Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2517–2519, April 1984
Medical Sciences

Detection of cerebral lactate *in vivo* during hypoxemia by ^{1}H NMR at relatively low field strengths (1.9 T)

(spin echo/surface coil/electroencephalogram/brain metabolism)

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Contributed by R. G. Shulman, December 12, 1983

ABSTRACT **^{1}H NMR was used to monitor lactate production and clearance during hypoxemia and recovery in the rabbit brain at the relatively low magnetic-field strength of 1.89 T. An array of conventional physiological variables were recorded simultaneously with spectrum acquisition, including the electroencephalogram and electrocardiogram. The sensitivity and spectral resolution achieved at this field strength should be applicable to studies of human brain pathophysiology in the large-bore magnets now available.**

The application of nuclear magnetic resonance spectroscopy to studies of brain pathophysiology and metabolism is in its infancy. Most of the studies of human brain have been devoted to ^{1}H imaging based on the ^{1}H resonance of tissue water (1, 2). ^{31}P spectroscopy has been applied to the study of cerebral pH and high-energy phosphate metabolism under normal and pathological conditions in animals (3–7), and very recently similar measurements have been made in human infants suffering from various anoxic–ischemic encephalopathies (8).

Recently we showed the feasibility of *in vivo* cerebral metabolic studies in rats by high resolution ^{1}H NMR spectroscopy (9). Suppression of the tissue water signal and resolution enhancement of the resulting spectrum permitted resonances of numerous metabolites to be observed. Lactate formation was monitored in the brains of rats breathing low-oxygen gas mixtures and shown to depend on the length and depth of hypoxia. The 7.2-cm diameter bore of the 8.4-T magnet used in that study will not accommodate animals larger than the rat. The large bores of magnets that are now available (20–60 cm diameter) will admit large animals as well as humans. A potentially severe problem with these large-bore magnets is their field strength which is 1/4.4 that used in our previous study of the rat brain. The lower spectral resolution at these magnetic-field strengths places uncertain limits on the usefulness of ^{1}H NMR for metabolic studies.

We describe in this report the use of high-resolution ^{1}H NMR spectroscopy at the relatively low field strength of 1.89 T to follow lactate production and clearance during hypoxemia in the rabbit brain. The spectral resolution we achieved should be obtainable for ^{1}H NMR studies of human brain metabolism in the 60-cm bore magnets now available.

METHODS

NMR Parameters. NMR measurements were made with an Oxford Research Systems TMR-32/200 spectrometer (clear bore, 200 mm.; B_0 field, 1.89 T; ^{1}H resonance frequency, 80.285 MHz) operating in the pulsed Fourier-transform mode. A two-turn 1.7-cm surface coil placed against the exposed dura mater was used for transmission and reception of radiofrequency signals. The B_0 field homogeneity was optimized by shimming on the tissue water resonance using a pulse duration that gave a signal of maximum intensity. Spectra were acquired using a Hahn spin-echo sequence $(\theta\text{-}\tau\text{-}2\theta\text{-}\tau\text{-A-D})_n$ with a τ delay of 120 msec. The θ pulse duration was 7 μsec. The acquisition time (A) was 0.41 sec (2500 Hz spectral width; 2048 time domain points). An additional relaxation delay (D) of 0.85 sec was placed after each acquisition. Spectra were normally acquired in blocks of 100 free-induction decays (n), resulting in a total time of 2.5 min per accumulation. The spin-echo sequence suppressed the H_2O resonance by a factor of about 10 relative to the conventional acquisition sequence $(\theta\text{-A-D})_n$. In addition, the spin-echo method eliminated lipid and other broad (short T_2) resonances from the spectrum. Phase artifacts due to ^{1}H field inhomogeneity were not observed. It has recently been shown that cycling the phases of rf pulses can eliminate artifacts from a spin-echo spectrum obtained by a surface coil (10). We have found that by judicious selection of θ and τ values these phase artifacts can be eliminated without cycling of the pulse phases (unpublished data).

The chemical shifts of pure compounds and acid extracts of rabbit brain (not shown; prepared according to ref. 9) were referenced to internal sodium 3-trimethylsilyl[2,2,3,3-^{2}H]propionate at δ = 0.00 ppm in a 0.1 M potassium phosphate buffer in ^2H_2O (p^2H = 7.4); *N*-acetylaspartate (δ = 2.023 ppm) served as an intracellular chemical shift reference in the *in vivo* ^{1}H spectra of the rabbit brain.

Animal Preparation. Female New Zealand white rabbits weighing 1.9–2.1 kg were anesthetized with enflurane for placement of a tracheal cannula and femoral arterial catheter. Subcutaneous stainless-steel wire leads were placed on the left extremities and scalp for recording the electrocardiogram and electroencephalogram. Electrical variables were amplified and recorded with a Grass model 7 polygraph. Millihenry rf chokes were placed in series on all electrical connections with the animal. A 1.8-cm-diameter disk of skull centered on the bregma was removed with a trephine, care being taken to ensure an intact dura mater. The animal was then placed on a plastic restraining board that maintained the head in a fixed position, paralyzed with pancuronium bromide (1 mg·kg^{-1}, s.c.) and D-tubocurarine chloride (1.0–1.5 mg·kg^{-1}, s.c.), and ventilated with a Harvard model 661 respirator. Enflurane was discontinued on completion of surgery. Analgesia was provided by ventilation with 70% nitrous oxide in oxygen. Blood pressure was monitored with a Statham P50 transducer. The animal was arranged in the bore of the magnet so as to center the cranial cavity on the volume of the homogeneous magnetic field of the instrument. Arterial pH, P_{CO_2}, and P_{O_2} were measured with a Radiometer BGA3 blood gas analyzer. Blood glucose was measured on arterial samples using Dextrostixs and an Ames Glucometer. Animal temperature was maintained at 37.5 ± 0.5°C by a thermal heating pad.

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RESULTS

Several resonances were observed in the low-field, high-resolution ^1H spectrum of the rabbit brain *in vivo*. Assignments of the ^1H resonances were based on the chemical shifts of the pure compounds, the presence of similar resonances in rat brain extracts (9), and extract spectra of the excised rabbit brain frozen *in situ* (not shown). The unresolved doublet (J = 7.4 Hz) of the lactate resonance at 1.32 ppm was inverted using a spin-echo delay of 60 msec, both in the postmortem rabbit brain *in situ* and in the excised brain as an additional confirmation of the lactate assignment (11). Assigned resonances in the ^1H spectra in Fig. 1 include lactate; *N*-acetylaspartate; amino acids, which include the resonances of glutamate, glutamine, and the aspartyl moiety of *N*-acetylaspartate; phosphocreatine and creatine (unresolved); phosphorylcholine and other choline-containing molecules (unresolved).

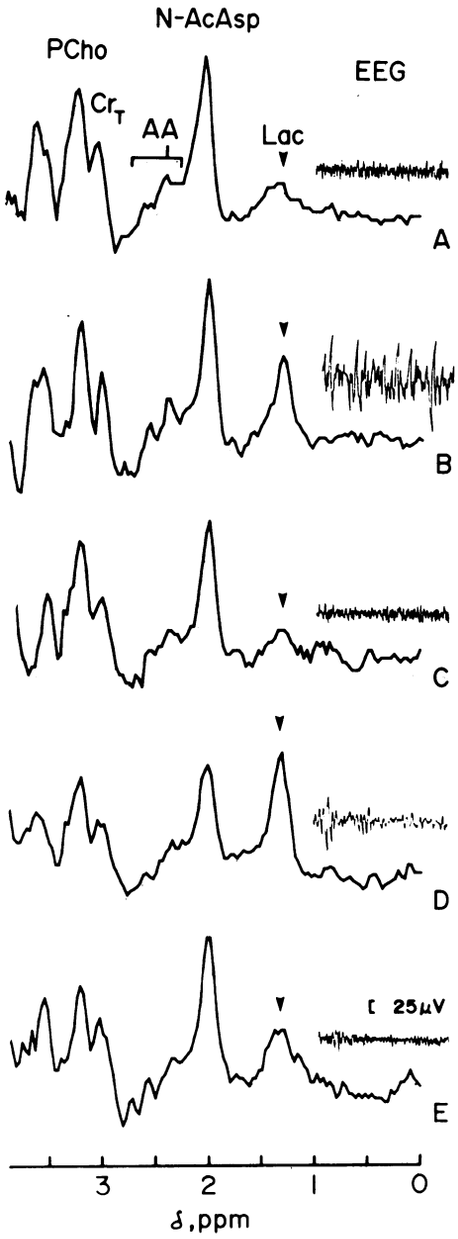

FIG. 1. *In vivo* ^1H NMR spectra of two successive hypoxic insults with recovery in the rabbit brain. Spectrum A, normoxic, 33% inspired O_2; spectrum B, hypoxic, 19 min at 7% O_2; spectrum C, recovery, 44 min at 33% O_2; spectrum D, hypoxic, 23 min at 4.8% O_2; spectrum E, recovery, 44 min at 33% O_2. A 10-sec trace of the electroencephalogram is shown to the right of each spectrum. Table 1 contains other pertinent physiological data for each spectrum. Labeled resonances are as follows: PCho, phosphorylcholine; Cr_T, phosphocreatine/creatine (total pool); AA, amino acids including resonances from aspartyl group of *N*-acetylaspartate, glutamate, and glutamine; N-AcAsp, *N*-acetylaspartate; Lac, lactate.

Table 1. Cerebral lactate levels and physiological data during hypoxia and recovery in the rabbit

	A	B	C	D	E
Inspired O_2, %	33	7	33	4.8	33
Lactate/*N*-AcAsp*	0.21 (±0.03)	0.57 (±0.04)	0.23 (±0.03)	1.0 (±0.07)	0.37 (±0.04)
Po_2†	185	37	153	24	125
Pco_2	23	22	24	18	22
pH_a	7.565	7.513	7.436	7.352	7.262
Blood glucose	8.9	7.6	13.7	13.5	18.9

A–E refer to the spectra shown in Fig. 1 and selected time points (open circles above letters) in Fig. 2. *N*-AcAsp, *N*-acetylaspartate; pH_a, arterial pH. Po_2 and Pco_2 values are in mm Hg (±2). Blood glucose is expressed as mM (±1.0).

*Resonance amplitudes for lactate and *N*-acetylaspartate in ^1H spectra were measured and expressed as a ratio. The values plotted in Fig. 2 and listed above were not corrected for the differences in the T_1 and T_2 relaxation times between lactate and *N*-acetylaspartate and cannot be used directly to compute concentrations.

†Corrected for pH and temperature effects.

Fig. 1 shows the ^1H spectra of the rabbit brain taken at various times during two successive hypoxic insults. The increase in the lactate resonance resulting from the transitions to low oxygen and its subsequent recovery after reoxygenation is clearly shown.

The electroencephalogram (shown to the right of each spectrum of Fig. 1) displayed high-amplitude slow-wave activity throughout the full 20-min period of the initial insult (spectrum B, 7% inspired O_2), while a progressive decrease in slow-wave amplitude was observed during the more intense hypoxic stress (spectrum D, 4.8% inspired O_2). Activity of lower amplitude and faster rhythm—characteristic of the prehypoxic state—was observed during each recovery period (spectra C and E).

The complete time course of lactate production and clearance during the two successive periods of hypoxia is presented in Fig. 2. The increase in arterial blood pressure with only a modest decrease in heart rate suggests that cerebral ischemia did not occur either time.

It was possible to calculate the apparent first-order rate constants for lactate clearance from the values of the lactate/*N*-acetylaspartate ratio (see Table 1) during the recovery periods after inspiration of 7% and 4.8% O_2. These values were found to be -0.064 and -0.043 min^{-1}, respectively.

DISCUSSION

These results show that brain lactate levels can be monitored continuously in the living animal at a magnetic field strength practical for studies of human cerebral pathophysiology. Lactate was observed with a good signal-to-noise ratio (0.4 $mM^{-1}\,scan^{-1/2}$), although the dynamic range limited the minimum concentration of cerebral metabolites observed to 1–2 mM in proton concentration. Because the spin-echo delay (τ) used here was optimized for the lactate coupling constant, the amino acid resonances were differentially suppressed due to J modulation. Determination of detection capability for cerebral amino acids will require further study.

The possible importance of lactate in the development of irreversible brain damage has been discussed in several recent studies (13–15). Nondestructive measurement of lactate levels and rates of accumulation and clearance in brains of human patients should be useful for diagnosis and monitor-

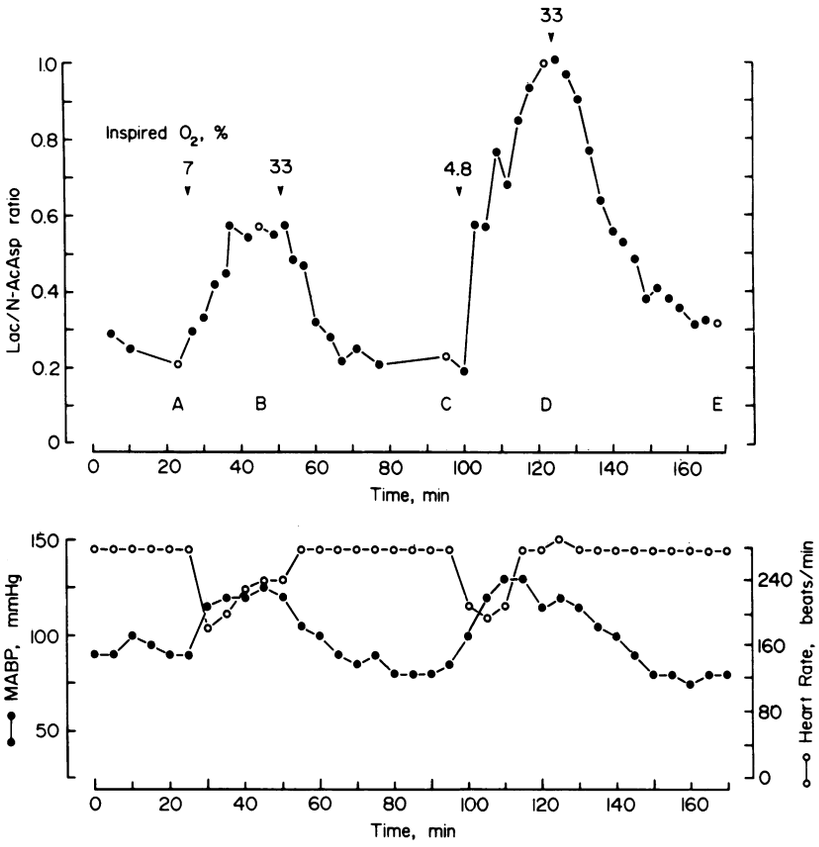

FIG. 2. Complete time course of cerebral lactate production and clearance, blood pressure, and heart rate during two successive hypoxic insults. The resonance amplitudes of lactate and *N*-acetylaspartate were determined from each spectrum and expressed as a ratio (*Upper*). *N*-acetylaspartate levels are relatively constant in adult rabbit brain tissue (6.7 μmol·g^{-1}, wet wt) and do not vary during ischemia (12) or after moderate postmortem periods. Since all resonances experienced a pronounced line broadening on the administration of hypoxic gas mixtures, the presentation of the data as ratios allows a more accurate determination of the changes in cerebral lactate levels. The letters A–E correspond to the spectra and data of Fig. 1 and Table 1, respectively. Numbers above arrows denote % inspired O_2; MABP, mean arterial blood pressure.

ing of therapy in stroke, perinatal asphyxia, and other anoxic–ischemic encephalopathies.

The relationship between intracellular pH and intracellular lactate levels is dependent on a number of respiratory and metabolic factors. The ability to measure both in the same brain by combining conventional ^{31}P NMR determinations of intracellular pH with the kind of lactate measurements reported here will lead to better understanding of the interplay of these two important variables under pathophysiological conditions.

We would like to acknowledge helpful discussions with J. Alger, M. Stromski, J. den Hollander, and M. Bendall. This work was supported by grants from the U.S. Public Health Service (GM 30287) and the Esther A. and Joseph Klingenstein Foundation.

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