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Spin-spin relaxation of the phosphodiester resonance in the ^{31}P NMR spectrum of human brain

The determination of the concentrations of phosphodiester components

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The phosphodiester peak in ^{31}P nuclear magnetic resonance spectra of human brain in vivo is often the most prominent feature of the spectrum. We have demonstrated that this resonance exhibits bi-exponential spin-spin relaxation, giving relaxation times of 2 and 10 ms. We interpret this in terms of the two components which make up the peak, bilayer lipids and the small cytosolic phosphates glycerophosphoethanolamine and glycerophosphocholine. Using the relaxation times and the relative peak heights of the two components we have also been able to quantitate the concentration of the bilayer lipids as 20–40 times that of ATP.

Nuclear magnetic resonance; Spin-spin relaxation; Human brain

1. INTRODUCTION

^{31}P NMR spectra of human brain at low field (1.5–2.0 T) show an intense resonance at 3 ppm (parts per million) relative to phosphocreatine which is the broadest, and often the tallest resonance [1,2]. This resonance, in animals, has been shown to originate mainly from bilayer phospholipids, and to a lesser extent from the small cytosolic phosphodiesters glycerophosphocholine and glycerophosphoethanolamine (Kilby, P.M., Bolas, N.M. and Radda, G.K., unpublished data).

The spin-spin relaxation time, T_2, has been determined and found to be 7 ms for a pure phospholipid bilayer and 3 ms for a pure lipid bilayer with added protein [3]. One would expect the T_2 of a cytosolic diester such as glycerophosphocholine to be larger than this, even in vivo, since a 3 ms T_2 would correspond to a resonance width of at least 100 Hz.

Detection of two components of a bi-exponential relaxation in vivo should allow determination of the concentrations of both phosphodiester components. The possibility that part of the phospholipid bilayer in vivo is invisible to NMR could then be investigated. In this paper we present data on the spin-spin relaxation times of the phosphodiester resonance in human brain using the spin-echo phase modulated rotating frame imaging method developed in this laboratory [4].

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2. METHODS

The spin-echo phase modulated rotating frame imaging method for one-dimensional chemical shift imaging was applied as described previously [4]. Spectra were acquired at 32.7 MHz in an Oxford Instruments 60 cm bore 1.9 T magnet interfaced to a Bruker Biospec spectrometer. A double concentric surface coil probe was used for both excitation and detection of the NMR signals [5]. The sweepwidth in the chemical shift (f_2) dimension was 7000 Hz, and the free induction decays were collected into 2 K of complex data points. Eight increments, of 32 scans each, were used for spatial encoding (f_1 dimension). The length of the amplitude encoding pulse varied from 160 to 1280 μs. Each image took 12.8 min to acquire, using an interpulse delay of 3 s. Two full sets of experiments were performed with echo-times used, either 20 μs, 1, 2, 4, 8 and 12 ms or 20 μs, 12, 1, 8, 2, 6, 3 and 10 ms in that order so the whole protocol was completed in 1.7 and 2.25 h, respectively.

Data were processed by complex Fourier transformation in both dimensions, with suitable data manipulation to remove any dispersion mode signals [6]. Images were scaled relative to the image acquired with the shortest echo-time. An exponential line-broadening of 15 Hz in the chemical shift dimension and a Gaussian aposidation function in the f_1 dimension were applied. The f_1 dimension was zero-filled to 64 rows. Chemical shifts were referenced to phosphocreatine at 0 ppm.

Data from sets of three sequential rows was extracted from the 2-D matrix and added together to improve the signal-to-noise ratio. These summed spectra were plotted over a wide chemical shift range (± 50 ppm) and the baseline positions were estimated by eye. Peak heights were then measured manually. Peak areas were determined by cutting out and weighing the plotted peaks.

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For bi-exponential spin-spin relaxation, the decaying magnetisation may be written as:

$$M(t) = M_A \times \exp(-t/T_{2A}) + M_B \times \exp(-t/T_{2B})$$

where $M(t)$ is the total magnetisation decaying with echo-time, t, M_A and M_B are the equilibrium magnetisations of the slow and fast relaxing components, respectively, and T_{2A}, T_{2B} are their spin-spin relaxation times.
When $T_{2A} << T_{2B}$ it can be shown that where $t << T_{2B}$

$$\ln M(t) \approx \ln(M_A + M_B) - M_A \times t/((M_A + M_B) \times T_{2A}). \quad (1)$$

And when $t >> T_{2A}$

$$\ln M(t) \approx \ln M_B - t/T_{2B}. \quad (2)$$

A line of the form $y = m_1 \times x + c_1$ was fitted to the data from the shortest echo-times (up to 4 ms) so that from eqn 1 $m_1 = -M_A/((M_A + M_B) \times T_{2A})$ and $c_1 = \ln(M_A + M_B)$. Similarily a line $y = m_2 \times x + c_2$ was fitted to the longer echo-time (4 ms and greater) data and values for $\ln M_B$ and $-1/T_{2B}$ measured. The slopes and intercepts of the two straight lines generated were determined by linear regression using the SAS statistics package (SAS Institute, Cary, NC 27512, USA) on the university VAX computer. Error analysis was performed by determination of the propagation of the variances of the derived parameters in terms of those in the regression. The variances (σ^2) of the parameters propagate as follows:

$$\sigma^2(T_{2A}) = (M_A/(M_A + M_B)m_1^2)^2 \times \sigma^2(m_1) + (M_A^2 + M_B^2) \exp(2c_2) \times \sigma_2(c_2)/((M_A + M_B)^2 m_1)^2 + (M_B/(M_A + M_B)^2 m_1)^2 \times \exp(2c_1) \times \sigma^2(2_1)$$

$$\sigma^2(T_{2B}) = (1/m_2^4) \times \sigma_2(m_2)$$

$$\sigma^2(M_A) = \exp(2c_1) \times \sigma^2(c_1) + \exp(2c_2) \times \sigma^2(c_c)$$

$$\sigma^2(M_B) = \exp(2c_2) \times \sigma^2(c_2)$$

The area of the phosphodiester peak relative to the γ-ATP peak can be determined if the assumption is made that the short T_2 component of the phosphodiester peak has the same lineshape as a spectrum of a dispersion of dimyristoylphosphatidylcholine at 37°C in aqueous buffer acquired at the same field strength. If such a spectrum is plotted with the peak height M_A then its area can be directly compared to the γ-ATP peak of the 20 μs echo spectrum acquired in vivo plotted over the same chemical shift range.

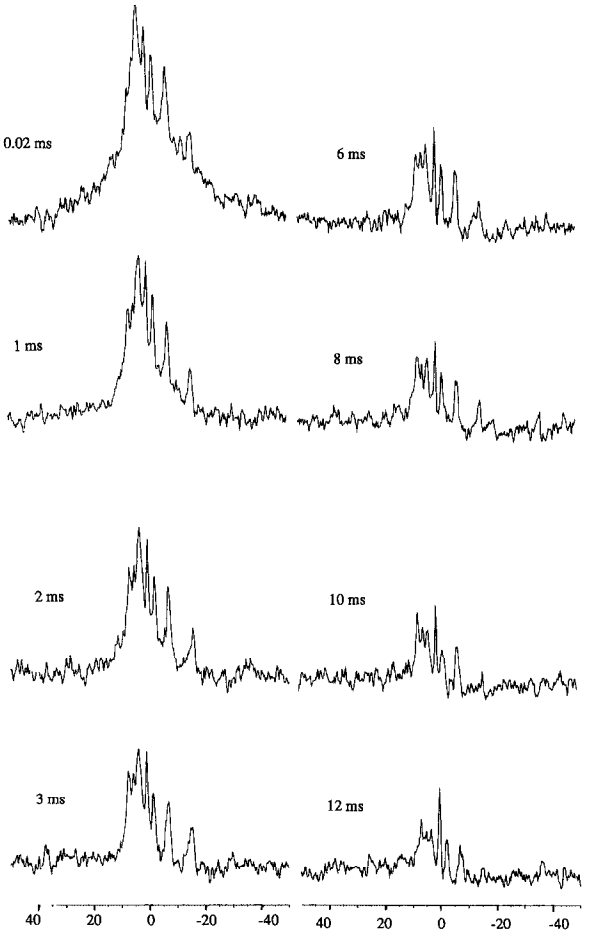

Fig. 1. Spectra of brain at different echo-times. Each is the sum of 3 rows in the data matrix and all correspond to a region of mixed grey and white matter.

3. RESULTS

A sequence of spectra at a depth which corresponds to a mixture of grey and white matter, with spin-echo times between 20 μs and 12 ms, is shown in Fig. 1. The decrease in the broad phosphodiester peak at 3 ppm is the most dramatic change with increasing echo-time. The appearance of a smaller narrow phosphodiester resonance can be seen clearly in the 8 ms echo spectrum.

Natural logarithms of peak heights plotted against echo-time are shown in Fig. 2 which clearly demonstrate the bi-exponential nature of the spin-spin relaxation. The values of T_2 for the fast and slow relaxing components of the phosphodiester resonance are shown in Table I. These show no significant changes with depth. The intensities of the component peaks are also shown and this indicates that the fast relaxing component has a much higher concentration comparable to that of bilayer phospholipids.

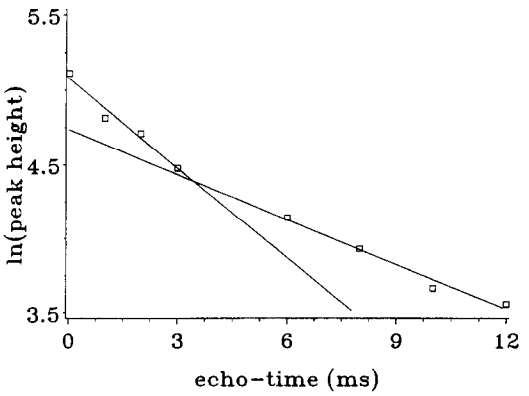

Fig. 2. Logarithmic plot of the peak heights of the diester peak at different echo-times for one depth in the brain. The steepest line is the regression line $y = m_1x + c_1$ and the other line $y = m_2X + c_2$.

Table I

Spin-spin relaxation times and peak heights of the components of the phosphodiester peak observed in the spin-echo phase modulated rotating frame experiment

Brain position	T_{2A}[a] ± SE[b]	T_{2B}[a] ± SE[c]	M_A ± SE[d]	M_B ± SE[d]	PDE/ATP[e]
First experiment					
1 mainly grey	1.6±0.2	8.9±1.8	43±7	57±11	19
2 mainly white	2.0±0.2	10.9±3.4	46±7	54±14	16
3 deeper	1.9±0.1	11.6±1.2	45±3	55±4	-
Second experiment					
1 grey + muscle	2.8±0.9	20.6±4.1	43±13	58±5	23
2 mainly grey	1.4±0.4	9.8±1.8	29±9	71±8	27
3 mainly white	3.0±0.2	19.6±4.8	54±5	46±10	39

Values of T_{2A}, the shorter spin-spin relaxation time, T_{2B}, the longer relaxation time, M_A, M_B, the peak heights calculated for the 0.02 ms echo-time and the area of the fast relaxing phosphodiester component relative to ATP. [a]Values in ms. [b]Standard errors in ms, $n=3$ in the first experiment and $n=4$ in the second experiment. [c]Standard errors in ms, $n=4$. [d]Standard errors in %, $n=3$ for M_A in the first experiment and $n=4$ for all others. [e]Intensity ratio of short T_2 PDE relative to γ-ATP

4. DISCUSSION

Determination of multiple relaxation values is notoriously difficult and normally requires a very high signal-to-noise ratio. A four parameter multi-exponential fit failed to converge on resonable values for the spin-spin relaxation times to the log analysis was used. The consistency of the data is encouraging considering the small number of data points used.

The origin of the two components of the phosphodiester peak in human brain is now clear. The fast relaxing component with a T_2 of around 2 ms is the bilayer lipid. This is consistent with the relaxation data measured by us and other workers [3] (Peter Kilby, unpublished results). The slower relaxing component is likely to be the small cytosolic compounds, glycerophosphocholine and glycerophosphoethanolamine. That this slow relaxing component originates from a molecule which gives a narrow NMR linewidth can be seen in the long echo-time spectra. This conclusion cannot exclude the possibility that this component, at least partly, originates from small vesicles which are tumbling fast in solution and thus give narrow linewidths.

Having measured the T_2s and peak heights of the two components of the phosphodiester peak it has proven possible to calculate the area of the bilayer peak in vivo. If the concentration of ATP in brain 3 μmol/g [7,8] then the NMR visible bilayer phospholipid concentration in the first experiment is around 50 μmol/g and 75–120 μmol/g in the second. The concentration of phosphate in phospholipids in human brain is 45 μmol/g in grey matter and 140 μmol/g in white matter [9]. Our values are all well within this range.

In conclusion this preliminary paper shows the presence of two components in the phosphodiester peak in human brain and allows the approximate quantitation of the bilayer lipid component relative to the ATP signal.

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