NUCLEAR MAGNETIC RESONANCE OF TISSUE ^{23}Na

I. ^{23}Na SIGNAL AND Na^+ ACTIVITY IN HOMOGENATE

H. MONOI

From the Department of Physiology, Tohoku University School of Medicine, Sendai, Japan

ABSTRACT The ability to depress the resonance intensity of ^{23}Na in rat liver tissue was not found in the supernatant fraction. It was exclusively localized in particulate fractions. The intensity and saturation behavior of the ^{23}Na signal was examined in suspensions containing various amounts of the particulate fraction of rat liver homogenate. The results strongly suggest that the ^{23}Na signal of tissue reflects quadrupole interactions and does not result from a slow exchange between the free and bound fractions of Na^+. The activity coefficient of Na^+ in rat liver homogenate (no medium was added) was 0.59, about 20% less than that in the isotonic saline. Available evidences and discussion indicate that the bound Na^+ in the homogenate is much less than the so-called "NMR-invisible" fraction of Na^+.

INTRODUCTION

Cope (1–4) and others (5–8) observed that the integrated intensity of the nuclear magnetic resonance (NMR) signal of ^{23}Na (spin $I = 3/2$) in most of the biological tissues examined is 30–50% of the intensity expected from the Na content. Pulse NMR studies by Cope (3, 9) and Berendsen and Edzes (10) showed that the ^{23}Na in rat and rabbit tissues has a single longitudinal relaxation time T_1 and two different transverse relaxation times T_2; the slow T_2 accounts for 30–50% of the total resonance intensity. An interpretation for these results, originally presented by Cope (1, 2, 9), is that the invisible steady-state signal and the fast T_2 of tissue ^{23}Na correspond to the bound ^{23}Na ("NMR-invisible" ^{23}Na), which is in slow exchange with the ^{23}Na in free solution; accordingly, the bulk of tissue Na^+ is in a bound state.

Shporer and Civan (11), however, observed small satellite bands in the ^{23}Na spectrum of liquid crystals of sodium linoleate in water; the intensity of the central band was 34–39% of the intensity expected from the Na content. The loss in the intensity of the ^{23}Na resonance of this system is evidently due to a quadrupole splitting. They offered an alternative interpretation that the observed signal of tissue ^{23}Na also reflects quadrupole interactions and does not imply the binding of the bulk of tissue Na^+. Edzes et al. (12) demonstrated a quadrupole splitting of ^{23}Na and ^{7}Li ($I = 3/2$) signals in hydrated oriented DNA fibers. Magnuson and Magnuson (13) examined the line

width and intensity of the ^{23}Na resonance of the suspensions of gram-negative bacterial cells, suggesting that the ^{23}Na resonance of this system also reflects quadrupole interaction, although they did not succeed in observing any satellites.

The main purpose of the present paper is to show that the ^{23}Na signal of tissue reflects quadrupole interactions and does not directly predict the amount of the bound Na^+ in tissue.

MATERIALS AND METHODS

Materials

Liver from adult female rats (Wistar) was used. Large pieces of tissue, tissue homogenate, and its centrifugal fractions were examined. Tissue was thoroughly homogenized in a glass and Teflon homogenizer without any added medium.

NMR Spectroscopy

The NMR signal (derivative of absorption mode) of ^{23}Na in 1.8 g samples was obtained at 22–24°C with a wide-line NMR spectrometer (Varian Associates, Palo Alto, Calif.; model V-4200B with a V-3606 electromagnet) operating at a radio frequency of 11.262 megacycles/s. The field swept with a modulation of 20 cycles/s. Unless otherwise stated, a sufficiently low level of rotatory radio frequency field H_1 was employed. In order to improve the signal-to-noise ratio, a relatively large amplitude of the field modulation was used. This gave practically identical line widths to the signals of all the samples examined at a sufficiently low H_1. The signal width of 5 M NaCl was about 30 mG at sufficiently small amplitudes of field modulation. Dilute NaCl solutions were used as the standard.

Determination of Na^+ Activity

The Na^+ activity was determined at 22–24°C with a NAS_{11-18} Na^+-selective glass electrode (Corning Glass Works, Corning, N.Y.; no. 476210). The reference electrode was a 3.3 M KCl-calomel half-cell with a junction of sleeve type. Care was taken to minimize the contamination of the sample with the KCl solution. The standard was NaCl solutions buffered with Tris-HCl (I = 0.1, pH 8.1). 0.77 was employed for the γ_{Na} at I = 0.1. The Na^+-electrode changed 57.1 mV (av) for each decade change in the Na^+ activity. The electrode exhibited a fairly longer transient in the homogenate before the final value was reached. After the transient period, the potential was stable for more than 1 h.

Na Content

The Na content was determined with the Na^+-electrode, after portions of the samples were dried at 105°C, ashed at 650°C in platinum crucibles, dissolved in 0.1 N HCl, and finally brought to pH 8 with Tris powder. The blank was run without sample.

RESULTS AND DISCUSSION

NMR Signal of ^{23}Na

The resonance intensity of ^{23}Na in rat liver tissue was 39% of the intensity expected from the Na content (Table I). When liver tissue was homogenized (no medium was added), the resonance intensity remained unchanged throughout all the range of H_1

TABLE I

NMR SIGNAL INTENSITY OF ^{23}Na IN RAT LIVER TISSUE AND ITS HOMOGENATE

The signal intensity was expressed in percentage of the intensity expected from the Na content. The values shown are the mean of three samples, followed by the range of the observation for an individual sample in brackets. Rats of the same group as in Table II were used.

Sample	Water (percent by wt)	Na content	Intensity
		mmol/kg sample	%
Tissue	70.6	26.5	39
	(70.1–70.9)	(25.9–27.0)	(38–41)
Homogenate	70.6	26.3	38
	(70.0–71.2)	(25.2–27.2)	(38–39)

available (Table I; Fig. 1 *a*, curve C). This suggests that the relaxation times for the visible ^{23}Na signal are similar in both liver tissue and its homogenate.

As shown in Fig. 1 *a* (curve B), no loss in the resonance intensity of ^{23}Na could be observed in the supernatant fraction of the homogenate. The ability to depress the resonance intensity of ^{23}Na was exclusively localized in the particulate fraction (Fig. 1 *b*, curved D). According to Cope (9), the free Na^+ in tissue is dissolved in *structured tissue water* because the T_2 for the visible ^{23}Na signal is four to five times shorter than for ^{23}Na in NaCl solution. However, Fig. 1 *a* (curve B) shows that the $T_1 \cdot T_2$ of the ^{23}Na in the supernatant fraction are much longer than the $T_1 \cdot T_2$ for the visible

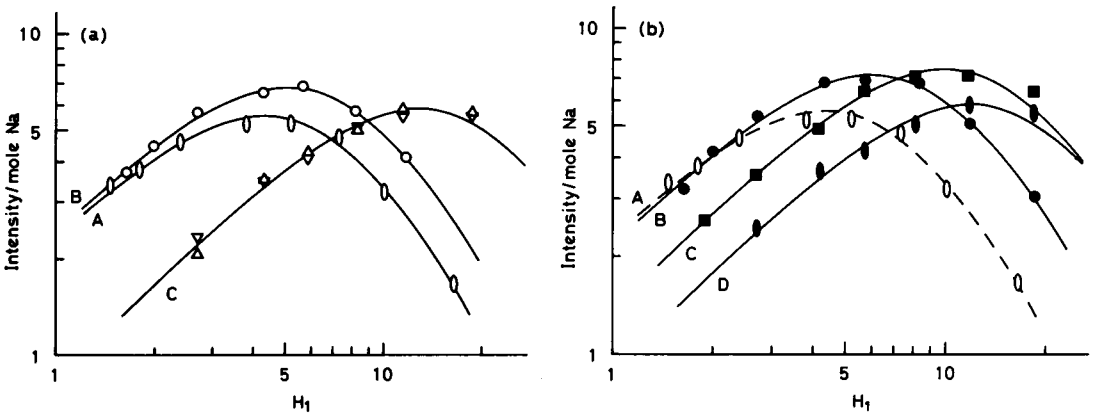

FIGURE 1 Peak-to-peak height of the ^{23}Na resonance plotted against the observed H_1. ▯, 30 mM NaCl; △, rat liver tissue; ▽, its homogenate (no medium added); ○, supernatant fraction (270,000 *g*, 15 h); ●, ■, ▮, various concentrations of the particulate fraction (3.7, 15.6, 22.7% as dry wt) in a Ringer's solution (28 mM Na^+, 127 mK K^+, 8 mM Mg^{++}, 0.5 mM Ca^{++}, 9 mM phosphate, the remaining anion being Cl^-; pH 6.8–7.0). H_1 and resonance intensity per 1 mol Na^+ are expressed in arbitrary units. Theoretical curves were fitted without any mathematical curve-fitting procedures.

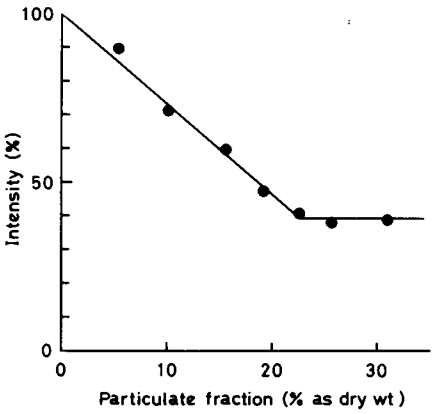

FIGURE 2 Effect on ^{23}Na resonance of varying concentrations of the particulate fraction of rat liver tissue. The particulate fraction was suspended in the same solution as in Fig. 1. Intensity is expressed in percentage of the intensity expected from the total Na^+. Concentration is expressed in grams (as dry wt) per 100 g suspension.

^{23}Na signals of the tissue and its homogenate; it is rather similar to the $T_1 \cdot T_2$ of the ^{23}Na in NaCl solution (cf. Appendix).

Figs. 1 *b* and 2 show experiments in which various amounts of the particulate fraction were suspended in a Ringer's solution containing 28 mM Na^+. The $T_1 \cdot T_2$ became shorter as the concentration of the particulate matter increased. At higher concentrations, the fractional intensity of ^{23}Na resonance exhibited a constant value (about 40%). It should be noted here that the transition $1/2 \leftrightarrow -1/2$ of spins $I = 3/2$ comprises 40% of the total resonance intensity, when quadrupole coupling occurs in the presence of strong magnetic field. These results are quite difficult to explain by means of the model of slowly exchanging free and bound ^{23}Na. They strongly suggest that the signal of ^{23}Na in homogenate and hence in tissue reflects quadrupole interactions.

Activity Coefficient of Na^+

The γ_{Na} of rat liver homogenate (no medium was added) was 0.59 (Table II, exp. A), about 20% less than that of the isotonic saline. A few times of freezing and thawing had no effect on the γ_{Na} of the homogenate. It has been reported (14–17) that the intracellular γ_{Na} of several giant cells is 0.19–0.46, as determined with Na^+-responsive glass microelectrodes. The liver homogenate prepared in the present study did not show such low level of γ_{Na}.

There is uncertainty concerning the observed Na^+ activity, for the liquid-junction potential at the reference electrode is quite difficult to estimate in the homogenate containing concentrated particulate matter. However, potentiometry on the centrifugal supernatant fraction of the homogenate gave practically the same γ_{Na} as of the original homogenate (Table II, exp. B). When roughly evaluated by Henderson's equation with appropriate parameters, the liquid-junction potential for the supernatant

TABLE II

ACTIVITY COEFFICIENT OF Na^+ IN RAT LIVER HOMOGENATE

The values shown are the mean of three samples, followed by the range of the observation for an individual sample in the brackets. Two (A) or four (B) rats of the same group as in Table I were sacrificed for each sample.

Exp.	Water (percent by wt)	Na content	Na^+ activity		γ_{Na}
			Fresh ($\times 10^{-3}$)	Freeze-stored* ($\times 10^{-3}$)	
		mmol/kg water			
A	70.4	37.9	22.4	22.5	0.59‡
	(70.3–70.4)	(36.5–40.6)	(20.9–24.2)	(20.7–24.6)	(0.57–0.60)
B	70.7	37.4	21.6§		0.58
	(70.5–71.0)	(36.7–38.6)	(21.0–22.1)		(0.57–0.59)

*After two to four times of freezing and thawing (at −20°C and room temperature, respectively).
‡(a_{Na} of fresh sample)/(Na content).
§Na^+ activity observed in the centrifugal supernatant fraction (270,000 *g*, 15 h) of the homogenate.

fraction may be expected to be 2–3 mV, at most, which corresponds to an error of some 10%, at most, in the activity determination (cf. ref. 16).

Another experiment confirmed this in a somewhat temporary way. After the homogenate packed in cellulose tubing was thoroughly dialyzed against a solution of nearly the same Na^+ activity as in the undialyzed sample, the electrochemical potential would be the same in both sides of the cellulose membrane. The observed Na^+ activity of the dialyzed sample was roughly 8% (which corresponds to 1.9 mV) larger than that of the dialyzing solution (Table III, exp. A). Considering the Donnan effect and the organic cations present in the sample, we may say that the liquid potential is roughly 1–3 mV, at most, in the dialyzed homogenate.

TABLE III

Na^+ ACTIVITY IN THOROUGHLY DIALYZED HOMOGENATE OF RAT LIVER

Homogenate packed in cellulose tubing (Visking Corp., Chicago, Ill.; no. 20/32) was dialyzed at 0–3°C for 60 h with nine exchanges of 10 vol of the solution: 28 (A) or 0 (B) mM Na^+, 127 (A) or 155 (B) mM K^+, 8 mM Mg^{++}, 0.5 mM Ca^{++}, 9 mM phosphate, the remaining anion being Cl^-; pH 6.8–7.0 at 20°C. The values shown are the mean of five samples, followed by the range of the observation for an individual sample in brackets. Two or three rats were sacrificed for each sample.

Exp.	Water	Na^+ activity		Ratio
		Homogenate ($\times 10^{-3}$)	Dialyzing solution ($\times 10^{-3}$)	
	%			
A	76.9	21.9	20.3	1.08
	(75.7–77.8)	(21.6–22.4)		(1.06–1.10)
B	76.8	0.51*		
	(75.8–78.7)	(0.46–0.53)		

*Not corrected for cations other than Na^+

Another source of uncertainty arises in the Na^+ activity determination, for the glass electrode may be regarded as being more or less responsive to the cationic groups of bio-organic molecules (18). Table III (exp. B) shows that after homogenate was dialyzed against a solution containing no added Na^+, the *apparent* Na^+ activity was 5×10^{-4}. Therefore, even if γ_{Na}, γ_K, and γ_H are assumed to be zero (the dialyzed sample still contained an appreciable amount of Na^+), the organic cations can explain only 2% of the Na^+ activity of the original homogenate. During dialysis, less than 2 g of organic substances per 100 g homogenate were lost through the cellulose membrane. If we assume that the lost organic substances have an average molecular weight of 100 and an average selectivity coefficient comparable to that of K^+ for the NAS_{11-18} glass, only 1% can be explained of the Na^+ activity in the original homogenate.

Thus, available data indicate that the error inherent in the experimental method is less than +10%. We may conclude that the bound Na^+ is 20–30% at most in rat liver homogenate.

This result is unfavorable to Cope's interpretation, according to which the ^{23}Na signal of rat liver homogenate implies that about 60% of the Na^+ is in a bound state. Of course, the amount of the bound Na^+ estimated from the γ_{Na} does not involve such type of binding that is accompanied by the concomitant binding of a large number of water molecules. However, in order to explain the observed signal in the homogenate by Cope's hypothesis, an unreasonably large amount of water (more than 30–40% of the total water in the homogenate) should be bound at the surface of, or within, the particulate components of the homogenate.

CONCLUDING REMARKS

The results presented in this paper indicate that the ^{23}Na signal of biological tissue reflects quadrupole interactions and does not result from a slow exchange between the free and bound fractions of Na^+. The visible signal of tissue ^{23}Na presumably represents the transition $1/2 \leftrightarrow -1/2$ of all the ^{23}Na in tissue. We cannot estimate the amount of the bound Na^+ from the resonance intensity of ^{23}Na. The potentiometry with a Na^+-electrode revealed that in rat liver homogenate, the *bound* Na^+ is 20–30% at most of the total Na^+.

The ability to depress the resonance intensity of tissue ^{23}Na was not found in the supernatant fraction. It was exclusively localized in particulate fractions. Consequently, we can suppose two states of tissue ^{23}Na. The ^{23}Na in one state will be present mostly or exclusively in the liquid phase of tissue and characterized by a single T_2 and a single Larmor frequency, if the exchange of the nuclei between the two states is absent. The ^{23}Na in the other state will interact with the membranous components (or other enormous assemblies of molecules or atoms) of tissue in such a way that two different T_2 occur of tissue ^{23}Na. Under these suppositions, the observed signal of tissue ^{23}Na can be well explained, as shown in the papers that follow.

The author thanks Dr. N. Miyamoto for his many suggestions and making the NMR spectrometer available for this study, and Dr. Y. Akama for his skillful help in operating the spectrometer.

Received for publication 28 May 1974.

APPENDIX

The peak-to-peak height of the derivative of the absorption curve of an assembly of identical spins obeying the Bloch equations is proportional to

$$[\gamma H_1 T_2^2/(1 + \gamma^2 H_1^2 T_1 T_2)^{3/2}] M_0, \tag{1}$$

where M_0 is the magnetization along the applied static magnetic field in the absence of the rotatory radio-frequency field H_1; γ is the gyromagnetic ratio; T_1 and T_2 are the relaxation times. The H_1 giving the maximum height, $H_{1,max}$, is proportional to $(T_1 T_2)^{-1/2}$. When a relatively large amplitude of field modulation is used in wide-line spectroscopy, the difference in the $H_{1,max}$ of two saturation curves tends to decrease.

REFERENCES

1. COPE, F. W. 1965. *Proc. Natl. Acad. Sci. U.S.A.* **54**:225.
2. COPE, F. W. 1967. *J. Gen. Physiol.* **50**:1353.
3. COPE, F. W. 1970. *Physiol. Chem. Phys.* **2**:545.
4. LING, G. N., and F. W. COPE. 1969. *Science* (Wash. D.C.). **163**:1335.
5. CZEISLER, J. L., O. G. FRITZ, JR., and T. J. SWIFT. 1970. *Biophys. J.* **10**:260.
6. ROTUNNO, C. A., V. KOWALEWSKY, and M. CEREIJIDO. 1967. *Biochim. Biophys. Acta.* **135**:170.
7. MARTINEZ, D., A. A. SILVIDI, and R. M. STOKES. 1969. *Biophys. J.* **9**:1256.
8. REISIN, I. L., C. A. ROTUNNO, L. CORCHS, V. KOWALEWSKI, and M. CEREIJIDO. 1970. *Physiol. Chem. Phys.* **2**:171.
9. COPE, F. W. 1970. *Biophys. J.* **10**:843.
10. BERENDSEN, H. J. C., and H. T. EDZES. 1973. *Ann. N. Y. Acad. Sci.* **204**:459.
11. SHPORER, M., and M. M. CIVAN. 1972. *Biophys. J.* **12**:114.
12. EDZES, H. T., A. RUPPRECHT, and H. J. C. BERENDSEN. 1972. *Biochem. Biophys. Res. Commun.* **46**: 790.
13. MAGNUSON, N. S., and J. A. MAGNUSON. 1973. *Biophys. J.* **13**:1117.
14. HINKE, J. A. M. 1959. *Nature (Lond.).* **184**:1257.
15. HINKE, J. A. M. 1961. *J. Physiol.* **156**:314.
16. LEV, A. A. 1964. *Nature (Lond.).* **201**:1132.
17. DICK, D. A. T., and S. G. A. MCLAUGHLIN. 1969. *J. Physiol.* **205**:61.
18. EISENMAN, G. 1965. *In* Advances in Analytical Chemistry and Instrumentation. C. N. Reilley, editor. Wiley-Interscience, New York. **4**:213.