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Localized ^{31}P NMR Spectroscopy of the Adult Human Brain *in Vivo* Using Stimulated-Echo (STEAM) Sequences

K. D. MERBOLDT, D. CHIEN, W. HÄNICKE, M. L. GYNGELL, H. BRUHN, AND J. FRAHM

Max-Planck-Institut für biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, Federal Republic of Germany

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Phosphorus NMR spectroscopy of the brain of healthy volunteers (20–25 years) has been performed with use of the stimulated-echo (STEAM) localization technique. Investigations were carried out on a 2.0 T whole-body MRI/MRS system (Siemens Magnetom). Phosphorus spectra with echo times of TE $\geq$ 3 ms were typically obtained in measuring times of about 10 min from a 125 ml volume-of-interest localized in the occipital part of the brain. The spectral resolution was optimized by localized shimming on the water proton signal (5–7 Hz) using the same sequence. Relaxation data were evaluated from spectra acquired under experimental conditions matched to the relaxation times of the individual metabolite resonances. Echo times ranged from 5 to 115 ms for T_2 determinations. For T_1 determinations repetition times were varied from 500 to 9000 ms. Broadband proton decoupling was achieved by means of a WALTZ-8 composite pulse sequence applied for the initial 68 ms of the 256 ms phosphorus acquisition period.

Due to its elegant access to the energy metabolism of a living cell, e.g., the direct observation of high-energy phosphates, inorganic phosphates, and the cytosolic pH, phosphorus NMR spectroscopy has become almost synonymous with *in vivo* NMR and clinical spectroscopy (MRS). Based on investigations of tissue extracts, perfused organs, and experimental animals, more recent studies focused on human subjects exploiting the increased availability of clinical whole-body MRI systems with field strengths of up to 2.0 T. Accordingly, the primary interest in ^{31}P NMR studies extended from skeletal muscle to other organs (*1–4*), including liver (*5–8*), heart (*9–11*), and brain (*12–15*) as well as pathological alterations such as tumors (*16, 17*) and stroke (*18*). Thus, proper localization of the spectroscopic acquisition, i.e., definition of the volume-of-interest (VOI), becomes increasingly important. Certainty about the origin of the spectrum plays a key role not only in medical diagnosis but also in basic biophysical and biochemical research attempting to correlate and quantify metabolite concentrations and their variations within a particular tissue.

Previous phosphorus studies employed a variety of techniques ranging from the application of surface coils (*19*) to more sophisticated approaches including rotating-frame imaging (*20*) or its Fourier-windowed (*21*) version, one-dimensional gradient techniques such as DRESS (*22*), three-dimensional gradient techniques such as ISIS (*23*), or chemical-shift imaging (CSI) (*24, 25*). Here we demonstrate the ability of the stimulated-echo (STEAM) spectroscopy technique (*26–28*) to obtain localized

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phosphorus NMR spectra from the human brain *in vivo*. Single-step STEAM localization sequences have already resulted in high-quality ^{1}H NMR spectra of human brain (*29–31*) and muscle (*32*), but were disregarded for phosphorus studies because of unavoidable signal losses for short T_2 species such as phosphate metabolites (*33*). However, the present work not only demonstrates the feasibility of phosphorus STEAM spectroscopy but also takes advantage of the stimulated echo to determine T_2 relaxation times in the adult human brain *in vivo*. Knowledge about T_1 and T_2 relaxation times is a prerequisite for quantitative evaluations of metabolite concentrations and in itself may be of interest for the study of molecular dynamics of the metabolites or the influence of tissue susceptibilities. In addition, the potential of broadband proton decoupling for improving both resolution and sensitivity of *in vivo* phosphorus spectroscopy is addressed.

METHODS

Figure 1 shows a schematic diagram of the stimulated-echo sequence employed for localized phosphorus NMR spectroscopy of human brain *in vivo*. STEAM sequences define the selected VOI by the intersection of three orthogonal slices that are excited within a single experimental run. When compared to previous proton sequences, the phosphorus version does not need a water suppression pulse and the gradient scheme was optimized to achieve the shortest echo time (TE = 3 ms), while eliminating unwanted signals. Residual effects due to the final FID after the third slice-selective RF pulse were further minimized by phase cycling the first and third RF pulse by 180° in alternate acquisitions. Gradient switching times were adjusted to 300 μs for a maximum strength of 10 mT m^{-1}. In the present study the middle interval was fixed to TM = 5 ms.

RF excitation was accomplished using Gaussian-shaped RF pulses of 640 μs duration in the presence of 3 mT m^{-1} gradients. The resulting bandwidth of 0.52 kHz/cm

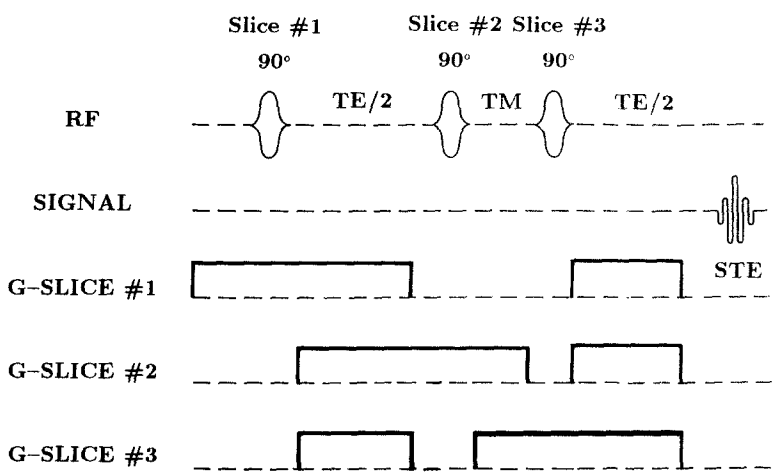

FIG. 1. Schematic diagram of the stimulated-echo (STEAM) RF pulse and magnetic field gradient sequence used for localized phosphorus NMR spectroscopy at echo times TE $\geq$ 3 ms (TM = 5 ms). For details see text.

corresponds to a ^{31}P chemical-shift uncertainty of about 0.06 cm/ppm at 2.0 T. In the present study the maximum displacement between the most outer resonances (β-ATP, PME) of about 1.5 cm represents 30% of the linear dimension of the $5 \times 5 \times 5$ cm^3 (125 ml) VOI. In some cases broadband proton decoupling was achieved by means of a composite pulse sequence applied for an initial period of 68 ms during the 256 ms acquisition interval (1K complex data points, 2 kHz receiver bandwidth). The individual pulse packages of the WALTZ-8 = $K—\bar{K}—\bar{K}—K—$ sequence consisted of $K = \bar{2}—4—\bar{2}—3—\bar{1}—$ sequences using "n" pulses of $n \times 500$ μs duration (equal amplitudes) with 500 μs spacings. "$\bar{K}$" and "$\bar{n}$" refer to RF pulses with a 180° phase shift. The proton RF pulses required for decoupling were transmitted via a second RF channel on the system. The decoupling was optimized by slowly increasing the power level of the second RF transmitter. Assuming total absorption of the RF power in the tissue, 4 kg of tissue, and a repetition time of 3000 ms, the maximum specific absorption rate corresponds to 0.25 W kg^{-1} for transmitter power levels that were sufficient for proton decoupling of phosphorus spectra from human brain *in vivo*.

Data processing consisted of zero filling of the data to 4K, Gaussian multiplication in the time domain, and Fourier transformation followed by zero- and first-order phase correction. For the evaluation of relaxation times from proton-coupled phosphorus spectra, Gaussian filtering was applied to give a line broadening of 4 Hz to optimize signal-to-noise at the expense of spectral resolution. On the other hand, a line broadening of only 2 Hz was applied for proton-decoupled spectra to maintain the improved resolution at the expense of signal-to-noise. No further resolution enhancement, baseline correction, or other fitting routines have been applied. Relaxation times and relative metabolite concentrations were determined using spectral peak areas as described previously (*30*).

The complete study protocol comprised (i) multislice FLASH NMR imaging (256^2 pixel resolution, TE = 6 ms, TR = 100 ms, flip angle 70°, nine slices, total measuring time 26 s) to determine the exact position of the VOI in all three orientations, (ii) localized shimming on the water resonance using the *same* STEAM sequence as that employed for phosphorus spectroscopy but with matched bandwidths for the proton RF pulses, and (iii) phosphorus acquisitions using variable echo times and repetition times. All studies were performed on a 2.0 T whole-body MRI/MRS system (Siemens Magnetom) equipped with a conventional 10 mT m^{-1} gradient system. Proton imaging, magnetic field homogeneity shimming, and proton decoupling were accomplished using a 23 cm Helmholtz transmit/receive coil below and above the head of the volunteers; phosphorus spectra were acquired using a 17 cm Helmholtz transmit/receive coil in the "earphone" position. Informed consent was obtained from the volunteers prior to investigation.

RESULTS

Chemical shifts, spectral resolution, and proton decoupling. The 125 ml VOI selected for phosphorus NMR spectroscopy was localized in the occipital part of the brain throughout this study. Figure 2a indicates a typical position. A corresponding localized phosphorus spectrum (256 acquisitions, TR = 3000 ms) with an echo time of TE = 3 ms is shown in Fig. 2b. Chemical shifts are in ppm units and referenced to PCr as

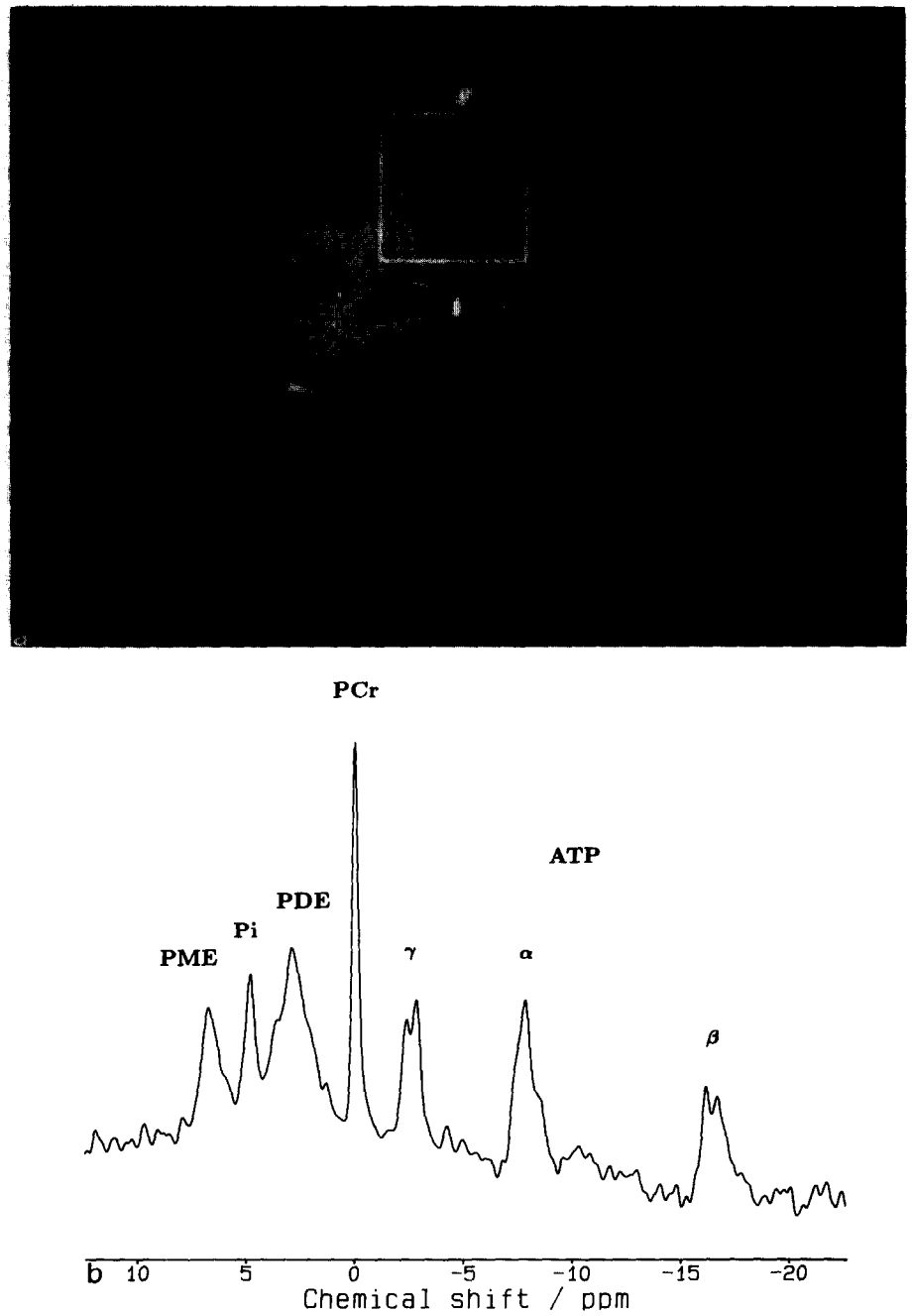

FIG. 2. (a) The 85 MHz (2.0 T) coronal FLASH NMR image (70°, TR = 100 ms, TE = 6 ms, 23 cm Helmholtz coil) of a young healthy volunteer indicating a $5 \times 5 \times 5$ cm^3 volume-of-interest (VOI) selected for localized phosphorus NMR spectroscopy of human brain *in vivo*. (b) Corresponding 34 MHz (2.0 T) localized phosphorus NMR spectrum (125 ml VOI, 256 scans, TR = 3000 ms, TE = 3 ms, TM = 5 ms, 17 cm Helmholtz coil in the earphone position). According to their chemical shifts resonance signals are assigned to the α-(−7.62 ppm), β-(−16.3 ppm), and γ-phosphates (−2.57 ppm) of adenosine triphosphate (ATP), phosphocreatine (PCr, reference 0 ppm), phosphodiesters (PDE, 2.90 ppm), inorganic (ortho-) phosphates (P_i, 4.83 ppm), and phosphomonoesters (PME, 6.75 ppm). The resonances exhibit a 4 Hz line broadening due to Gaussian multiplication of the time-domain data.

zero. The spectrum contains resonances from the γ- (−2.57 ppm), α- (−7.62 ppm), and β-phosphates (−16.30 ppm) of adenosine triphosphate (ATP), phosphocreatine (PCr, 0 ppm), phosphodiesters (PDE, 2.90 ppm), inorganic phosphate (P_i, 4.83 ppm), and phosphomonoesters (PME, 6.75 ppm). The chemical-shift values represent average values taken from proton-coupled spectra of different volunteers. Due to the excellent homogeneity achieved by localized proton shimming the spectral linewidth of PCr is about 9–10 Hz *including* a 4 Hz line broadening by Gaussian filtering. The homonuclear splitting of the γ-ATP resonance is routinely resolved.

The certainty of spectral assignments and the accuracy of chemical-shift determinations may be considerably improved using broadband proton decoupling to remove unresolved ^1H–^{31}P spin–spin couplings. This is demonstrated by the localized proton-decoupled phosphorus spectrum shown in Fig. 3. The spectral resolution is remarkably enhanced for the PME and PDE regions as well as for the α-ATP phosphate resonance rendering the unambiguous detection of nicotinamide adenine dinucleotides (NAD, −8.37 ppm) possible. The ATP resonances exhibit chemical shifts at −2.53 ppm (γ), −7.54 ppm (α), and −16.03 ppm (β) as well as a homonuclear J splitting with a coupling constant of $J \approx 17$ Hz. The PDE and PME regions are dominated by three

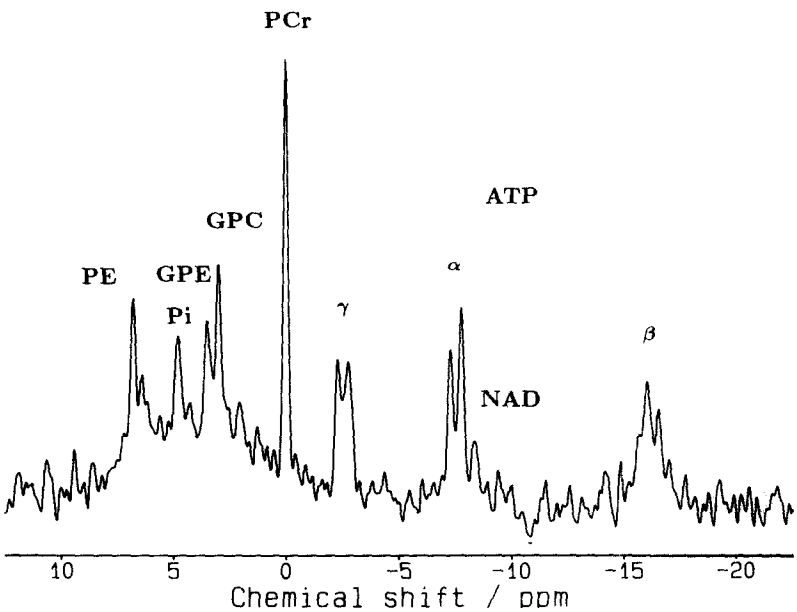

FIG. 3. The 34 MHz (2.0 T) localized proton-decoupled phosphorus NMR spectrum (125 ml, TE = 3 ms, TM = 5 ms, TR = 3000 ms, 256 scans, 2 Hz line broadening) from the occipital brain of a young healthy volunteer. Decoupling was achieved by means of a WALTZ-8 sequence applied during the initial 68 ms of the 256 ms phosphorus acquisition period. The calculated specific absorption rate was 0.25 W kg^{-1}. The spectrum comprises resonances due to the α- (−7.54 ppm), β- (−16.03 ppm), and γ-phosphates (−2.53 ppm) of adenosine triphosphate (ATP), phosphocreatine (PCr, reference 0 ppm), and inorganic (ortho-) phosphates (P_i, 4.80 ppm). Further resonances may be assigned to nicotinamide adenine dinucleotides (NAD, −8.37 ppm), glycerophosphocholine (GPC, 2.99 ppm), glycerophosphoethanolamine (GPE, 3.51 ppm), and phosphoethanolamine (PE, 6.78 ppm).

strong resonances that may be identified with glycerophosphocholine (GPC, 2.99 ppm), glycerophosphoethanolamine (GPE, 3.51 ppm), and phosphoethanolamine (PE, 6.78 ppm) even though the latter resonance may contain resonances from other sugar phosphates; see, e.g., (*34, 35*). Further resonances may be tentatively assigned to phosphatidylcholine and phosphoenolpyruvate (2.05 ppm) and phosphocholine (6.39 ppm). In general, proton decoupling also contributes to a better definition of the P_i resonance at 4.80 ppm and its peak area for quantitative evaluations.

In addition to the improved spectral resolution proton-decoupled phosphorus spectra of human brain *in vivo* routinely exhibited marked sensitivity enhancements. In particular, PCr showed a signal increase of about 70% at TR = 1500 ms and about 40% at TR = 3000 ms. A significant signal increase was maintained for very low RF power levels insufficient for proton decoupling. While such sensitivity effects may be formally assigned to a "transient" nuclear Overhauser enhancement, neither their specific nature *in vivo* nor their quantitative predictability is fully understood so far (see Discussion).

T_1 and T_2 relaxation times of phosphate metabolites. In order to avoid complications due to distorted resonance intensities by proton decoupling, all spectral recordings for the measurements of relaxation times were performed without proton decoupling. In fact, the existence of transient NOE effects may be misleading in the quantitative evaluation of (relative) metabolite concentrations and relaxation times under variable experimental conditions, e.g., for different durations, timings, or duty cycles of the decoupling sequence when changing the repetition time TR.

Phosphorus T_1 and T_2 relaxation times were obtained by varying the repetition time and the echo time of the STEAM sequences, respectively. Figure 4 shows two series of spectra recorded with different repetition times to determine T_1 values. In Fig. 4a the range of TR values from 500 to 3000 ms was chosen to optimize the experimental conditions for relatively short T_1 relaxation times (e.g., ATP), whereas the series of spectra in Fig. 4b matches the requirements for long T_1 values (e.g., PME and PCr).

Similar matched conditions were employed for the corresponding measurements of T_2 relaxation times that are easily possible using the STEAM method. Due to the large spread of values for the different metabolites, three ranges of echo times were found necessary to adequately sample the respective relaxation curves. Figure 5a shows a series of T_2-weighted spectra (TE = 5–25 ms) used to evaluate the relaxation times of compounds with small T_2 values such as ATP and PDE. For T_2 determinations of P_i the echo times were prolonged to intermediate values in Fig. 5b (TE = 5–55 ms), whereas PCr required even larger values as shown in Fig. 5c (TE = 5–115 ms).

DISCUSSION

Chemical shifts and spectral resolution. The present results prove the feasibility and ease of phosphorus STEAM spectroscopy. Well-resolved phosphorus spectra including ATP are obtainable even at echo times of TE = 10–15 ms. When compared to ISIS localization the single-step STEAM sequence offers the considerable advantage of proton shimming on the selected VOI *with the same sequence.* The resulting T_2 attenuation for the currently available minimum echo time of TE = 3 ms is already comparable to what is often applied in phosphorus CSI experiments (*3, 15, 37*) where

similar delay times are required for the application of phase-encoding gradients. However, while STEAM sequences acquire the data in the form of an *echo* with fully rephased magnetizations, CSI studies in most cases suffer from a *delayed* FID acquisition that causes severe phasing problems and eventually introduces uncertainties in the spectral interpretation. Zero- and first-order phase corrections of the STEAM spectra were straightforward and did not require additional baseline corrections or other data manipulations.

At the chosen echo times of TE $\geqslant$ 3 ms, it is mainly the PDE region that is rapidly attenuated when compared to FID acquisitions using simple surface coils (*9, 12, 18*) or ISIS techniques (*3, 4, 17*). A quantitative determination of the effect is difficult due to the uncertainties in the true PDE, P_i, and PME areas in FID spectra in particular when resolution enhancement techniques are applied to remove "broad" components. In that respect, the improved resolution due to differential T_2 losses in the PDE and PME regions may be considered an advantageous potential of phosphorus STEAM spectroscopy. Nonlocalized phosphorus spin-echo acquisitions have already been used for the same purpose (*38–40*). In particular, it is highly unlikely that the T_2-weighted spectra are contaminated with residual broad phosphate signals of, e.g., membrane-bound phospholipids due to their very short T_2 relaxation times. In fact, the surprisingly high signal intensities of some mobile PDE and PME resonances at echo times as long as TE = 15 ms (compare Figs. 5a and 5b) seem to indicate that reports on significant contributions from partially immobilized compounds in liver (*41*) and brain (*42*) may have overestimated the effect. From a technical viewpoint T_2 weighting provides an alternative to selective presaturation recently proposed by Thoma *et al.* (*43, 44*) and in any case should be preferable over post-processing manipulations.

The improvements in spectral resolution observed upon broadband proton decoupling are in excellent agreement with previous studies by Luyten *et al.* (*45*). At least as far as mobile metabolites are concerned proton decoupling allows the identification of PE and GPC as the major constituents of the broad PME and PDE resonances in proton-coupled phosphorus spectra of the adult human brain. The elimination of unresolved proton splittings from the ATP resonances as observed for the α-ATP phosphate unravels a different kind of line broadening for the broader β- and γ-ATP resonances. This finding, together with the chemical shift of β-ATP (*34, 46*), not only confirms that under normal conditions ATP in the adult human brain is complexed with magnesium (Mg^{2+}) but also indicates its position in between the β- and γ-phosphates of the molecule. The latter finding is in agreement with similar observations for the binding of manganese to ATP in aqueous solution (*47*). An estimation of the free Mg^{2+} concentration from the chemical-shift different $\delta_{\alpha\beta}$ of the α- and β-ATP resonances, i.e., 7.54 and 16.03 ppm in proton-decoupled spectra, respectively, yields 680 μM in agreement with data reported for rat brain (*48*). This concentration has been calculated using the equation described by Gupta *et al.* (*49, 50*)

$$[Mg^{2+}] = K_D \frac{\delta_{ATP} - \delta_{\alpha\beta}}{\delta_{\alpha\beta} - \delta_{MgATP}} \quad [1]$$

with chemical-shift values of δ_{ATP} = 10.81 ppm and δ_{MgATP} = 8.32 ppm for the pure and complexed form of ATP, respectively, and assuming a dissociation constant of K_D = 50 μM. The chemical-shift difference between P_i and PCr varied slightly from

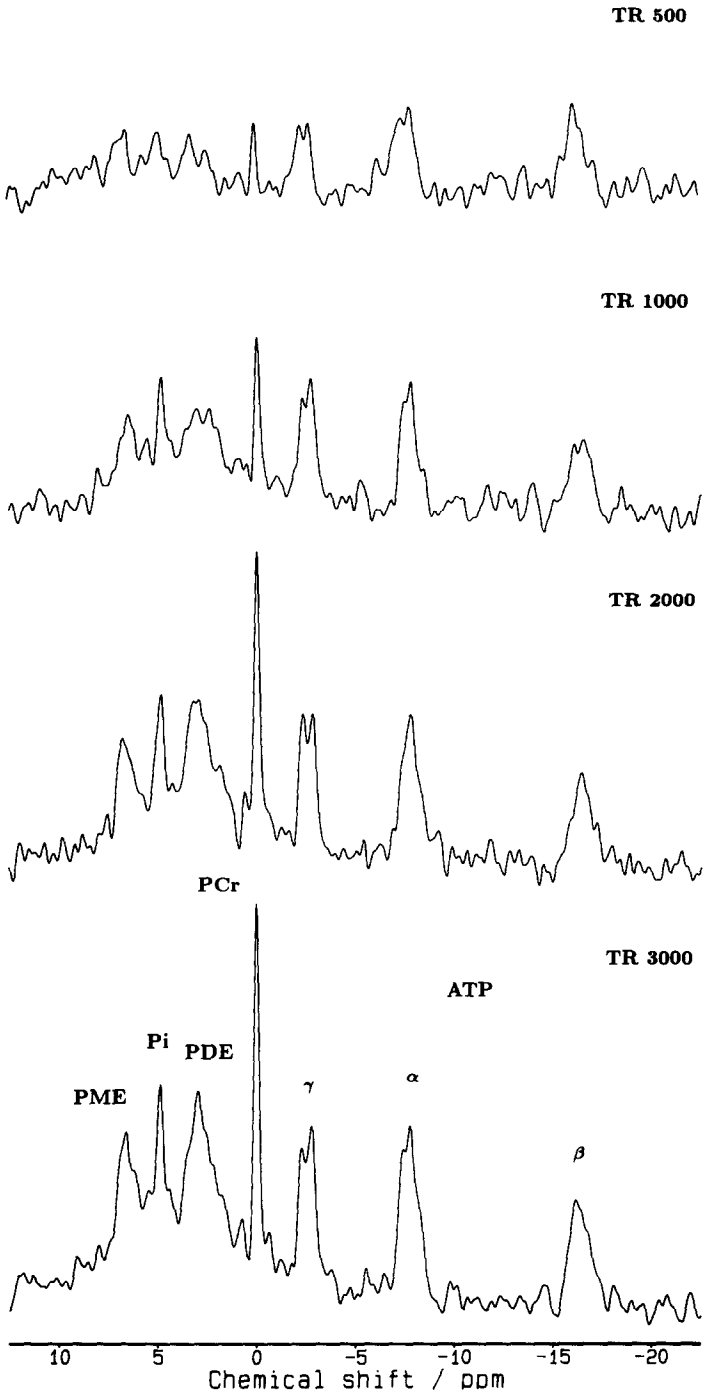

FIG. 4. The 34 MHz (2.0 T) localized phosphorus NMR spectra (125 ml, TE = 3 ms, TM = 5 ms, 4 Hz line broadening) from the occipital brain of young healthy volunteers. The spectra were recorded at repetition times (a) TR = 500, 1000, 2000, and 3000 ms using 512 scans, and (b) TR = 1500, 3000, 6000, and 9000 ms using 128 scans, respectively. The series of spectra demonstrate the T_1 dependence of the metabolite resonances for short and long relaxation times. The intensities have been scaled for direct comparability.

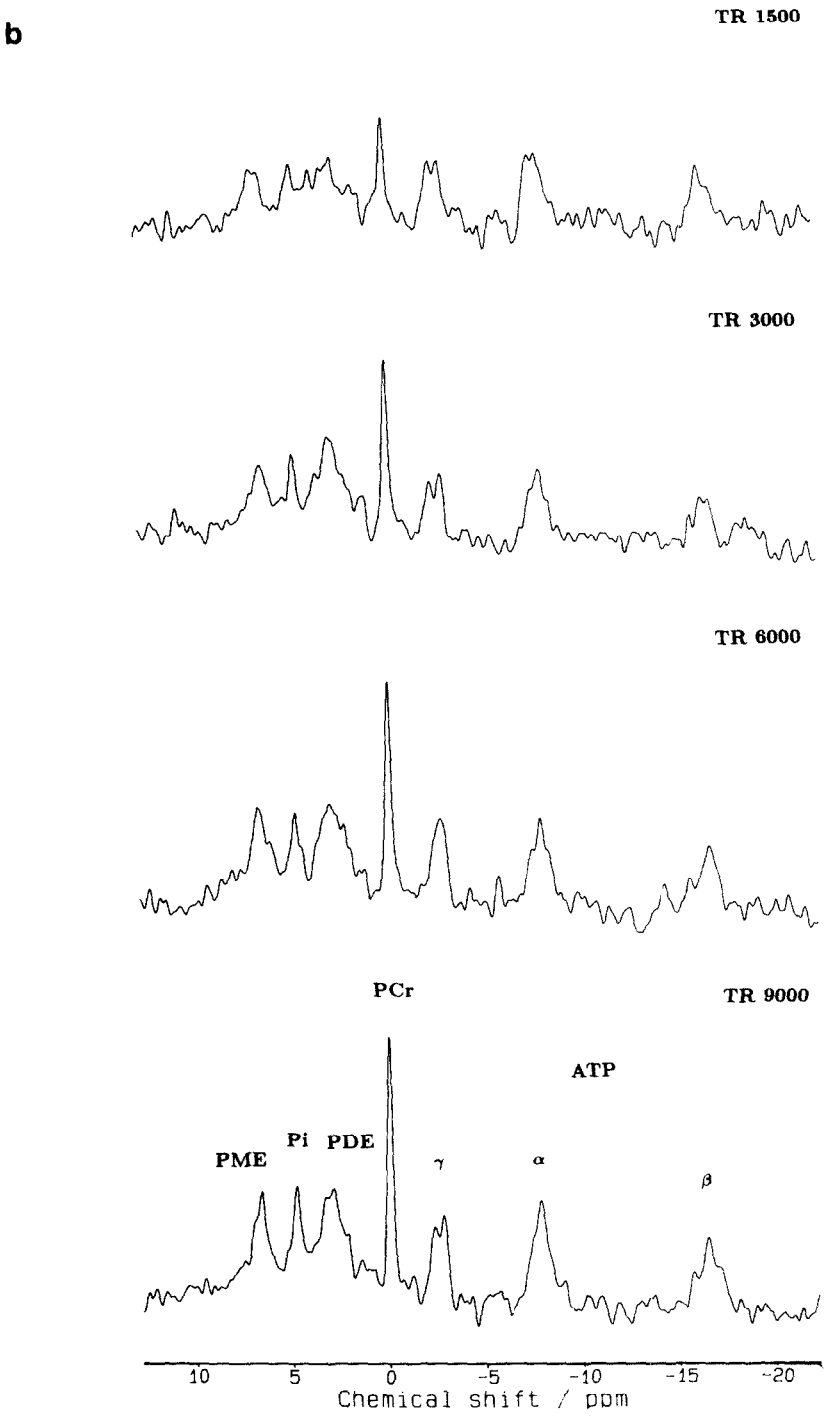

FIG. 4—*Continued*

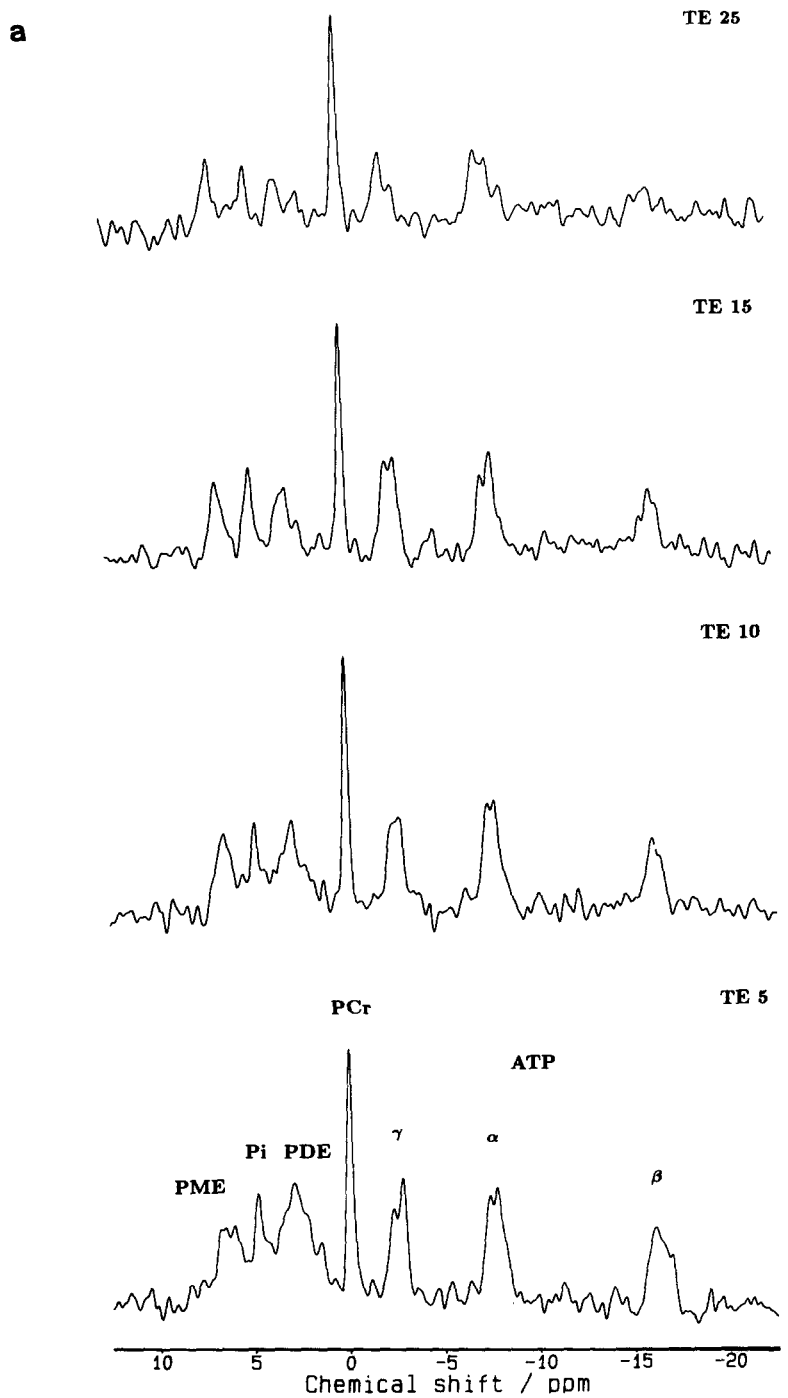

FIG. 5. The 34 MHz (2.0 T) localized phosphorus NMR spectra (125 ml, TM = 5 ms, 4 Hz line broadening) from the occipital brain of young healthy volunteers. The spectra were recorded at echo times (a) TE = 5, 10, 15, and 25 ms using 512 scans and TR = 2000 ms, (b) TE = 5, 15, 35, and 55 ms using 256 scans and TR = 3000 ms, and (c) TE = 5, 35, 75, and 115 ms using 256 scans and TR = 3000 ms, respectively. The series of spectra demonstrate the T_2 dependence of the metabolite resonances for short, medium, and long relaxation times. The intensities have been scaled for direct comparability.

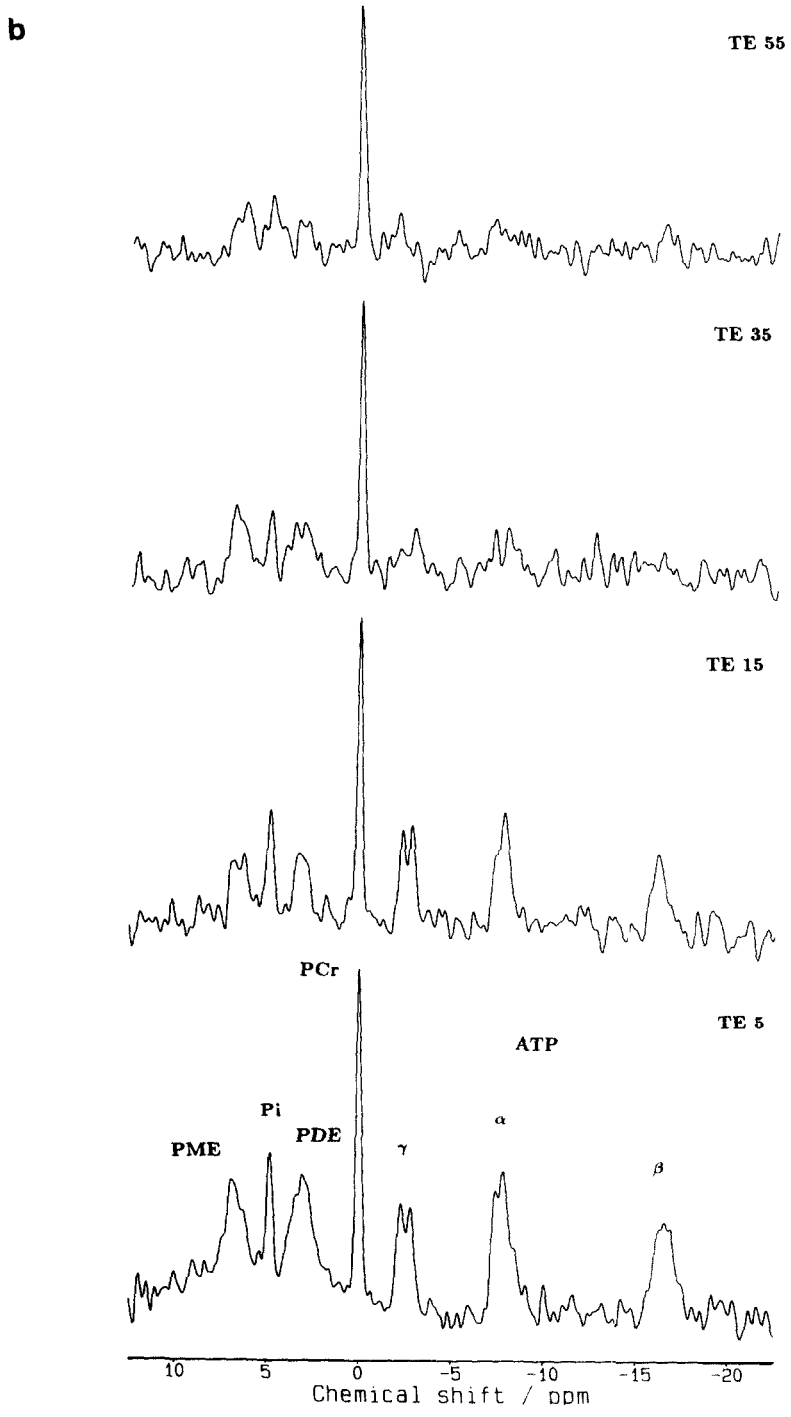

FIG. 5—*Continued*

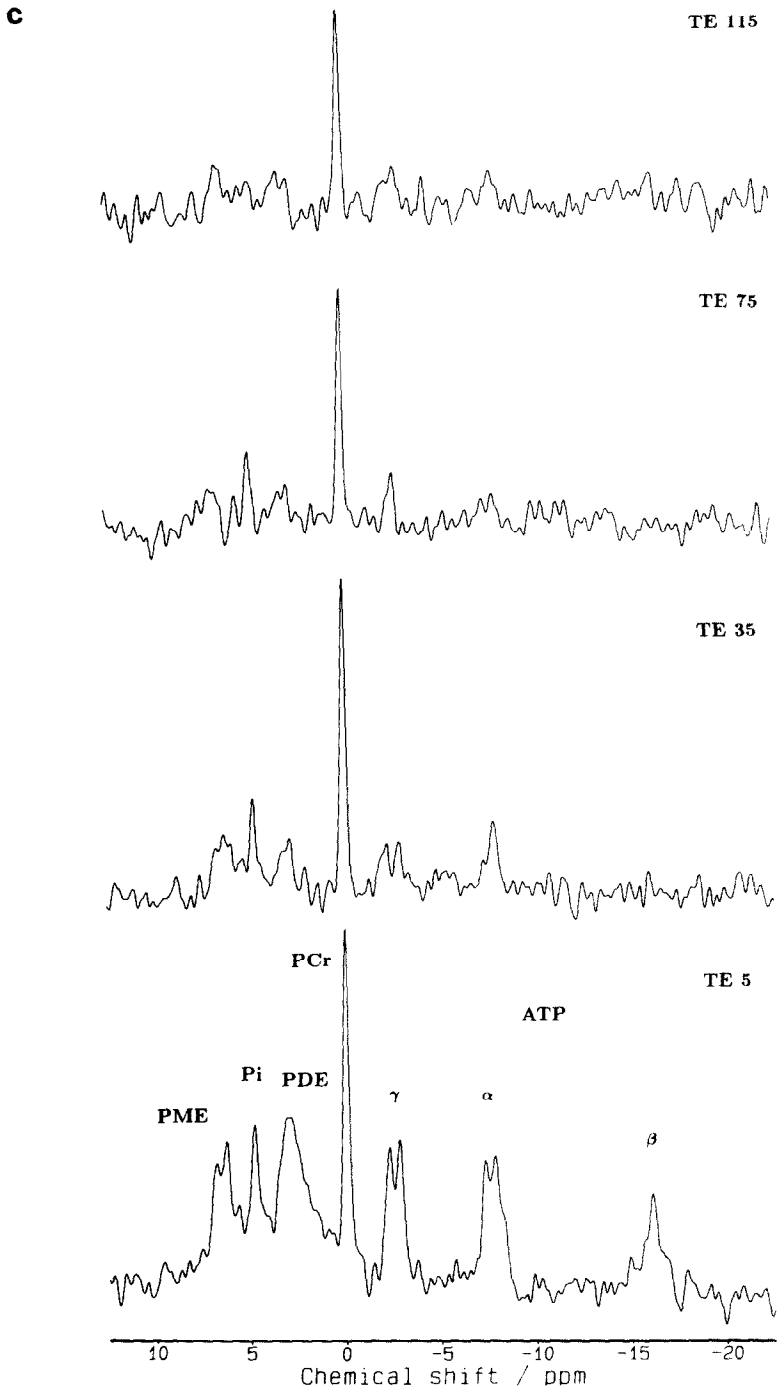

FIG. 5—*Continued*

4.80 to 4.85 ppm for the individual studies. According to the titration formula derived by Petroff *et al.* (*51*)

$$\mathrm{pH} = 6.77 + \log \frac{\delta - 3.29}{5.68 - \delta} \qquad [2]$$

this range corresponds to cytosolic pH values of 7.00–7.04.

NOE effects and broadband proton decoupling in vivo. Although well reproducible, and also observed by a variety of other groups (*45, 52, 53*), the signal increase upon broadband proton decoupling lacks a quantitative description. In fact, the actual implementation of the WALTZ-8 sequence deviates from classical high-resolution NMR experiments in using considerably longer RF pulses and delays inside the composite pulse packages. It was also surprising that decoupling of the phosphorus spectra could be achieved with power levels that were well below 90° for a "1" pulse in the WALTZ-8 sequence.

In the form used for the recording of the proton-decoupled spectrum shown in Fig. 3, the STEAM sequence represents an *inverse gated decoupling* experiment which for fully relaxed acquisitions would result in heteronuclear decoupling but not in a NOE. A formal assignment of the observed effects for TR = 1500–3000 ms to a "transient" NOE remains unsatisfactory as long as there is no understanding of the relevant mechanisms. In addition, the exact behavior of the spin system subjected to the actual pulse sequence is not known, and the proton–phosphorus cross-relaxation rates are not available.

In agreement with expectations from classical NOE theories, marked signal increases for PCr could already be observed at low power levels insufficient for proton decoupling. The enhancement increased with decreasing repetition time of the sequence, but did not shown any significant variations for different durations (68–272 ms) of the decoupling sequence during the phosphorus acquisition. The *gated decoupling* experiment, in which the WALTZ-8 sequence is applied immediately *before* application of the STEAM sequence but not during data acquisition, yielded a similar signal increase, of course without proton decoupling. In fact, for this purpose even a simple 180° proton inversion pulse turned out to be sufficient when preceding the phosphorus STEAM sequence at a suitable delay. All these findings were confirmed by related *in vitro* experiments on aqueous inorganic phosphate phantoms.

T_1 relaxation times. The evaluated T_1 and T_2 relaxation times of phosphate metabolites in the adult human brain are summarized in Table 1. The reliability of the data benefits from accurate localization as well as from experimental conditions that were matched to the measurement of either long or short relaxation times. T_1 relaxation times were calculated from four different repetition times, while T_2 evaluations were performed using five different echo times. Standard deviations are ±20%. Relaxation times for resonances in the PME and PDE regions of the phosphorus spectra must remain ambiguous due to their unknown multicomponent contributions. Proton decoupling would solve this problem provided the signal variations *in vivo* become predictable.

The evaluated T_1 relaxation times are in general agreement with recent phosphorus relaxation studies of the normal human brain by other (*4, 54–56*). Literature T_1 ranges at 1.5 T are 2.5–3.0 s for PME, 1.5–2.4 s for P_i, 1.6–1.7 s for PDE, 2.7–4.0 s

TABLE 1

Spin–Lattice and Spin–Spin Relaxation Times (2.0 T) and Relative Metabolite Concentrations of Phosphate Metabolites Detected in Localized, Proton-Coupled Phosphorus NMR Spectra of the Adult Human Brain *in Vivo*

Compound	δ (ppm)	T_1 (s)	T_2 (ms)	T_1/T_2	Relative concentration (mM)
PME	6.75	4.0[a]	70[a]	60	—
P_i	4.83	2.5	80	30	1.0
PDE	2.90	2.0[a]	20[a]	100	—
PCr	0	3.0	150	20	5.0
γ-ATP	−2.57	0.7	30	20	3.3
α-ATP	−7.62	0.7	30	20	4.0
β-ATP	−16.3	1.0	20	50	3.3

Note. The relaxation times represent mean values (n = 6 for both T_1 and T_2 determinations, respectively) with standard deviations of ±20%. Chemical shifts are referenced to PCr. They represent mean values from about 50 spectra (n = 12) recorded under different experimental conditions.

[a] The data for the PDE and PME resonances are compromised by their multicomponent nature. The given chemical shifts refer to their major components, respectively. Relative metabolite concentrations are normalized to 5.0 (mM) for PCr.

for PCr, and 0.6–1.4 s for the three ATP phosphates using IR ISIS and IR rotating-frame imaging, respectively. Surprisingly good agreement was obtained with data from proton-decoupled phosphorus spectra (*57*). Relaxation times obtained from rat brain studies at higher fields of 4.7–8.5 T (*58, 59*) may be affected by increasing contributions from chemical-shift anisotropy in addition to dipolar proton–phosphorus interactions as has been shown by relaxation studies in solution (*60*). Phosphorus T_1 relaxation times in human skeletal muscle have been reported to possess considerably larger values, e.g., 6.9 s for PCr and 3–5 s for ATP phosphates (*4*).

T_2 relaxation times. Measurements of T_2 relaxation times of homonuclear spin–spin coupled resonances are affected by J modulation. In STEAM sequences a first nulling of the signal amplitudes of a doublet occurs at TE = $1/J$, i.e., about 60 ms for the ATP phosphates. In order to minimize the influence of J modulation, T_2 relaxation times of ATP were only evaluated from spectra recorded at echo times of TE $\leqslant$ 20 ms. Other methodological means to eliminate J modulation such as spin-locking $T_{1\rho}$ measurements or related techniques using (selective) refocusing (*61–63*) were not considered throughout this study.

To our knowledge the data in Table 1 represent the first T_2 determinations localized in human brain *in vivo*. The T_2 values are close to those reported by Rémy *et al.* (*59*) for rat brain at 4.7 T. Potential relaxation effects due to chemical exchange or diffusion cannot be excluded using the single-echo STEAM sequence. CPMG measurements of PCr in rat brain at 4.7 T yielded T_2 = 240 ms (*64*) as opposed to 150 ms for human brain found here. These relatively long relaxation times will allow more accurate assessments of PCr in cerebral tumors using STEAM localization with improved RF pulses and echo times of about 10 ms. Similar arguments apply to P_i and even to ATP when corrected for T_2 attenuation.

When compared to human skeletal muscle, brain ATP T_2 values show no significant differences, whereas T_2 relaxation times of PCr are considerably longer in muscle (*65*). Together with the prolonged T_1 relaxation times this finding may reflect a greater overall mobility (or less binding) of PCr in muscle cells than in neurons. It is also interesting to note that the T_1/T_2 ratios for phosphate metabolites in human brain, i.e., 20–30 for ATP, PCr, and P_i, are significantly larger than those reported for proton resonances (*30*). For example, proton studies resulted in a value of about 7.5 for the *N*-methyl proton of Cr + PCr as compared to 20 for the less mobile phosphate group of PCr using phosphorus NMR.

The T_2 value obtained for PCr corresponds to a "natural" linewidth of about 2 Hz in contrast to the observed linewidths of 5 Hz (without line broadening) in proton-decoupled phosphorus spectra. The latter values are identical to the water proton linewidths obtained after localized shimming on the 125 ml VOI. The instrumental linewidth is about 1 Hz as determined by applications of the same STEAM sequence (TR, TE, VOI, system) to aqueous model solutions of metabolites. Supported by a large number of proton NMR studies in various regions of the human brain the linewidth must be predominantly attributed to (microscopic) tissue susceptibility differences. A surprisingly larger linewidth of 9–10 Hz (without line broadening) holds for the P_i resonance. Since the corresponding T_2 value of 80 ms represents a natural linewidth of 4 Hz, this broadening beyond the homogeneity limits may also be due to the overlap of slightly shifted P_i resonances that reflect tissue compartments with different intracellular pH values. In fact, a variation of the pH from 6.98 to 7.06 already corresponds to a chemical-shift difference of 0.1 ppm or 3.44 Hz (34.4 MHz phosphorus frequency at 2.0 T) that may add to the apparent *in vivo* linewidth. The observed linewidths (without line broadening) of the α-ATP resonance in proton-decoupled phosphorus spectra are about 9 Hz in close agreement with the value calculated from the respective T_2 relaxation times of 30 ms.

Metabolite concentrations. Quantification of metabolite concentrations is the ultimate goal of localized *in vivo* NMR spectroscopy. An important step on the way is the determination of relaxation times. While the present study reports data for the normal adult brain, there is no certainty about the validity of these relaxation times in tissues with pathological alterations (*55, 59, 66*). Clinical applications, therefore, will find it necessary to confirm relaxation data in patient studies by *multiple* recordings of differently T_1-weighted spectra which, in our opinion, are still more rapidly obtained with single-voxel spectra than with chemical-shift images. The time penalty encountered with CSI techniques generally leads to the acquisition of only one data set with a strong preference for short TR values and concomitantly strong saturation of compounds with long T_1 relaxation times.

Relative metabolite concentrations were calculated by measuring spectral peak areas and correcting the values by means of the measured T_1 and T_2 relaxation times. Neglecting the less meaningful data for the PME and PDE resonances, the results for P_i, PCr, and ATP are summarized in the last column of Table 1. The values are normalized to PCr. When PCr is taken as an internal reference with an absolute concentration of about 5.0 m*M* the numbers may be read as absolute concentrations in millimolar. A direct determination of absolute concentrations with use of an external standard was not the subject of the present study.

The absolute concentrations of phosphate metabolites in normal human brain reported in three recent studies using ISIS sequences (*54, 67, 68*) range from 1.0 to 2.6 mM for P_i, 2.7 to 4.3 mM for PCr, 2.1 to 5.7 mM for α-ATP, and 1.8 to 3.5 mM for β- and γ-ATP in rough agreement with the present results. More accurate data based on a technique that is less susceptible to systematic errors are due to an approach by Bottomley *et al.* (*69*) using fully relaxed FID spectra of transverse sections of the total brain including an external reference and MRI correlations of muscle and brain contributions. The corresponding concentrations yield 1.5–1.7 mM for P_i, 5.1–5.2 mM for PCr, and 3.0 mM for ATP in close relation to the relative concentrations in Table 1. The 20% increase in the apparent ATP concentration calculated from the α-ATP resonance may be explained by a corresponding signal contribution of dinucleotide resonances (NAD) as demonstrated in the proton-decoupled spectrum shown in Fig. 3.

In general, phosphorus STEAM spectra offer a variety of advantages for quantitative studies. While spectra calculated from delayed FIDs with missing data points suffer from a typical hole centered around the PCr resonance, and the intensities of true FID spectra are affected by a broad hump in that region (*70*), STEAM sequences provide a solution to these problems in the form of echo acquisition. In particular, highly accurate peak areas for P_i are available at a certain degree of T_2 attenuation where its long T_2 relaxation time allows a complete removal of overlap with resonances from the PME and PDE chemical-shift ranges. The data from TE = 5–10 ms spectra yield a concentration of 1.0 mM (relative to 5.0 mM for PCr). The corresponding metabolite ratios (corrected for relaxation effects) are $PCr/P_i = 5$, $PCr/ATP = 1.5$, and $P_i/ATP = 0.3$, respectively. On the other hand, most reports using ISIS sequences seem to overestimate the P_i concentrations in human brain. In fact, if the evaluation of our data were entirely based on TE = 3 ms spectra, then the relative P_i concentration would be calculated to be 1.5 mM.

CONCLUDING REMARKS

This work demonstrates the feasibility and potential of phosphorus STEAM spectroscopy for localized investigations of phosphate metabolites *in vivo*. In general, good quality spectra of the human brain can be obtained from a 100 ml VOI in 5–10 minutes, making clinical applications of localized phosphorus spectroscopy more suitable for studies of "global" degenerative diseases rather than for focal lesions. Quantitative evaluations of metabolite concentrations benefit from the accurate determination of T_1 and T_2 relaxation times *in vivo*. In this respect, deliberate T_2 attenuation may improve the assessment of P_i and other mobile components in the PME and PDE spectral regions.

The practical advantages of broadband proton decoupling are the access to PE, GPE, and GPC (as well as other phosphate metabolites if present) and the removal of unresolved proton splittings from the ATP resonances. The latter finding separates contributions from adenine dinucleotides to the α-ATP resonance and may give further information on the ATP–Mg^{2+} complexation. The not yet clarified sensitivity improvements by "transient" NOE effects represent a complication that may affect quantitative determinations of relaxation times and metabolite concentrations.

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REFERENCES

1. G. K. RADDA, B. RAJAGOPALAN, AND D. J. TAYLOR, *Magn. Reson. Q.* **5,** 122 (1989).
2. P. A. BOTTOMLEY, *Radiology* **170,** 1 (1989).
3. D. B. TWIEG, D. J. MEYERHOFF, B. HUBESCH, K. ROTH, D. SAPPEY-MARINIER, M. D. BOSKA, J. R. GOBER, S. SCHAEFER, AND M. W. WEINER, *Magn. Reson. Med.* **12,** 291 (1989).
4. P. R. LUYTEN, J. P. GROEN, J. W. A. H. VERMEULEN, AND J. A. DEN HOLLANDER, *Magn. Reson. Med.* **11,** 1 (1989).
5. R. D. OBERHÄNSLI, G. J. GALLOWAY, D. J. TAYLOR, P. J. BORE, B. RAJAGOPALAN, AND G. K. RADDA, *Brit. J. Radiol.* **59,** 695 (1986).
6. C. SEGEBARTH, A. GRIVIGNÉE, P. R. LUYTEN, AND J. A. DEN HOLLANDER, *Magn. Reson. Med. Biol.* **1,** 7 (1988).
7. B. D. ROSS, *Magn. Reson. Med. Biol.* **1,** 81 (1988).
8. D. J. MEYERHOFF, M. D. BOSKA, A. M. THOMAS, AND M. W. WEINER, *Radiology* **173,** 393 (1989).
9. P. A. BOTTOMLEY, *Science* **229,** 769 (1985).
10. B. RAJAGOPALAN, M. J. BLACKLEDGE, W. J. MCKENNA, N. BOLAS, AND G. K. RADDA, *Ann. N. Y. Acad. Sci.* **508,** 321 (1987).
11. P. A. BOTTOMLEY, R. J. HERFKENS, L. S. SMITH, AND T. M. BASHORE, *Radiology* **165,** 703 (1987).
12. P. A. BOTTOMLEY, H. R. HART, W. A. EDELSTEIN, J. F. SCHENCK, L. S. SMITH, W. M. LEUE, O. M. MÜLLER, AND R. W. REDINGTON, *Radiology* **150,** 441 (1984).
13. G. K. RADDA, P. J. BORE, AND B. RAJAGOPALAN, *Brit. Med. Bull.* **40,** 155 (1984).
14. J. W. PRICHARD AND R. G. SHULMAN, *Annu. Rev. Neurosci.* **9,** 61 (1986).
15. T. R. BROWN, J. S. TAYLOR, J. MURPHY-BOESCH, D. VIGNERON, T. HEDDEMA, E. KOSINSKI, AND S. J. NELSON, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, Works-in-Progress, p. 1031, Amsterdam, August 12–18, 1989.
16. R. D. OBERHÄNSLI, D. HILTON-JONES, P. J. BORE, L. J. HANDS, B. P. RAMPLING, AND G. K. RADDA, *Lancet* **2,** 8 (1986).
17. C. M. SEGEBARTH, D. F. BALÉRIAUX, D. L. ARNOLD, P. R. LUYTEN, AND J. A. DEN HOLLANDER, *Radiology* **165,** 215 (1987).
18. P. A. BOTTOMLEY, B. P. DRAYER, AND L. S. SMITH, *Radiology* **160,** 763 (1986).
19. J. J. H. ACKERMAN, T. H. GROVE, G. G. WONG, D. G. GADIAN, AND G. K. RADDA, *Nature (London)* **283,** 167 (1980).
20. P. STYLES, C. A. SCOTT, AND G. K. RADDA, *Magn. Reson. Med.* **2,** 402 (1985).
21. K. R. METZ AND R. W. BRIGGS, *J. Magn. Reson.* **64,** 172 (1985).
22. P. A. BOTTOMLEY, T. B. FOSTER, AND R. D. DARROW, *J. Magn. Reson.* **59,** 338 (1984).
23. R. J. ORDIDGE, A. CONNELLY, AND J. A. B. LOHMAN, *J. Magn. Reson.* **66,** 283 (1986).
24. A. A. MAUDSLEY, A. OPPELT, AND A. GANSSEN, *Siemens Forsch. Entwicklungsber.* **8,** 326 (1979).
25. T. R. BROWN, B. M. KINCAID, AND K. UĞURBIL, *Proc. Natl. Acad. Sci. USA* **79,** 3523 (1982).
26. J. FRAHM, K. D. MERBOLDT, AND W. HÄNICKE, *J. Magn. Reson.* **72,** 502 (1987).
27. M. L. GYNGELL, J. FRAHM, K. D. MERBOLDT, W. HÄNICKE, AND H. BRUHN, *J. Magn. Reson.* **77,** 596 (1988).
28. J. FRAHM, H. BRUHN, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **9,** 79 (1989).
29. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **9,** 126 (1989).
30. J. FRAHM, H. BRUHN, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.* **11,** 47 (1989).
31. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Radiology* **172,** 541 (1989).

32. H. BRUHN, J. FRAHM, M. L. GYNGELL, K. D. MERBOLDT, W. HÄNICKE, AND R. SAUTER, *Magn. Reson. Med.*, in press (1990).
33. A. J. S. DE CRESPIGNY, T. A. CARPENTER, AND L. D. HALL, *J. Magn. Reson.* **85,** 595 (1989).
34. C. T. BURT, S. M. COHEN, AND M. BÁRNY, *Annu. Rev. Biophys. Bioeng.* **8,** 1 (1979).
35. T. GLONEK, S. J. KOPP, E. KOT, J. W. PETTEGREW, W. H. HARRISON, AND M. M. COHEN, *J. Neurosci.* **39,** 1210 (1982).
36. D. R. BAILES, D. J. BRYANT, G. M. BYDDER, H. A. CASE, A. G. COLLINS, I. J. COX, P. R. EVANS, R. R. HARMAN, A. S. HALL, S. KHENIA, P. MCARTHUR, A. OLIVER, M. R. ROSE, B. D. ROSS, AND I. R. YOUNG, *J. Magn. Reson.* **74,** 158 (1987).
37. T. R. BROWN, S. D. BUCHTHAL, J. MURPHY-BOESCH, S. J. NELSON, AND J. S. TAYLOR, *J. Magn. Reson.* **82,** 629 (1989).
38. J. J. H. ACKERMAN, J. L. EVELHOCH, B. A. BERKOWITZ, G. M. KICHURA, R. K. DEUEL, AND K. S. LOWN, *J. Magn. Reson.* **56,** 318 (1984).
39. K. M. BRINDLE, M. B. SMITH, B. RAJAGOPALAN, AND G. K. RADDA, *J. Magn. Reson.* **61,** 559 (1985).
40. G. S. KARCZMAR, N. J. TAVARES, AND M. W. WEINER, *Magn. Reson. Med.* **9,** 8 (1989).
41. T. E. BATES, S. R. WILLIAMS, AND D. G. GADIAN, *Magn. Reson. Med.* **12,** 145 (1989).
42. S. CERDAN, V. H. SUBRAMANIAN, M. HILBERMAN, J. CONE, J. EGAN, B. CHANCE, AND J. R. WILLIAMSON, *Magn. Reson. Med.* **3,** 432 (1986).
43. W. J. THOMA, L. M. HENDERSON, AND K. UĞURBIL, *J. Magn. Reson.* **61,** 141 (1985).
44. W. J. THOMA, J. A. SNYDER, AND G. A. PEARSON, *NMR Biomed.* **2,** 112 (1989).
45. P. R. LUYTEN, G. BRUNTINK, F. M. SLOFF, J. W. A. H. VERMEULEN, J. I. VAN DER HEIJDEN, J. A. DEN HOLLANDER, AND A. HEERSCHAP, *NMR Biomed.* **1,** 177 (1989).
46. M. J. DAWSON, D. G. GADIAN, AND D. R. WILKIE, *J. Physiol.* **267,** 703 (1977).
47. F. F. BROWN, I. D. CAMPBELL, R. HENSON, C. W. J. HIRST, AND R. R. RICHARDS, *Eur. J. Biochem.* **38,** 54 (1973).
48. W. R. ADAM, D. J. CRAIK, J. G. HALL, M. M. KNEEN, AND R. M. WELLARD, *Magn. Reson. Med.* **7,** 300 (1988).
49. R. K. GUPTA, J. L. BNOVIC, AND Z. B. ROSE, *J. Biol. Chem.* **253,** 6172 (1978).
50. R. K. GUPTA, P. GUPTA, AND R. D. MOORE, *Annu. Rev. Biophys.* **13,** 221 (1984).
51. O. A. C. PETROFF, J. W. PRICHARD, K. L. BEHAR, J. R. ALGER, J. A. DEN HOLLANDER, AND R. G. SHULMAN, *Neurology* **35,** 781 (1985).
52. C. M. STREAR, L. BOLINGER, AND J. S. LEIGH, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 187, Amsterdam, August 12–18, 1989.
53. P. BACHERT-BAUMANN, F. ERMARK, H. J. ZABEL, R. SAUTER, W. SEMMLER, AND W. J. LORENZ, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 1092, Amsterdam, August 12–18, 1989.
54. J. VERMEULEN, P. R. LUYTEN, AND J. A. DEN HOLLANDER, 6th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 136, New York, August 17–21, 1987; J. A. DEN HOLLANDER, P. R. LUYTEN, A. J. H. MARIËN, C. M. SEGEBARTH, D. F. BALÉRIAUX, R. DE BEER, AND D. VAN ORMONDT, *Magn. Reson. Q.* **5,** 152 (1989).
55. B. HUBESCH, D. SAPPEY-MARINIER, J. E. HODES, AND M. W. WEINER, 7th Annual Meeting, Society of Magnetic Resonance in Medicine, Works-in-Progress, p. 54, San Francisco, August 20–26, 1988.
56. M. J. BLACKLEDGE AND P. STYLES, *J. Magn. Reson.* **83,** 390 (1989).
57. D. VAN ORMONDT, R. DE BEER, A. J. H. MARIËN, J. A. DEN HOLLANDER, P. R. LUYTEN, AND J. W. A. H. VERMEULEN, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 600, Amsterdam, August 12–18, 1989; private communication.
58. J. L. EVELHOCH, C. S. EWY, B. A. SIEGFRIED, J. J. H. ACKERMAN, D. W. RICE, AND R. W. BRIGGS, *Magn. Reson. Med.* **2,** 410 (1985).
59. C. RÉMY, J. P. ALBRAND, A. L. BENABID, M. DÉCORPS, M. JACROT, J. RIONDEL, AND M. F. FORAY, *Magn. Reson. Med.* **4,** 144 (1987).
60. E. R. ANDREW, W. S. BREY, R. GASPAR, AND A. QIU, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 769, Amsterdam, August 12–18, 1989.
61. R. FREEMAN, S. WITTEKOEK, AND R. R. ERNST, *J. Chem. Phys.* **52,** 1529 (1970).
62. A. A. BOTHNER-BY AND R. SHUKLA, *J. Magn. Reson.* **77,** 524 (1988).

63. P. C. M. VAN ZIJL, C. T. W. MOONEN, AND M. VON KIENLIN, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 646, Amsterdam, August 12–18, 1989.
64. A. ZIEGLER, J. P. ALBRAND, C. RÉMY, AND M. DÉCORPS, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 768, Amsterdam, August 12–18, 1989.
65. C. THOMSEN, K. E. JENSEN, AND O. HENRIKSEN, *Magn. Reson. Imaging* **7**, 557 (1989).
66. I. J. COX, D. J. BRYANT, G. A. COUTTS, D. G. GADIAN, C. J. PEDEN, J. SARGENTONI, AND I. R. YOUNG, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 599, Amsterdam, August 12–18, 1989.
67. G. B. MATSON, B. HUBESCH, T. J. LAWRY, D. J. MEYERHOFF, D. SAPPEY-MARINIER, AND M. W. WEINER, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 596, Amsterdam, August 12–18, 1989.
68. R. BUCHLI AND P. BOESIGER, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 598, Amsterdam, August 12–18, 1989.
69. P. A. BOTTOMLEY, C. J. HARDY, J. COUSINS, M. ARMSTRONG, AND W. WAGLE, 8th Annual Meeting, Society of Magnetic Resonance in Medicine, p. 369, Amsterdam, August 12–18, 1989; P. A. BOTTOMLEY AND C. J. HARDY, *Clin. Chem.* **35**, 392 (1989).
70. P. A. BOTTOMLEY, H. C. CHARLES, P. B. ROEMER, D. FLAMIG, H. ENGESETH, W. A. EDELSTEIN, AND O. M. MÜLLER, *Magn. Reson. Med.* **7**, 319 (1988).