A PROTON SPIN-ECHO STUDY OF THE STATE OF WATER IN FROG NERVES

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ABSTRACT A proton spin-echo study of the water in the sciatic nerve trunk of the bullfrog reveals two distinct types of water as distinguished by their spin-spin relaxation times. The relative concentrations of these two types were determined for nerves which had been treated with normal Ringer's solution and also for nerves which had been treated with potassium-doped (0.15 M) Ringer's solution. In both cases the relative concentrations are the same as those previously determined through signal integration of high resolution proton magnetic resonance spectra obtained for the same systems, although the high resolution studies were performed on nerves doped with paramagnetic ions and the spin-echo studies were performed on undoped nerves. The doping procedure would appear to be a valid and very useful way of studying water in this neural system.

INTRODUCTION

High resolution proton magnetic resonance techniques have been recently employed to study the effect on nerve trunk water of various types of treatment of the trunk. These treatments included soaking in normal Ringer's solution (1) the passage of a sustained direct current along the axis of the trunk in an anaerobic environment (1), soaking in potassium-doped Ringer's solution (1), and soaking in Ringer's solution doped with one of several drugs (2).

Certain of these procedures, notably the treatment by potassium-doped (0.15 M) Ringer's solution are considered to produce depolarization and hence we shall subsequently refer to nerve trunks which were so treated as being depolarized. It was found that whereas the water inaccessible to added paramagnetic ions constitutes about 65% of the total water in a polarized nerve trunk, this percentage is decreased to about 43% in the depolarized case. In addition, the proton spin-spin relaxation rate is increased by a factor of approximately 1.5 upon depolarization. The difficulty with these experiments is that doping of the nerve by paramagnetic ions was required in order to isolate the signal from inaccessible water. The presence of these ions could have markedly influenced the observed phenomena and it is important to determine whether or not the changes observed occur in undoped nerves.

Proton spin-echo experiments performed on undoped nerve trunks of *Rana*

catesbeiana provide such a determination; the results clearly establish that the percentage of inaccessible water does not depend on the presence of the paramagnetic ions, and that this percentage does indeed decrease markedly upon depolarization. Previously (1) we have termed the accessible and inaccessible water as extracellular and intracellular, respectively. Since we do not know if water such as that associated with myelin is accessible or inaccessible to paramagnetic ions we have changed our terminology and will employ the terms accessible and inaccessible. In addition, the spin-echo data reveal that the proton spin-spin relaxation rate is about an order of magnitude larger for the inaccessible water of the high resolution study than for accessible, and that both rates are increased by a factor of approximately 1.5 upon depolarization.

EXPERIMENTAL

All of the experiments reported here were performed at 60 M Hz on a PS-60 spectrometer produced by NMR Specialties Inc., New Kensington, Pa. The assembly was equipped with a 12 inch Varian (Varian Associates, Palo Alto, Calif.) electromagnet, a Varian Mark II Fieldial control, and a sample temperature control unit modeled after the Varian V-6049 unit. The spectrometer probe was modified to accept a special sample tube of the type used for the high resolution proton magnetic resonance experiments (1) on the sciatic nerve trunk. A Carr-Purcell (3) sequence of one 90° pulse followed by a series of 180° pulses was used. This type of pulse sequence provides a direct measurement of the effective spin-spin relaxation time according to

$$A(t) = A(0)e^{-t/T_2^*} \qquad (1)$$

where $A(t)$ is the amplitude of the spin-echo envelope at time t, $A(0)$ is the initial amplitude, and T_2^* is the effective spin-spin relaxation time. It can be shown (4) that in general T_2^* includes effects of diffusion and spin-exchange but that for a sufficiently rapid pulse repetition rate, a, these effects approach zero and T_2^* approaches T_2, the true spin-spin relaxation time.

It was found in the experiments reported here that for pulse repetition rates greater than about 200/sec, T_2^* is independent of repetition rate and hence diffusion and exchange effects may be neglected. The data reported here were obtained using a rate of 2500/sec.

All of the nerve trunk segments used in these experiments were obtained from the sciatic nerve trunk of 6–8 inch *Rana catesbeiana*. After dissection the nerve trunks were kept in a well-aerated Ringer's[1] solution at room temperature until they were used. A portion of the nerve trunk 3–4 cm in length was then cut off and placed in a sample tube whose inner surface had been coated with a Dow Corning (Dow Corning Corp., Midland, Mich.) silicone lubricant. The sample was then placed in the spectrometer so that the nerve trunk segment extended at least 1 cm above and below the receiver coil. It had been determined that a sample of this length was magnetically equivalent to a sample of infinite length. It had been previously (1) determined that such treatment even accompanied by paramagnetic ion doping left the nerve in a functional state capable of reversible polarization and depolarization.

The spin-echo T_2 experiment was then performed at various temperatures between 0°C and 37°C. The temperature was measured with a copper-constantan thermocouple and a Honey-

[1] The Ringer's solution employed was 111 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 0.1 mM NaH_2PO_4, and 11 mM glucose.

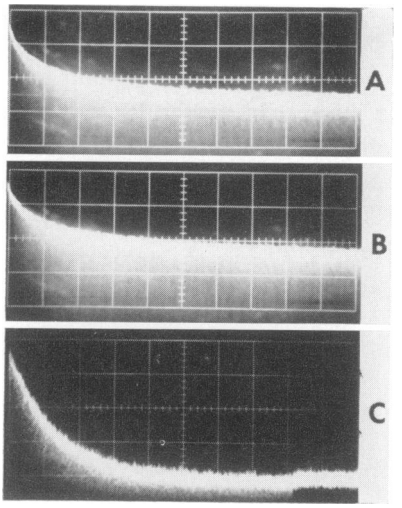

FIGURE 1 (*A*) Spin-echo envelope for a polarized frog sciatic nerve trunk segment. (*B*) Spin-echo envelope for a depolarized frog sciatic nerve trunk segment. (*C*) Spin-echo envelope for a 0.08 M $CoCl_2$ aqueous solution in the same sample tube used for the sciatic nerve trunk segment. The time scale for each photograph is 50 msec per large division. Photographs (*A*) and (*B*) are for 10.0°C and 9.9°C, respectively. Photograph (*C*) is for room temperature.

well Model 2746 potentiometer (Honeywell, Inc., Minneapolis, Minn.) Neither the sample tube nor the thermocouple were moved during the course of the entire temperature sequence. A time of at least 10 min was allowed for the sample to equilibrate at each temperature and the temperature was measured before and after each spectrum to ensure that there was no drift.

The sample was then removed from the spectrometer and placed in a Ringer's solution that was 0.15 M in KCl. It was allowed to remain in this solution for at least 10 min and then was replaced in the sample tube. The entire measurement procedure was repeated for the depolarized nerve trunk.

The spin-echo envelope was displayed on a Tektronix oscilloscope (Tektronix, Inc., Beaverton, Ore.) and was photographed with a Polaroid camera. Three separate oscilloscope sweep times, 20, 50, and 100 msec/cm were employed for all samples at all temperatures. The amplitude of the envelope above the baseline was measured at various times after the 90° pulse and plot of log $A(t)$ vs. t was constructed for each temperature.

RESULTS AND DISCUSSION

Fig. 1 *A* is a photograph of the spin-echo envelope for a polarized nerve at $T = 10.0$°C. Fig. 1 *B* is a similar photograph for a depolarized nerve trunk at $T = 9.9$°C and Fig. 1 *C* is the corresponding photograph of an aqueous cobaltous chloride solution whose high resolution full-line width is 16.2 sec^{-1}. The $CoCl_2$ solution was studied in the same sample tube employed with the nerve segments and was used as a control. The time scale in all three photographs of Fig. 1 is 50 msec per large division.

The semilog plots corresponding to Figs. 1 *A* and 1 *B* are shown in Figs. 2 *A* and 2 *B*, respectively. The envelope of the cobaltous chloride solution spin-echoes decays according to equation 1. The T_2^{-1} value for the $CoCl_2$ solution is 14.4 sec^{-1} in satisfactory agreement with the high resolution value.

Figs. 2 *A* and 2 *B* obviously do not follow the decay predicted by equation 1.

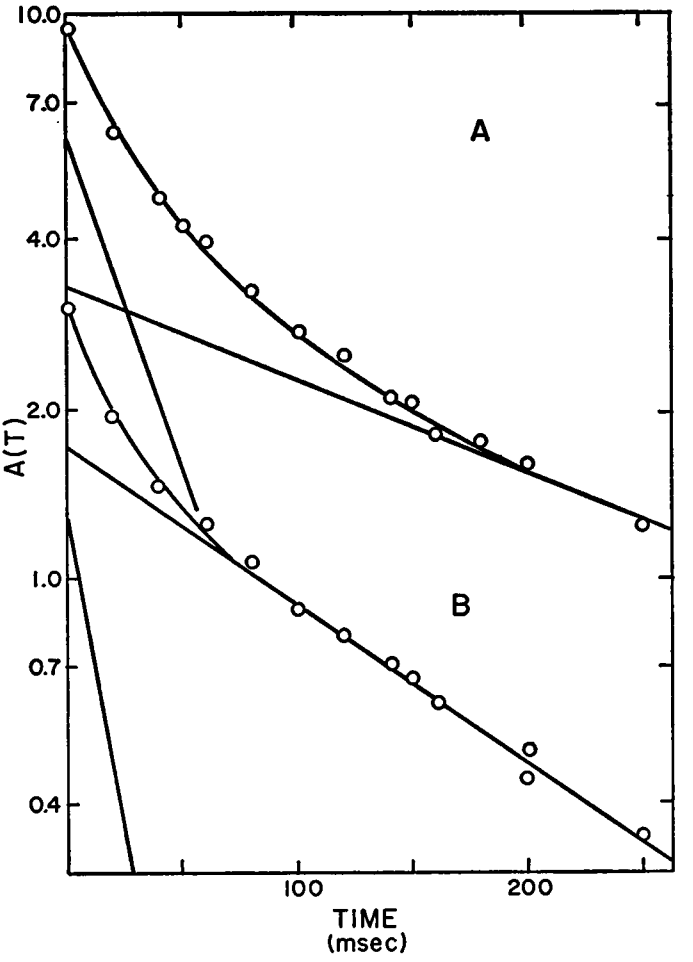

FIGURE 2 Plots of log $A(t)$ vs. t taken from the photographs shown in Fig. 1. The circles are experimental points. The curves in Figs. 2 *A* and 2 *B* have been resolved into two exponential functions according to equation 2. The top curve is for the polarized nerve and the bottom curve is for the depolarized nerve. The exponential functions of equation 2 are represented by the straight lines in the figure and the solid curves through the data points are sums of these straight lines. For clarity of presentation the top curve has been displaced upward by a factor of about 3. The value of $A(o)$ is actually equal for the same nerve in the polarized and depolarized states.

However, these decay curves can be fit within experimental uncertainty by a funciton of the type

$$A(t) = A_A(0)e^{-t/T_{2A}} + A_B(0)e^{-t/T_{2B}} \qquad (2)$$

as shown by the two straight lines in each figure. The solid curves through the data points are the sum of the two straight lines in both cases.

Equation 2 is characteristic of echo decay for two superimposed high resolution signals of different line widths, the case revealed for nerve by the high resolution study (1). When T_{2A} and T_{2B} differ sufficiently, as they do in this case, the curve-fitting procedure is relatively easy and values of the four parameters are obtained within relatively narrow limits. These parameters are then the minimum number required to characterize the echo decay.

In Table I are presented T_{2A} and T_{2B} values for both polarized and depolarized nerves as functions of temperatures. The percentage of water of type A is $100[A_A(0)/(A_A(0) + A_B(0))]$ and the values for both polarized and depolarized nerves are given in Table I. The water percentages are temperature independent within experimental uncertainty and equal to the values obtained in the high resolution study for type A being that previously (1) termed inaccessible.

The T_{2A} values for the polarized nerve are a factor of approximately 1.5 larger than the T_{2A} values for the depolarized nerve, a result which is also in agreement with the high resolution result. The T_{2A} values in both cases are smaller by about a factor of one-half than the corresponding high resolution values and the source of this discrepancy is being investigated further.

TABLE I

RELAXATION TIMES T_{2A} FOR TYPE A WATER, T_{2B} FOR TYPE B WATER, AND PERCENTAGES OF TYPE A WATER AS FUNCTIONS OF TEMPERATURE FOR POLARIZED AND DEPOLARIZED NERVES

	Polarized		
Temperature	T_{2B}	T_{2A}	%A water
°C	*sec*	*msec*	
36.6	0.20	38	65
31.5	0.24	36	65
25.6	0.26	34	65
16.7	0.26	34	66
13.0	0.26	34	65
10.0	0.26	36	64
4.6	0.24	36	65
0.8	0.26	31	65
	Depolarized		
Temperature	T_{2B}	T_{2A}	%A water
°C	*sec*	*msec*	
35.2	0.14	25	44
30.5	0.18	24	43
23.5	0.15	26	43
19.0	0.17	25	47
14.0	0.13	22	43
9.9	0.15	20	43
3.7	0.14	22	43

With the exception of the numerical values of T_{2A} the agreement between spin-echo and high resolution studies is excellent and provides strong support for the applicability of paramagnetic ion doping in the study of nerves and for the conclusions reached through this application. This is quite important since for these systems a wider range of information may be obtained through high resolution spectroscopy than through spin-echo studies. An example is exchange rate information which is easily obtained from the high resolution line shapes but is not easily obtainable from spin-echo data for the relatively slow exchange rates encountered (1). The studies presented in this paper were repeated twice with two other nerve trunks and the data of Table I are reproducible within the uncertainties indicated by the numbers in the table.

As a final note it is interesting to observe the decrease in T_{2B} upon depolarization. This indicates something that was not open to investigation by high resolution techniques (1), namely that the state of the accessible water is affected by depolarization and this observation also will be subject for further study.

We wish to express our appreciation to Professor Eli Namanworth of this department for technical assistance and several helpful discussions, and to Professor B. D. Lindley of the Physiology Department of this University for several helpful suggestions.

This investigation was supported by Public Health Service Research Grant No. CA-37668, from the National Cancer Institute, and by Public Health Service Grant No. NB 07758-01.

Received for publication 28 June 1968.

REFERENCES

1. FRITZ, O. G., JR., and T. J. SWIFT. 1967. *Biophys. J.* **7**:675.
2. FRITZ, O. G., JR., A. C. SCOTT, and T. J. SWIFT. 1968. *Nature.* **218**:1051.
3. CARR, A. Y., and E. M. PURCELL. 1954. *Physiol. Rev.* **94**:630.
4. LUZ, Z., and S. MEIBOOM. 1963. *J. Chem. Phys.* **39**:366.