原著

^{31}P Spin-Lattice Relaxation Time Measurements in Biological Systems: Heart, Liver, Kidney and Erythrocytes of Rat.

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Spin-lattice relaxation time (T_1) of phosphorus compounds in the perfused heart, liver, kidney and erythrocytes of rats were measured by the DESPOT (Driven-equilibrium single-pulse observation of T_1) method at 8.45 T. This method is a rapid and accurate technique for the measurement of T_1 values. T_1 values of phosphomonoesters (PME), 2, 3-diphosphoglycerate (DPG), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr) and three phosphates of ATP were ranged from 0.15±0.02 sec (ß-ATP in the liver) to 8.5±1.6 sec (PDE in the kidney). T_1 value of ß-ATP in the liver was 1/4-1/5 of those in the mandibular gland, heart, erythrocytes and kidney. T_1 values obtained from biological materials are useful for selecting the optimal pulse repetition times and pulse angles to maximize the signal-to-noise ratio of ^{31}P spectra, and for correcting distortions of signal intensities in the spectra.

1. Introduction

To overcome the low sensitivity of ^{31}P nucleus, acquisition parameters, a pulse angle and a pulse repetition time, should be chosen so as to maximize the signal-to-noise ratio (S/N) of the spectrum. Under the steady state, signal intensities of resonances are suppressed by the factor[1]:

$$[1-\exp(-t/T_1)]\cdot\sin\theta/\ [1-\cos\theta\cdot\exp(-t/T_1)]$$

where θ is a pulse angle, T_1 is a spin-lattice

キーワード　phosphorus nuclear magnetic resonance, longitudinal relaxation time, ATP, creatine phosphate, phosphodiesters

relaxation time, and t is a pulse repetition time. Figure 1 shows ^{31}P NMR spectra of the perfused rat kidney collected by pulse angles of 15°, 45° and 75° with a constant pulse repetition time of 1.0 sec. Because T_1 values of three phosphates of ATP were approximately 1.0 sec, apparent S/N of the resonances increased when the pulse angle was increased. However, relative intensities of resonances were significantly distorted: the ratios of signal intensities of phosphodiesters to those of ß-ATP with pulse angles of 15°, 45° and 75° were 1.0, 0.45 and 0.31, respectively. Knowledge of the individual T_1 values is necessary to correct for this effect and to determine the tissue contents of different phosphorus compounds. Reports of T_1 measurements of phosphorus compounds in biological systems have been relatively few[2~6], since the low sensitivity of ^{31}P nucleus requires the excessive time for T_1 measurement by conventional methods. Recently, the DESPOT method (Driven-equilibrium single-pulse observation of T_1) was reported to reduce the time required for T_1 measurement to 10% of that using the inversion recovery method[1,7,8]. In addition, actual performance of the inversion recovery method was shown to be somewhat worse than the DESPOT method[1,7]. We have applied this method to the perfused heart, liver and kidney of the rat and also to the suspension of rat erythrocytes, in order to determine T_1 values of phosphorus compounds in these tissues at 8.45T.

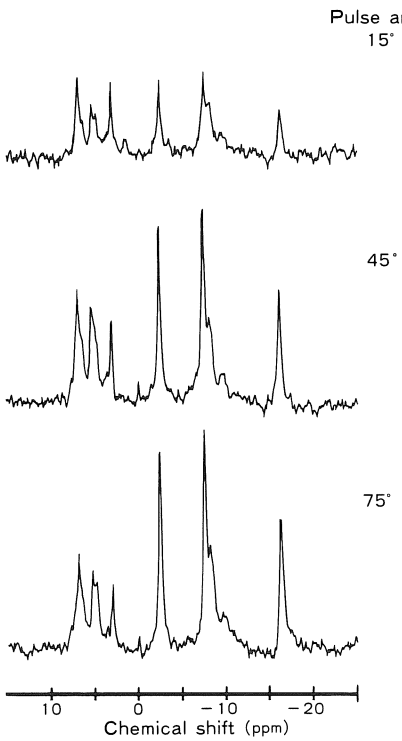

Figure 1

A series of ^{31}p NMR spectra of the perfused rat kidney at 31°C. Each spectrum is an average spectrum from 4 experiments. In each experiment, a pulse repetition time of 1.0 sec, three pulse angles from 15° to 75° in 30° steps, 1024 accumulations and a line broadening factor of 20 Hz were used. From the lower magnetic field, the resonances were assigned as PME (phosphomonoesters, 6.8 ppm), PDE (phosphodiesters, 3.0 ppm), PCr (phosphocreatine, 0 ppm), γ-ATP/ß-ADP (−2.5 ppm), α-ATP/α-ADP (−7.6 ppm) and ß-ATP (-16.2 ppm).

2. Materials and Methods

Organs were isolated from rats anestetized with sodium pentobarbital (50 mg/kg

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body weight) which was given intraperitoneally. The isolated perfused heart (male Sprague-Dawley rats, 150-200g)[9], liver (male Wistar rats, 80-120g)[10], kidney (female Sprague-Dawley rats, 90-130g)[11] and erythrocytes (male Wistar rats, 250-350g) suspended in the Krebs solution (with a hematocrit of 60-70%) of rat were placed in an NMR tube (10 or 15 mm outer diameter). Temperature of the perfusion was 37°C, except for the heart (33°C) and the kidney (31°C). ^{31}P NMR spectra were collected using a Bruker WM-360wb spectrometer (8.45T) with broad-band probes tuned to 145.8MHz for phosphorus. Six pulse angles (θ), from 15° to 90° in 15° steps, were used with constant pulse repetition time (t) of 0.3, 0.6 or 1.0 sec. The number of scans was chosen so as to yield spectra with sufficient signal-to-noise ratio. Data acquisition was completed within 1-2 hours, which ensure a reasonably constant level of organ viability. T_1 values were determined for ead of phosphomonoesters (PME), 2, 3-diphosphoglycerate (DPG), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr), γ-phosphate of ATP, α-phosphate of ATP and ß-phosphate of ATP, using the linear regression procedure of signal intensity (M(θ)) assuming the following theoretical relationship[1] :

$$M(\theta)/\sin\theta = \cos\theta \cdot M(\theta)/\sin\theta \cdot \exp(-t/T_1) + Mo\,[1-\exp(-t/T_1)] \quad [1]$$

where θ is the pulse angle, Mo is the equilibrium magnetization, T_1 is the spin-lattice relaxation time, and t is the pulse repetition time. As reported previously[8], the detectable range of T_1 values was between 0.2t and 10t. Pulse repetition times selected for each organs were : 0.3 sec for the liver, 0.6 sec for the erythrocytes, and 1.0 sec for the heart and

Table 1. T_1 values (in seconds) of phosphorus compounds in perfused organs of the rat.

Contents	Liver	Mandibular gland[8]	Heart	Erythrocyte	Kidney
β-ATP	0.15±0.02	0.81±0.05	1.04±0.13	1.05±0.07	1.13±0.09
α-ATP	0.24±0.02	0.96±0.07	1.08±0.16	0.95±0.06	1.10±0.18
γ-ATP	0.19±0.01	0.92±0.04	1.20±0.17	1.06±0.07	1.06±0.12
PCr	—	4.78±0.88	3.00±0.56	—	—
PDE	—	—	—	—	8.50±1.57
Pi	0.57±0.01	2.00±0.39	—	—	—
2-DPG	—	—	—	1.76±0.03	—
3-DPG	—	—	—	1.50±0.02	—
PME	1.10±0.07	5.21±0.94	—	—	4.13±0.45
Number of organs	(5)	(4-7)	(5)	(5)	(5)
Temperature	37°C	37°C	33°C	37°C	31°C

Note. PCr (phosphocreatine), PDE (phosphodiesters), Pi (inorganic phosphate), DPG (2,3-diphosphoglycerate), PME (phosphomonoesters). Values are means and standard errors of the mean.

the kidney.

3. Results and Discussion

T_1 values were obtained for PME, 2.3-DPG, Pi, PDE, PCr, γ-phosphate of ATP, α-phosphate of ATP and ß-phosphate of ATP. The results are listed in Table 1. T_1 values show a broad distribution from 0.15 sec (ß-phosphate of ATP in the liver) to 8.5 sec (PDE in the kidney). T_1 relaxation of phosphorus compounds depends on temperature. In these experiments, we used 37, 33 and 31°C for different organs, and differences of approximately 10% in the relaxation rates would be expected over this range of temperature. However, T_1 value of ß-phosphate of ATP in the liver was much shorter than in the mandibular gland, heart, erythrocytes and kidney.

In the liver, McLaughlin[4] and Iles[5] have reported short T_1 values for three phosphates of ATP (approximately 0.1 sec) and have speculated about the possible acceleration of spin-lattice relaxation by paramagnetic cations, such as Mn. In the present experiment, T_1 values of three phosphates of ATP in the liver were 1/4-1/5 of those in other organs. Since PME and Pi in the liver had similarly reduced T_1 values, this acceleration of spin-lattice relaxation might not be due to specific metal-ion binding to ATP[12,13]. Because the content of Fe (22 mg/kg tissue) is higher than that of Mn (1.3 mg/kg tissue)[14], the acceleration of spin-lattice relaxation could be explained by a non-specific effect due to Fe ion.

PME, PDE and PCr have relatively long T_1 values (3.0−8.5 sec) in the heart, mandibular gland and kidney. Previous investigators, using conventional methods, reported shorter T_1 values: 2.5 sec for PDE in the kidney[2] and 1.6 sec for PCr in the heart[3]. These significant differences suggest that an improper relaxation delay may have been used for the measurement of T_1 values in these studies.

T_1 values obtained by the DESPOT method allow us to choose acquisition parameters for optimizing the signal-to-noise ratio of the spectrum[15]. An optimal pulse repetition time t_{op} with a pulse angle of θ is given by:

$$t_{op}/T_1 = -\log_e(\cos\theta) \qquad [2]$$

The relative signal-to-noise ratio obtained using an optimal repetition time (t_{op}) is:

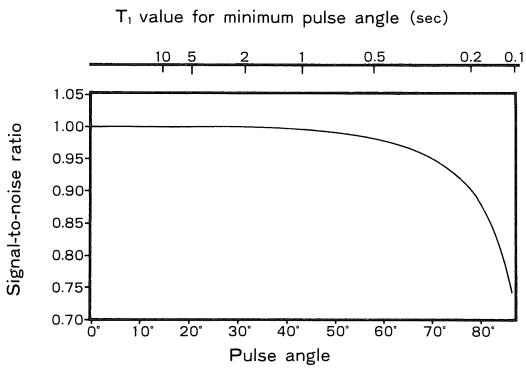

Figure 2

Relationship between pulse angle, using an optimal pulse repetition time, and relative signal-to-noise ratio. From the upper X-axis, the minimum pulse angle $\theta(\cos^{-1}(\exp(-0.3/T_1))$, which allows the separation of the discrete frequency component of RF pulse by 3.3 Hz, may be determined for a given T_1 value.

$$(1-\cos\theta)/\sin\theta \cdot (-1/t_{op})^{1/2} \qquad [3]$$

The relationship between pulse angle with an optimal pulse repetition time and relative signal-to-noise ratio is shown in Fig. 2. The signal-to-noise ratio increases when the pulse angle is reduced, and shows a plateau level below a pulse angle of 45°.

The other considerations in choosing the pulse repetition time is that RF power has discrete frequency components when applied as periodic sampling pulse. The frequency separation is inversely proportional to the pulse repetition time. Since the line widths of phosphorus compounds were observed to be approximately 15 Hz in the biological materials, we chose 0.3 sec as the minimum pulse repetition time. From the equation [2], the corresponding minimum pulse angle is $\cos^{-1}(\exp(-0.3/T_1))$ for a given T_1 value. The relationship between the minimum pulse angle, using the optimal pulse repetition time, and T_1 value is shown in Fig. 2. Thus, from the measured T_1 values and the relationship shown in Fig. 2, we can select optimal acquisition parameters maximizing the signal-to-noise ratio of ^{31}P spectra.

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