to avoid spectral complications due to complex J modulation, the echo time was set to TE = 20 ms (TM = 30 ms) in all cases. Fully relaxed proton NMR spectra were acquired from a $5 \times 5 \times 5$-mm^3 (125-μl) volume-of-interest (VOI) positioned centrally in the brain. Water suppression resulted in a ratio of residual water/*N*-acetyl aspartate (NAA) in the range 2–10, thus avoiding baseline artifacts in the spectra. Data processing consisted of zero filling to 8K complex points, 2.0 Hz Gaussian multiplication in the time domain, and Fourier transformation followed by zero- and first-order phase correction. Measuring times of individual spectra were 6.4 and 12.8 min using 128 scans and repetition times of TR = 3000 and 6000 ms, respectively.

The study included 14 Wistar albino rats (350–500 g body wt) and 2 Sprague–Dawley rats (180–200 g body wt). Moreover, glucose resonances were frequently observed in other rats ($n \approx 70$) investigated in this lab. The animals were anesthetized using intraperitonial chloralhydrate (360 mg/kg body wt) and placed in a prone position with the receiver coil positioned over the head without surgery. More recently, halothane anesthesia (1.0% in 7:3 N_2O:O_2) has been employed. No change in the detectability of glucose was observed. Arterial plasma glucose levels were determined enzymatically (*8*).

RESULTS

The assignment of the strongly coupled resonances of glucose at a field strength of 2.0 T as well as their observation in localized proton NMR spectra of gray matter in human diabetics has been reported in preceding papers (*9, 10*). The appearance of the respective resonances at a field strength of 2.35 T is demonstrated in Fig. 1. It demonstrates the influence of strong coupling at the lower field strength which effectively simplifies the multiplet structure observed at 7.0 T. Following line broadening to mimic the *in vivo* linewidths of 6–7 Hz in proton NMR spectra of rat brain at 2.35 T, three prominent resonances remain at 3.43, 3.80, and 3.86 ppm. In Fig. 1d glucose is best appreciated by its 3.43 ppm resonance even though it overlaps the outermost up-field resonance of taurine (see below). Depending on the glucose concentration the other two resonances contribute to a shoulder on the 3.75 ppm CH resonance of glutamate.

Localized proton NMR spectra of model solutions with different concentrations of glucose are shown in Fig. 2. The model solutions contain mixtures of cerebral metabolites with *relative* concentrations that resemble the spectral pattern observed in rat brain *in vivo*: 12 m*M* NAA and glutamate, 10 m*M* creatine, 2 m*M* choline, 3 m*M* taurine, and 8 m*M* *myo*-inositol. The known ratios and the resulting spectral patterns can be used to establish *relative* glucose concentrations under fully relaxed conditions.

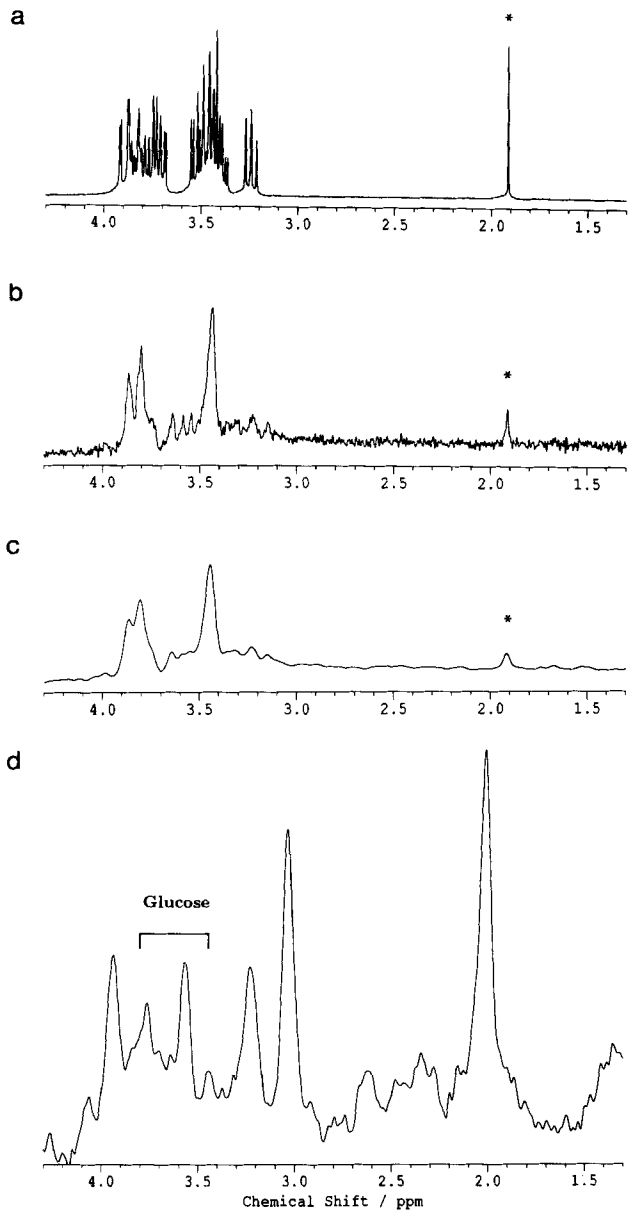

FIG. 1. Proton NMR spectra of an aqueous solution of glucose at (a) 7.0 T and (b,c) 2.35 T in comparison to (d) a 2.35-T proton NMR spectrum of rat brain *in vivo*. (a) Fully relaxed FID spectrum of glucose (7.0 T, pH 7.2, 50 m*M*, *acetate reference at 1.915 ppm); (b) fully relaxed, localized proton spectrum of glucose (2.35 T, STEAM, 125-μl VOI, TE = 20 ms, pH 7.2, 50 m*M* in a 10-ml spherical flask, *reference); (c) line-broadened version of spectrum (b) yielding major resonances at 3.43, 3.80, and 3.86 ppm; and (d) localized proton NMR spectrum of rat brain *in vivo* (2.35 T, STEAM, 125-μl VOI, TE = 20 ms, TR = 3000 ms, 128 scans).

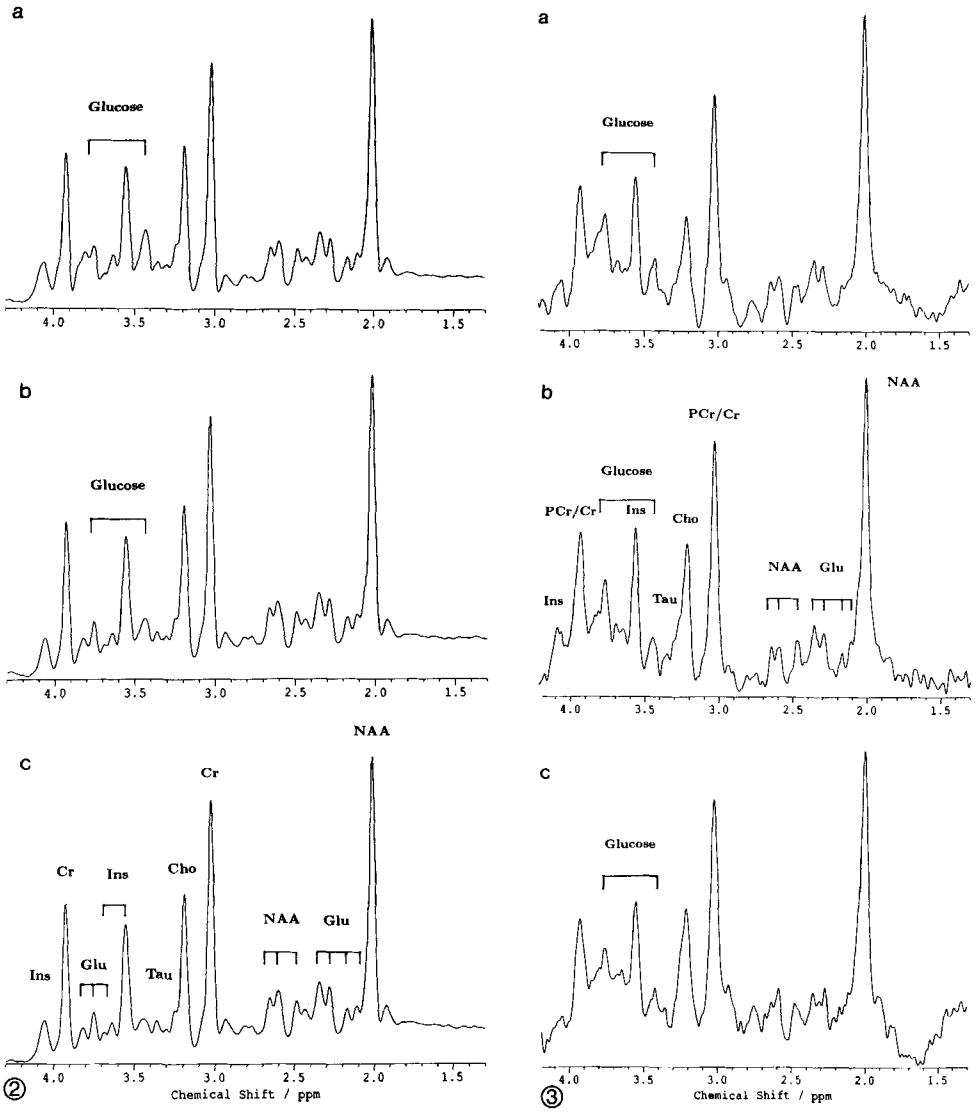

FIG. 2. Localized proton NMR spectra of metabolite model solutions (2.35 T, STEAM, 125-μl VOI, TR = 6000 ms, TE = 20 ms, TM = 30 ms, 64 scans, 6.4 min, pH 7.1) comprising 10 mM creatine (Cr), 12 mM *N*-acetyl aspartate (NAA), 12 mM glutamate (Glu), 2 mM choline (Cho), 3 mM taurine (Tau), and 8 mM *myo*-inositol (Ins). The spectra represent the metabolic pattern of normal rat brain for three different concentrations of glucose and are significantly line-broadened to mimic the *in vivo* resolution. (a) 6 mM glucose (50% of NAA), (b) 2 mM glucose (17% of NAA), and (c) no glucose.

FIG. 3. Localized proton NMR spectra of rat brain *in vivo* (2.35 T, STEAM, TR = 6000 ms, TE = 20 ms, TM = 30 ms, 128 scans, 12.8 min, 2 Hz line broadening) acquired from a centrally located 125-μl VOI as shown in Fig. 1. The spectra originate from three different animals and represent the range of glucose levels observed in this study. Resonances are due to *N*-acetyl aspartate (NAA), glutamate (Glu), phosphocreatine and creatine (PCr/Cr), cholines (Cho), taurine (Tau), *myo*-inositol (Ins), and glucose.

In Fig. 2 the glucose concentration is zero in the bottom spectrum (c), 2 m*M* in the middle spectrum (b), and 6 m*M* in the top spectrum (a). This corresponds to 17 and 50% of the NAA concentration for spectra (b) and (a), respectively.

Figure 3 shows *in vivo* brain spectra obtained from three different rats. The spectra cover the range of glucose signal intensities observed in this study. In general, the interindividual reproducibility of the metabolite resonances is excellent. Resonance assignments follow the data presented in Fig. 2 as well as a previous study on proton NMR spectroscopy of rat brain *in vivo* at 2.35 T (*7*). Minor signal variations were observed for choline-containing compounds, whereas the glucose intensity shows a certain degree of variability. The relative brain tissue concentrations of glucose are summarized in Table 1 and compared to the mean arterial blood glucose level determined for the same group of animals. Cerebral glucose levels are given in units of the NAA and total creatine concentrations relative to the model solutions shown in Fig. 2. Absolute concentrations are discussed below.

To further confirm the NMR assignment of glucose resonances, Fig. 4 shows the metabolic alterations obtained for two rats before and 25 min after death. Sacrifice of the animals was accomplished by intraperitoneal potassium cyanide. While the *in vivo* spectra show glucose resonances within the range described by Fig. 3, the postmortem spectra exhibit no detectable glucose signals but strongly elevated levels of lactate. Assuming a 7 m*M* concentration of NAA (see below), the accumulation of about 20 m*M* lactate originates from continued glycogenolysis and anaerobic glycolysis. Depletion of the steady-state level of cerebral glucose removes spectral contributions at 3.43, 3.80, and 3.86 ppm and allows three out of four strongly coupled resonances of taurine (3.26, 3.32, 3.38, and 3.43 ppm) to be resolved. The overall reduction in peak amplitudes postmortem is due to a slightly poorer magnetic field homogeneity leading to line broadening.

DISCUSSION

The feasibility of direct and noninvasive observation of glucose in localized proton NMR spectra of rat brain *in vivo* provides a unique means for monitoring glucose concentration in tissues. For this purpose the identification of glucose resonances must be followed by a quantification of cerebral concentrations. The relative concentration

TABLE 1

Concentrations of Arterial Plasma Glucose and Brain Glucose in Normal 24-h-Fasted Rats (n = 14) as Determined by Enzymatic Blood Analysis and Proton NMR Spectroscopy *in Vivo*, Respectively

	Brain		
Plasma (m*M*)	% [PCr/Cr]	% [NAA]	m*M*[a]
7.7 ± 0.8	20–50	15–40	1.1–2.8

[a] Absolute concentrations of cerebral glucose are based on the assumption of a 7 m*M* concentration of *N*-acetyl aspartate.

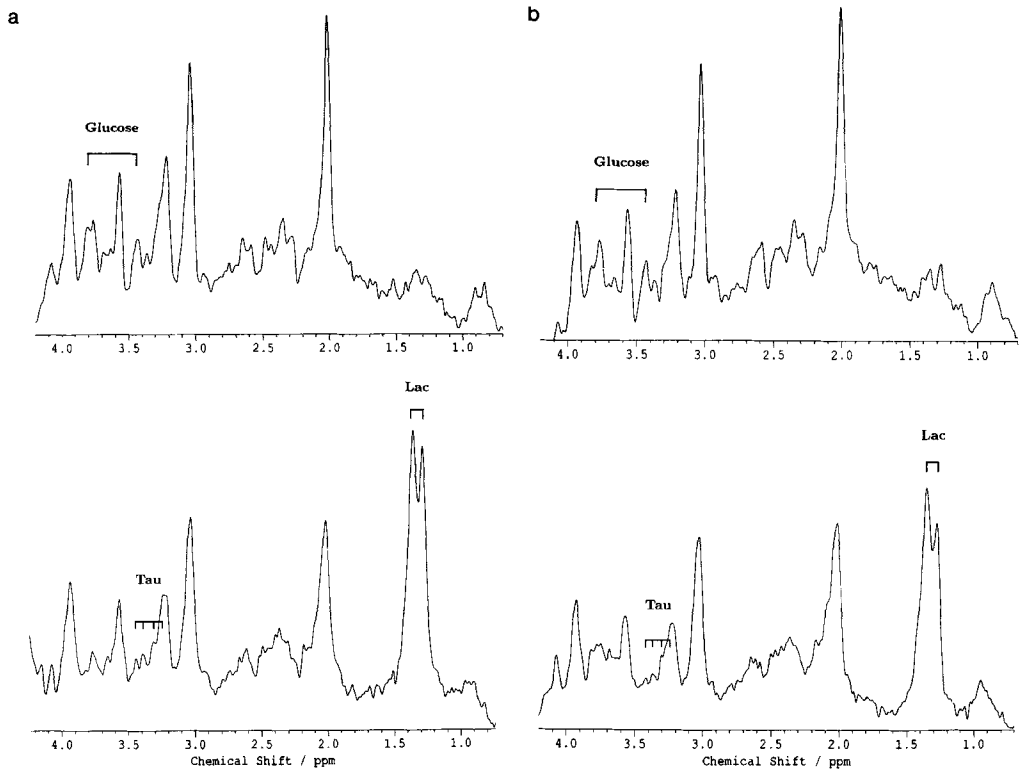

FIG. 4. Localized proton NMR spectra of rat brain before and 25 min after death (2.35 T, STEAM, TR = 3000 ms, TE = 20 ms, TM = 30 ms, 128 scans, 6.4 min, 2 Hz line broadening). The spectra in (a) and (b) originate from two different animals. The postmortem spectra show elevated levels of lactate (Lac), a depletion of glucose, and a concomitant resolution of three out of four taurine (Tau) resonances (3.26, 3.32, 3.38, and 3.43 ppm). Magnetic field homogeneity is compromised in the postmortem spectra.

of glucose was estimated by comparing the *in vivo* spectra with those of model solutions with various glucose concentrations (e.g., see Fig. 2). The results are given in Table 1.

Absolute concentrations require an internal reference. For example, the total creatine concentration is generally assumed to be 10 mM (*11*) which would result in cerebral glucose concentrations of 2.0–5.0 mM. However, the creatine data from tissue extracts contradict our recent findings in human brain *in vivo*. Following three different strategies for absolute quantification (water reference, external phantom, separate phantom studies) the NAA concentration turns out to be about 10 mM, yielding a total creatine concentration of only 5–6 mM (*12*). Assuming the same creatine concentration in rat brain, the NAA concentration will be 7 mM in agreement with a large number of biochemical determinations (*13*).

Assuming a 7 mM NAA concentration the cerebral glucose is in the range 1.1 to 2.8 mM as indicated in the last column of Table 1. This value corresponds to a ratio of brain glucose to arterial blood glucose in anesthetized rats of 0.26 as opposed to

0.45 if 10 mM creatine is assumed. More detailed insights into glucose transport require certainty about the determination of *absolute* metabolite concentrations by proton NMR and will be pursued in subsequent glucose clamp studies. Moreover, the known effects of anesthesia on blood and brain glucose levels (*2*) will be the subject of further investigations.

Obviously, the relative cerebral glucose concentration in rats is higher than that in human brain. In a very large number of proton NMR studies of human volunteers, we have never observed glucose resonances under normoglycemia. This finding limits the human brain glucose concentration to a value below the level of detectability, i.e., to <1 mM (or 10% of the NAA concentration). Using an arterial glucose value of 4.5 mM, also the human brain/blood glucose ratio of <0.22 is lower than that in rats.

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