EFFECTS OF ALKALI CATIONS ON THE NUCLEAR MAGNETIC RESONANCE INTENSITY OF ^{23}Na IN RAT LIVER HOMOGENATE

HIROSHI MONOI

From the Department of Physiology, Tohoku University School of Medicine, Sendai, Japan 980

ABSTRACT Effects of alkali cations on the nuclear magnetic resonance intensity of ^{23}Na were studied in rat liver homogenate. The loss in the resonance intensity of ^{23}Na in the homogenate was able to be divided into two components, one of which is abolished by the addition of Cs^+ ("Cs-sensitive component"), the other being insensitive to Cs^+ ("Cs-insensitive component"). Both components were sensitive to guanidinium ion. In a pH range of 7.4–4.9, the Cs-sensitive component varied remarkably, but the Cs-insensitive component remained virtually unchanged. The sequence of effectiveness of alkali cations (300 mmol/kg sample) in restoring the fractional intensity of ^{23}Na was: Cs ~ Na > Li ~ Rb > K. It was suggested that the sequences of effectiveness of alkali cations in abolishing the two components are quite different from each other. The present results were examined within the framework of a simple model. Within this framework, the results suggest that there occur, in particulate fractions, sites whose affinity for Cs^+ is sufficiently lower than that for Na^+.

INTRODUCTION

The main features of the nuclear magnetic resonance (NMR) signal of tissue ^{23}Na are: (*a*) the (integrated) resonance intensity is 30–50% (in most tissues examined) of the intensity expected from the Na content (Cope, 1967; Czeisler et al., 1970; other references can be found in Monoi, 1974*a*), (*b*) tissue ^{23}Na possesses two different transverse relaxation times T_2 (Cope, 1970*a*, *b*; Berendsen and Edzes, 1973; Shporer and Civan, 1974), (*c*) it possesses a single longitudinal relaxation time T_1 (Berendsen and Edzes, 1973; Shporer and Civan, 1974).

According to the current interpretation of tissue ^{23}Na signals (Shporer and Civan, 1972; Berendsen and Edzes, 1973; Monoi, 1974*a*, *b*, 1976; Monoi and Katsukura, 1976), the longer and the shorter T_2 of tissue ^{23}Na correspond to the respective T_2 of the transition $1/2 \leftrightarrow -1/2$ and of the transitions $\pm 3/2 \leftrightarrow \pm 1/2$ of the whole ^{23}Na nuclei in tissue. Macromolecules dissolved in liquid phase of tissue make practically no contribution to the loss in the resonance intensity (and hence the occurrence of two different T_2) of tissue ^{23}Na; the loss in the resonance intensity of tissue ^{23}Na is due to the presence of subcellular membranes (or other components of the particulate fraction) including cell membrane (Monoi, 1974*a*; Monoi and Katsukura, 1976).

In this paper, the effects of alkali cations on the resonance intensity of ^{23}Na are studied in rat liver homogenate. The results indicate that the loss in the resonance intensity of ^{23}Na in the homogenate consists of two components, which differ from each other in several respects. The results were examined within the framework of a simple model. Within this framework, the results strongly suggest that there occur, in particulate fractions, sites whose affinity for Cs^+ is sufficiently lower than that for Na^+.

MATERIALS AND METHODS

Materials

Liver from adult rats (Wistar strain) was used. Excised tissue was cut into small pieces, mixed with 0.28 or 0.60 part by wt of maleate-NaOH-NaCl buffer, pH 6.8–7.0, and homogenized in a glass homogenizer with a loose-fitting Teflon pestle. 1,440-mg aliquots of the homogenate were suspended in 360 mg of pure water ("basic suspension"); the final concentration of Na^+ and maleate were 136.0–137.6 mmol/kg sample and 10 mmol/kg sample, respectively. The concentration of homogenized tissue in suspension was 50 or 62.5% as wet wt. Further addition was indicated in figures. Cations were added as chloride salts. After allowed to stand at 0–3°C for 5–6 h, the suspensions were frozen in dry ice-alcohol and stored at about −20°C for a few days until they were examined by NMR spectroscopy. This procedure of freezing and thawing had no effect on the resonance intensity of ^{23}Na.

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 was modulated at 20 cycles/s. A sufficiently low level of radio-frequency field and a relatively large amplitude of field modulation were used. The modulation amplitude employed gave practically identical peak-to-peak widths (~220 mG) and practically maximum peak-to-peak heights to the signals of all the samples examined including NaCl solutions. At sufficiently small modulation amplitudes, the peak-to-peak width of dilute NaCl solutions was about

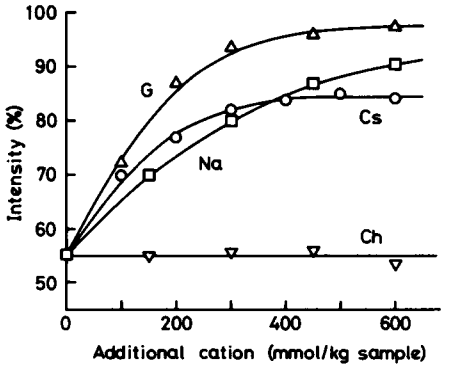

FIGURE 1 Effect of the additional cation concentration on the resonance intensity of ^{23}Na in rat liver homogenate. The concentration of homogenized tissue in suspension is 62.5% as wet wt. Resonance intensity is expressed in percentage of the intensity expected from the Na content. Each point is the mean of four or five samples. Ch, choline ion; G, guanidine-HCl.

45 mG. This large value of the signal width of NaCl solution is predominantly due to the inhomogeneity of the magnetic field. Dilute NaCl solutions were used as the standard.

pH

The pH of suspensions of homogenized tissue was determined at 22–24°C with a conventional glass pH electrode. The reference electrode was a 3.3 M KCl-calomel half-cell with a junction of sleeve type.

Na Content

The Na content of liver tissue was determined with a NAS_{11-18} Na^+-sensitive glass electrode (Corning Glass Works, Corning, N.Y.; cat. no. 476210), after tissue was homogenized, diluted by a sufficient amount of 0.1 N HCl, stirred, and brought to pH 8 with Tris powder. The standard was NaCl solutions buffered with Tris-HCl (I = 0.1, pH 8.1). The blank was run without sample.

RESULTS

Effect of Cation Concentration

The resonance intensity of ^{23}Na in the basic suspension (containing 62.5% as wet wt of homogenized tissue) was 55.2% of the intensity expected from the Na content (Fig. 1). When increasing amounts of Na^+ were added to the basic suspension, the fractional resonance intensity of ^{23}Na increased towards the 100% level. Choline ion (up to 600 mmol/kg sample) had no appreciable effect. Guanidinium ion was more effective than Na^+; at a concentration of 400 mmol/kg sample or more, the fractional intensity of ^{23}Na was restored to near 100%. As for Cs^+, the case was quite different. At a Cs^+ concentration of 400 mmol/kg sample or more, the resonance intensity reached a maximum, which was definitely below 100%.

The result indicates that the loss in the resonance intensity of ^{23}Na in the basic suspension can be divided into two components, one of which is abolished by the addition of Cs^+ (Cs-sensitive component), the other being insensitive to Cs^+ (Cs-insensitive component). Both components are sensitive to guanidinium ion.

It should be noted here that after *additional* ^{23}Na is applied to the basic suspension and a new equilibrium state is attained, the fractional intensity of the *intrinsic* ^{23}Na previously present in the basic suspension will be the same as the fractional intensity of the whole (i.e., *additional* + *intrinsic*) ^{23}Na in the suspension. Consequently, even when the signal of ^{23}Na is being observed, the effects of additional Na^+ and other cations can be directly compared.

Effect of pH

The two components of the loss in the resonance intensity of ^{23}Na exhibited quite different pH dependencies (Fig. 2). In a pH range of 7.4–4.9, the Cs-insensitive loss in intensity (i.e., the loss in intensity in the presence of a sufficient amount (600 mmol/kg sample) of Cs^+) remained virtually unchanged. On the other hand, the Cs-sensitive loss in intensity (i.e., the difference between the intensities in the presence of a sufficient amount of Cs^+ and in the absence of additional cations) varied remarkably in that pH range.

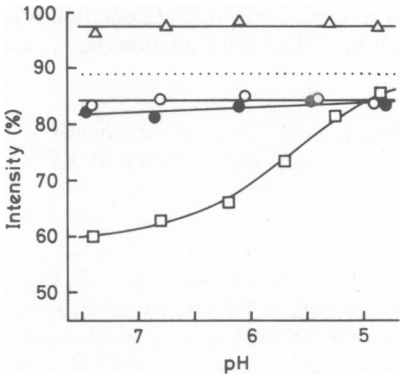

FIGURE 2 Effect of pH on the resonance intensity of ^{23}Na in rat liver homogenate in the presence or absence of Cs^+. pH was adjusted by adding HCl or by replacing some part of NaCl with equimolar NaOH. ● and ○, 62.5% homogenate containing Cs^+ (300 and 600 mmol/kg sample, respectively); □, 50% homogenate containing no additional cations; △, 62.5% homogenate containing guanidine-HCl (600 mmol/kg sample). The dotted line represents the resonance intensity for 50% homogenate containing Cs^+ (600 mmol/kg sample). Each point is the mean of three or four samples.

In the presence of guanidinium ion (600 mmol/kg sample), the fractional intensity of ^{23}Na was around 100% and virtually independent of pH.

Relative Effects of Alkali Cations

When one of the alkali cations was added to the basic suspension, the fractional resonance intensity of ^{23}Na increased (Fig. 3). At an additional cation concentration of 300 mmol/kg sample, Cs^+ and Na^+ were most effective; the sequence of effectiveness in restoring the fractional intensity of ^{23}Na was: Cs ~ Na > Li ~ Rb > K.

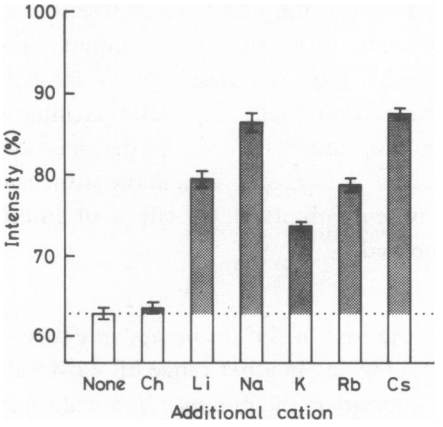

FIGURE 3 Relative effects of alkali cations on the resonance intensity of ^{23}Na in rat liver homogenate. The concentration of additional cations is 300 mmol/kg sample. The concentration of homogenized tissue is 50% as wet wt. Vertical bars denote the standard error of the mean (of six samples). Ch, choline ion.

This sequence of selectivity cannot be found among Eisenman's 11 sequences of selectivity (Eisenman, 1962), which have proved to be the selectivity sequences actually observed in most biological and nonbiological phenomena (see, for example, Diamond and Wright, 1969). One possible and, probably, most reasonable explanation for this discrepancy is that the loss in the resonance intensity of ^{23}Na consists of at least two components which have different sequences of selectivity from each other. The sequences of effectiveness of alkali cations in abolishing the Cs-sensitive and the Cs-insensitive losses in intensity are most likely quite different from each other.

DISCUSSION

The present results will be examined within the framework of a somewhat simplified model presented in a previous paper (Monoi, 1974*b*). The model is composed of four conditions: (*I*) there occur two states, A and B, of ^{23}Na in the system, (*II*) the exchange of ^{23}Na between the two states is rapid (but not too rapid), (*III*) (for state A) $\tau_c \ll \omega_L^{-1}$ (τ_c, the correlation time for the fluctuation of the electric field gradients at the position of the ^{23}Na nucleus; ω_L, the Larmor angular frequency), and the fluctuating field gradients average to zero during a period sufficiently shorter than T_2, (*IV*) (for state B) (*a*) $\tau_c \gtrsim \omega_L^{-1}$, and/or (*b*) the fluctuating field gradients at the nucleus do not average to zero during a period sufficiently longer than ω_L^{-1}. In the first order effect of the perturbation by quadrupole interactions, the theoretical line shape of the ^{23}Na resonance of tissue (or, at least, tissue composed of unoriented cells) consists of two Lorentzian components, both characterized by the same Larmor frequency. The narrower component comprises 40% of the total integrated intensity.

The ability to depress the resonance intensity of tissue ^{23}Na is localized in particulate fractions (Monoi, 1974*a*). When increasing amounts of the particulate fraction are added to a Ringer's solution, the resonance intensity of ^{23}Na decreases approximately linearly with the increase of the concentration of the particulate matter; at higher concentrations, the intensity exhibits a constant value (~40%) (Monoi, 1974*a*). Since the activity coefficient of Na^+ in liver homogenate (no homogenizing medium added) is only 20–30% less than that in the isotonic saline (Monoi, 1974*a*), the fractional population, p_B, of ^{23}Na in state B in liver homogenate may be regarded as approximately proportional to the concentration of the particulate matter. Therefore, in a first approximation, the loss in the resonance intensity of ^{23}Na in liver homogenate can be regarded as proportional to p_B in the range of the fractional loss in intensity between 0 and ~50%.

Within the framework of the model, it is reasonable to assume that ionic (or nonionic polar) groups on the surface of (or within) subcellular membranes (or other components of the particulate fraction) interact, in the sense defined by condition *IV*, with ^{23}Na nuclei present in their neighborhoods (or in contact with them) and hence are responsible for the loss in the resonance intensity of ^{23}Na. (Such groups will be referred to as "site N".) Additional cations increase the fractional intensity of ^{23}Na because they will shield site N through transient ion pair formation or complexing

(and hence decrease the number of available site N per one ^{23}Na nucleus) and consequently decrease p_B.

Under these assumptions, the following consideration indicates that (at least) some fraction of the sites N possesses a sufficiently low affinity for Cs^+ as compared with that for Na^+. If there occurs only one species of site N in liver homogenate, then, when one of the alkali cations M_i^+ is added to the basic suspension at a constant pH, p_B is proportional to the parameter p:

$$p \equiv \frac{[N]}{K^*_{Na} + [Na]_{bas} + (K_{Na}/K_i)[M_i]_{add}}, \tag{1}$$

where K_i is the dissociation constant of M_i^+ for site N, $[Na]_{bas}$ is the concentration of Na^+ in the basic suspension, $[M_i]_{add}$ is the concentration of the additional cation M_i^+ extrinsically applied to the basic suspension, [N] is the concentration of site N in the suspension, and K^*_{Na} may be regarded as constant ($\geq K_{Na}$). When increasing amounts of the additional cation are applied to the basic suspension, p_B decreases towards zero (in the case that K_i is not sufficiently larger than K_{Na}), and, consequently, the fractional intensity of ^{23}Na increases towards the 100% level, irrespective of parameters other than p_B affecting the relaxation times of ^{23}Na. This is not consistent with the present data for Cs^+ (see Fig. 1). This discrepancy is quite difficult to explain without assuming that (at least) some fraction of the sites N possesses a sufficiently large K_{Cs} as compared with K_{Na}.

On the other hand, almost all the NMR studies of tissue water suggest, or are compatible with, the occurrence of a large fraction of "free" water having a very short correlation time and one or more small fractions of "immobilized" water having longer correlation times. Accordingly, there is also a possibility that ^{23}Na dissolved in immobilized water interacts, in the sense defined by condition *IV*, with the electric-field gradients produced by immobilized water molecules. Additional cations could alter directly the structure of the immobilized water (and hence alter the nuclear magnetic parameters of ^{23}Na dissolved in it) and consequently alter the fractional intensity of ^{23}Na. In this situation, the effectiveness of additional cations in restoring the fractional intensity of ^{23}Na could be related to their ability to alter the water structure. However, we cannot find any correlation between them, for (*a*) the sequence of effectiveness in abolishing the Cs-insensitive loss in intensity is: Na > Cs ~ Mg (~0), and the sequence of effectiveness in abolishing the Cs-sensitive loss is: Mg > Cs > Na (data for Mg^{++}, in preparation), whereas (*b*) Mg^{++} and Na^+ are "structure-formers" (Mg ≫ Na), and Cs^+ is a "structure-breaker" (Frank and Evans, 1945; Frank and Wen, 1957); it is also noted that guanidinium ion is moderately "structure-making" (see Subramanian et al., 1971), but virtually abolished the loss in the resonance intensity of ^{23}Na. These suggest that the change of water structure brought about directly by additional cations makes little, or small, contribution to the increase of the fractional intensity of ^{23}Na in liver homogenate.

Therefore, within the framework of the model, the present results suggest that there occur, in particulate fractions, sites whose affinity for Cs^+ is sufficiently lower than that

for Na^+. The simplest explanation for the present results is as follows. There will occur at least two species of site N, one of which possesses a sufficiently low affinity for Cs^+ as compared with that for Na^+ ("Cs-disfavoring site"), whereas the other favors Cs^+ ("Cs-favoring site"). The former is independent of pH in the range of 7.4–4.9. The latter seems to contain weakly acidic groups. The two species of site N possess quite different sequences of preference for alkali cations. It is interesting that the Cs-disfavoring site seems to contain no weakly acidic group whose pK_a is larger than 4.5–5.

I thank Dr. N. Miyamoto (Research Institute of Electrical communication, Tohoku University, Sendai, Japan) for his suggestions and making the NMR spectrometer available for this study.

Received for publication 30 September 1975 and in revised form 9 August 1976.

REFERENCES

BERENDSEN, H. J. C., and H. T. EDZES. 1973. The observation and general interpretation of sodium magnetic resonance in biological material. *Ann. N. Y. Acad. Sci.* **204**:459.

COPE, F. W. 1967. NMR evidence for complexing of Na^+ in muscle, kidney, and brain, and by actomyosin. The relation of cellular complexing of Na^+ to water structure and to transport kinetics. *J. Gen. Physiol.* **50**:1353.

COPE, F. W. 1970*a*. Complexing of sodium ions in myelinated nerve by nuclear magnetic resonance. *Physiol. Chem. Phys.* **2**:545.

COPE, F. W. 1970*b*. Spin-echo nuclear magnetic resonance evidence for complexing of sodium ions in muscle, brain, and kidney. *Biophys. J.* **10**:843.

CZEISLER, J. L., O. G. FRITZ, JR., and T. J. SWIFT. 1970. Direct evidence from nuclear magnetic resonance studies for bound sodium in frog skeletal muscle. *Biophys. J.* **10**:260.

DIAMOND, J. M., and E. M. WRIGHT. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Ann. Rev. Physiol.* **31**:581.

EISENMAN, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2** (Suppl.): 259.

FRANK, H. S., and M. W. EVANS. 1945. Free volume and entropy in condensed systems. III. Entropy in binary liquid mixtures; partial molal entropy in dilute solutions; structure and thermodynamics in aqueous electrolytes. *J. Chem. Phys.* **13**:507.

FRANK, H. S., and W.-Y. WEN. 1957. Structural aspects of ion-solvent interaction in aqueous solutions: a suggested picture of water structure. *Discuss. Faraday Soc.* **24**:133.

MONOI, H. 1974*a*. Nuclear magnetic resonance of tissue ^{23}Na. I. ^{23}Na signal and Na^+ activity in homogenate. *Biophys. J.* **14**:645.

MONOI, H. 1974*b*. Nuclear magnetic resonance of tissue ^{23}Na. II. Theoretical line shape. *Biophys. J.* **14**: 653.

MONOI, H. 1976. Nuclear magnetic resonance of tissue ^{23}Na. Correlation time. *Biochim. Biophys. Acta.* In press.

MONOI, H., and Y. KATSUKURA. 1976. Nuclear magnetic resonance of ^{23}Na in suspensions of pig erythrocyte ghosts: a comment on the interpretation of tissue ^{23}Na signals. *Biophys. J.* **16**:979.

SHPORER, M., and M. M. CIVAN. 1972. Nuclear magnetic resonance of sodium-23 linoleate-water. Basis for an alternative interpretation of sodium-23 spectra within cells. *Biophys. J.* **12**:114.

SHPORER, M., and M. M. CIVAN. 1974. Effects of temperature and field strength on the NMR relaxation times of ^{23}Na in frog striated muscle. *Biochim. Biophys. Acta.* **354**:291.

SUBRAMANIAN, S., T. S. SARMA, D. BALASUBRAMANIAN, and J. C. AHLUWALIA. 1971. Effects of the urea-guanidinium class of protein denaturants on water structure: heats of solution and proton chemical shift studies. *J. Phys. Chem.* **75**:815.