NUCLEAR MAGNETIC RESONANCE STUDIES OF SODIUM IONS IN ISOLATED FROG MUSCLE AND LIVER

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ABSTRACT Nuclear magnetic resonance (NMR) was used to determine Na^+ complexing in muscle and liver (at 23°C) from bullfrogs (*Rana catesbeiana*) and to study the influence of temperature on Na^+ complexing in muscle from leopard frogs (*Rana pipiens*). The Na^+ complexed in muscle and liver was found to be 36.6 ± 4.6% and 66.1 ± 3.5% respectively. A temperature decrease from +34°C to −2°C results in a 20% decrease in the mobility of the free Na^+ in the fresh muscle. This 20% decrease in mobility results in about 50% of the free Na^+ at 34°C being complexed at the lower temperature.

INTRODUCTION

An accurate estimation of Na^+ mobility is critical to any explanation of compartmental Na^+ flux in living tissue. A common assumption that Na^+ exists primarily in free solution throughout tissue has not been conclusively supported experimentally. Values for complexed or nonmobile Na^+ in isolated fresh tissue range from approximately 30% in squid axon (1) to 60–85% in bullfrog muscle (2, 3). It has not been resolved whether the variations in estimations of Na^+ complexing result from differences between species, tissues, analytical techniques, etc.

Our studies,[1] using NMR techniques similar to those of Cope (2), were designed to characterize Na^+ complexing in muscle as influenced by temperature and in liver which has not been studied. In addition, our preliminary procedures, which involved repeating earlier muscle measurements, did not produce anticipated results. These differences, whether natural or experimental, exist and should be considered by interested investigators.

METHODS

Equipment

A Varian (Varian Associates, Palo Alto, Calif.) model No. VF-16C wide-line spectrometer set to give the derivative of the resonance curve with a magnetic field strength of approxi-

[1] For abstract of preliminary report see 1968. *Bull. Amer. Phys. Soc.* **13**:960.

mately 7000 G and a radio frequency of approximately 7880 kc/sec was used to obtain all experimental data. The 4–8 Mc probe was used throughout.

Room Temperature Studies

Frogs (*Rana catesbeiana*) were decapitated and pieces of either muscle from the upper hind leg or liver were removed, blotted, and packed tightly into an NMR tube of 1.4 cm diameter to a height of approximately 6.0 cm. Initial NMR measurements of tissue were obtained about 15 min after decapitation. Two to four traces of the NMR spectra of each sample were recorded and the average peak-to-peak height of the derivative curve was obtained in order to reduce error due to instrumental noise.

NMR spectra of a standard solution of 0.05 N NaCl plus 0.17 N KCl were recorded before and after each tissue was analyzed. The KCl was included to approximate the intracellular K levels. It has been shown by Cope (3) and confirmed by our studies that 0.10–0.17 N KCl changes the peak height of the NMR spectrum of Na^+ by about 5%. The NMR apparatus was retuned after inserting each tissue and standard sample.

Calculations of free Na^+ were based on the experimental evidence (obtained using NaCl standards) of Jardetsky and Wertz (4) and Cope (3) that the peak-to-peak height of the derivative of the Na^+ resonance curve is directly proportional to the concentration of free Na^+ in the sample. This relationship was verified by our measurements. Jardetsky and Wertz (4) also demonstrated that when Na^+ is complexed with an ion exchange resin, the NMR spectrum of Na^+ is broadened so greatly that it becomes invisible. We are, therefore, assuming that the only Na^+ in the tissue which gives rise to a resonance curve is the Na^+ in free solution. Such assumptions have been made in other NMR studies of Na^+ complexing in living cells (2, 3, 5, 6).

After initial measurements, each tissue sample was transferred to a 40 ml platinum crucible and ashed at 700°C for 12–15 hr. Porcelain and quartz crucibles are unsuitable because of problems with sodium adsorption (3). Similar ashing procedures have been used by Cope (2, 3). The ashed samples were redissolved in 0.10 N HCl and washed into the original NMR tubes used for fresh tissue analysis. Care was taken to assure that the volume of the ashed sample in HCl was the same as the volume of the fresh tissue before ashing. The free Na^+ in

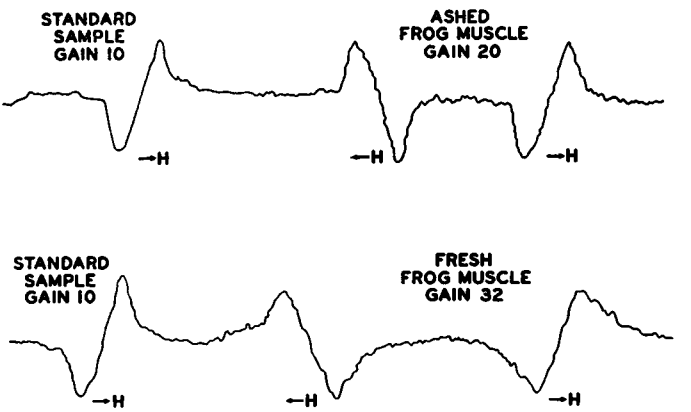

FIGURE 1 Representative NMR traces from measurements of Na^+ resonance in bullfrog muscle. Gain settings are listed on the curves. The standard sample was 0.05 N NaCl + KCl. ←H represents a decreasing magnetic field. →H represents an increasing magnetic field.

the ashed sample as determined by NMR analysis was assumed to represent the total Na^+ in fresh tissue.

Representative NMR traces from our measurements using frog muscle are shown in Fig. 1. The signal to noise ratio was approximately 12:1. The accuracy of our experimental procedures as evaluated by NMR analysis of the 0.05 N NaCl standard before and after ashing is comparable to that obtained by Cope (2, 3).

Temperature Variation Studies

The influence of temperature on Na^+ complexing in muscle was measured using fresh muscle from leopard frogs (*Rana pipiens*). All procedures were the same as those used for fresh bullfrog muscle with the following exceptions: The Varian V-4540 Variable Temperature Controller was used for sample temperature variation. When the controller was placed between the magnetic pole faces, the area available for a test tube containing the sample was decreased by approximately one-half. This caused a decrease by a factor of four in the number of Na nuclei which were available for resonance between the pole faces. Therefore, resonance signals were lower in our temperature dependent studies.

NMR analysis for Na^+ was carried out on a 0.05 N NaCl standard and fresh muscle samples (two samples for each temperature) at four different temperatures: −2°, 6°, 23°, and 34°C. This temperature range was chosen so as not to deviate too greatly from the temperature at which the cells could live. The signal to noise ratio for these muscle curves was approximately 7:1.

RESULTS AND DISCUSSION

Na^+ Complexing in Muscle and Liver

Our results (Table I) indicate that the total Na^+ in bullfrog muscle was 22.9 mM ± 1.2 (4). This is slightly lower than values given by Cope (3) using the same method and by Ling (7) using different methods. However, Na^+ losses encountered when standards were ashed in our experiments were similar to those encountered by Cope (3). The largest discrepancy between the two NMR studies occurred in estimates of per cent Na complexed in skeletal muscle: 36.6 ± 4.6 (4) % (Table I) as compared with 65–80% given by Cope (2, 3). The reason for such large differences are un-

TABLE I

SODIUM COMPLEXING IN ISOLATED BULLFROG MUSCLE AND LIVER*

Sample	Number of samples	Sodium		Complexed
		Free	Total	
		mM	*mM*	%
Muscle	4	14.5 ± 1.4	22.9 ± 1.2	36.6 ± 4.6
Liver	3	9.3 ± 1.2	27.2 ± 1.0	66.1 ± 3.5

* All Na concentrations were determined from standardized NMR signals at 23°C. Free Na^+ represents the signal in fresh tissue; Total Na^+ represents the signal in the same sample after ashing. Values represent the Mean ± SE.

known since instrumentation and analytical procedures were apparently the same in both studies, but it may be due to nutritional and seasonal differences of frogs used in the two studies. Cope's original work did not indicate these parameters and therefore, differences could have existed. Sodium losses during ashing cannot account for the difference in Na^+ complexing. Differences in the amount of blood, lymph, and interstitial fluid in different samples of the same tissue could result in large differences in NMR estimation of Na^+ complexing if this complexing was not the same in extracellular and intracellular compartments. This idea is supported by the work of Jardetsky and Wertz (6) and our most recent observations which indicate that Na^+ complexing in blood is negligible. Lower values for Na^+ complexing have also been reported for nervous tissue. Hinke (1), using microelectrodes, estimated that about 30% of the Na^+ in squid axon was complexed.

Estimates of total and bound Na^+ from the NMR spectra of Na^+ in bullfrog liver (Table I) are much higher than our data for muscle but are extremely close to Cope's (2) data for muscle. Sodium complexing in liver was not expected to be high considering that liver blood volume is high relative to that in muscle and Na^+ complexing in blood is low (8). Such data suggest that intracellular complexing of Na^+ in liver is extremely high.

Temperature-Dependent Studies

The results of the temperature-dependent studies are shown in Fig. 2. It should be noted that the scale on the curves for the standard is 10 times the scale for the fresh muscle curves. Each curve shown here represents one-half of the derivative of the resonance curve. Four separate NMR traces were averaged to obtain each curve. No appreciable change in NMR line width is seen in the NaCl standard, which in-

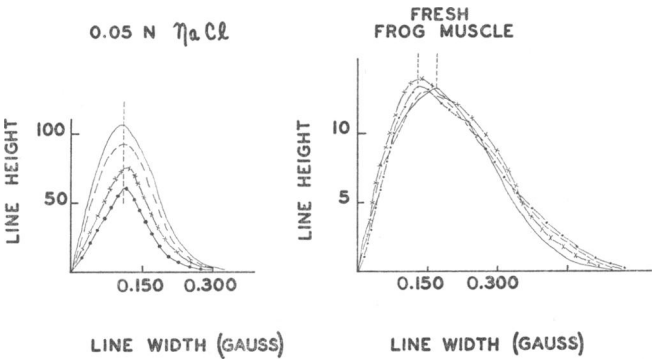

FIGURE 2 One-half of derivative of Na^+ resonance curve obtained from 0.05 N NaCl standard and fresh leopard frog muscle as a function of temperature: —— −2°C; — 6°C; —x—x— 23°C; —·—·— 34°C. Each curve is an average of four separate NMR tracings. NMR line heights are given in arbitrary units. Vertical dotted lines bisect peaks of curves at temperature extremes.

dicates that the temperature changes from −2°–34°C do not affect the reorientation or diffusion of the Na^+ appreciably in aqueous NaCl. The area under the NaCl curves is increasing as the temperature decreases. The ratio of the amplitudes of the NaCl derivative curves at the two extremes of temperature is approximately 2. This is in qualitative agreement with the theory that as the temperature decreases the density of states in the lower energy level increases and, therefore, one expects an increase in the absorption signal which is manifested in one way by an increase in amplitude (9).

Temperature curves for muscle show a change in line width as measured by the peak-to-peak separation of the curves of 0.030 ± 0.015 G (SD) between the extremes of temperature. Since the line width itself is about 0.150 G, this represents a 20% change in line width due to temperature effects. The line width is increasing as temperature decreases. This could be interpreted as a 20% decrease in the mobility of the Na^+. Cope (3) has found evidence of this line broadening with a decrease in temperature when Na^+ is in solution with organic anions such as citrate. The areas, as well as the amplitudes, under the fresh muscle curves are essentially the same. Since the ratio of amplitudes in the standard solution was 2:1 at the two temperature extremes, we might expect the same ratio when observing free Na^+ in the muscle unless there were fewer Na nuclei available for resonance at the lower temperature. A decrease in half of the nuclei would accomplish this. In conclusion, this temperature study shows that when the temperature is changed from +34° to −2°C in frog muscle, there is a 20% decrease in the mobility of the free Na^+. This 20% decrease in mobility results in about 50% of the free Na^+ at 34°C being complexed at the lower temperature.

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