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^{23}Na-NMR STUDIES OF Na^+ INTERACTION WITH HUMAN RED CELL MEMBRANES FROM NORMOTENSIVES AND HYPERTENSIVES

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Na^+ interaction with unsealed human red cell ghosts has been studied by ^{23}Na-NMR relaxation rate (R_1) measurements. Data on a total of nine subjects including seven volunteer normotensives (NBP) and two untreated hypertensives (HBP) are presented. Qualitative treatment of the data gives information on the dynamic behavior of Na^+ undergoing fast exchange between the free and bound states. The excess longitudinal relaxation rate $(\Delta R)^{-1}$ plotted against total $[Na^+]$, known as the James-Noggle plot, exhibits different behavior for NBP and HBP ghosts, with a relatively low binding constant of approx. 100 M^{-1} for HBP ($p < 0.025$) compared to a high constant of 500–1000 M^{-1} for NBP. To associate our NMR data with membrane-bound ($Na^+ + K^+$)-ATPase, ^{23}Na relaxation rates were measured in the presence of 5 mM ouabain. James-Noggle plots constructed for ouabain-sensitive excess relaxation rates show the binding for NBP to be even high affinity ($> 10^3$ M^{-1}) but low capacity. These data may suggest that for a given amount of intracellular Na^+, the binding affinity could determine the distribution of Na^+ between the membrane and cytoplasm, and that the ($Na^+ + K^+$)-ATPase which is primarily responsible for the Na^+ affinity might assume an abnormal transport mechanism in HBP membranes.

1. Introduction

Hypertension is a complicated disorder, and over 90% is so-called essential hypertension for which there are no known causes [1]. Clinical studies have suggested that it could be genetically determined. Investigations at the molecular level, thus, become imperative to support this view. A number of reports have demonstrated altered ion permeabilities [2–4] in vascular smooth muscle and red cells of essential hypertensive patients. Subsequently, the Na^+,K^+ pump in human red cells elicited a great deal of interest in research, and its alteration has been found and related to hypertension in recent studies [5–9]. Direct evidence is however lacking, as the purified enzyme from red cells is not yet available.

NMR techniques have been extensively applied to biological systems. Relaxation data are especially useful in probing the dynamic properties of ions. $^{23}Na^+$, though a quadrupole-relaxed nucleus with a spin of 3/2, has an advantage in that as a cation it is always under conditions of extreme narrowing, which results in a single peak in the spectrum. Bull [10] derived expressions for a two-state model, undergoing chemical exchange, in which longitudinal relaxation ($R_1 = 1/T_1$) data are readily obtained for analysis. The validity for a single time-averaged T_1 has been discussed in a recent study by Shinar and Navon [11]. For the data analysis, James and Noggle [12] previously presented a time-averaged expression for the two-

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state, free and bound, model system undergoing fast exchanges:

$$R_1 = (1/T_1) = R_{1F}X_F + R_{1B}X_B \quad (1)$$

where R_1 is the experimental relaxation rate, R_{1F} and R_{1B} the respective relaxation rates of free and bound Na^+, and X_F and X_B the corresponding mole fractions of free and bound Na^+. With the introduction of C_m and C_s ($C_s \ll C_m$) for the total concentrations of ions and interaction sites, respectively, and K_b for the ion-site formation constant, eq. 1 can be rearranged to give the James-Noggle expression:

$$(\Delta R)^{-1} = (R_1 - R_{1F})^{-1} = K_b^{-1}[C_s(R_{1B} - R_{1F})]^{-1} + C_m[C_s(R_{1B} - R_{1F})]^{-1} \quad (2)$$

Since $(\Delta R)^{-1}$ can be experimentally measured, the apparent binding affinity of Na^+ is readily estimated from the James-Noggle plot, $(\Delta R)^{-1}$ vs. C_m, the total [Na^+], by extrapolating the line onto the negative [Na^+] axis.

A study by Urry et al. [13] using ^{35}Cl- and ^{23}Na-NMR has indicated that Na^+ interaction sites with human red cell ghosts are detectable on the cytoplasmic side of the membrane, and that samples from hypertensive (HBP) patients might have a different Na^+ affinity than those from normotensive (NBP) control subjects. However, the behavior of NBP Na^+ binding was poorly defined, and the total [Na^+] that was critical in the determination of K_b was not corrected for the residual [Na^+] in the samples. It is, therefore, the objective of the present study using ^{23}Na-NMR to continue the efforts in establishing the difference in terms of Na^+-binding behavior between red cell samples from hypertensive and normotensive subjects, and to characterize the difference by their responses to ouabain, an inhibitor of the Na^+,K^+ pump [14,15] but having no direct effects on T_{1F} of Na^+. Thus, an ouabain-sensitive relaxation $(\Delta R)_{ou} = R_1 - R_{1,ou}$, where R_1 and $R_{1,ou}$ are the respective experimental relaxation rates determined in the absence and presence of ouabain can be extracted. By replacing R_{1F} in eq. 2 with $R_{1,ou}$, the James-Noggle plots can be similarly applied. Since the binding in a number of HBP samples has been found to be relatively uncomplicated in a previous study [13], the emphasis of our study is on the presentation and delineation of the NBP data.

2. Experimental

2.1. Ghost preparation

Venous blood was freshly drawn from untreated hypertensive patients and normotensive volunteers. In addition to CPDA anticoagulant, a small amount of EDTA was added to the blood prior to ghost preparation. As little as 50 ml of blood could be used to provide sufficient samples for the NMR studies. Unsealed ghosts were prepared by a modified procedure of Dodge et al. [16]. Buffer of 172 mM Tris-HCl at pH 7.4 was used to wash the cells, and the diluted buffer of 11.4 mM Tris was used to lyse the cells and store the resulting ghosts. All preparations were carried out at 0–4°C. The slightly pink color of the ghosts indicated the minimal presence of hemoglobin. However, ^{23}Na T_1 measurements did not show any significant interference from hemoglobin (Sigma, type IV) alone at 0.02% (w/v), consistent with a previous report [11]. Table 1 lists the pertinent information for normotensive volunteers and hypertensive patients. Some of the normotensive

Table 1

Information on subjects' age, sex, blood pressure (BP), and protein concentration in the ghost preparation

Subject no.	Age	Sex	BP (systolic/diastolic)	Protein concentration (mg/ml)
1	23	M	120/62	6.8
2	20	M	130/72	6.3
3	18	M	130/70	6.2
4	20	M	120/70	5.3
5	53	M	110/86	3.9
6	31	M	102/66	3.9
7	55	F	160/92	3.4
8	28	M	150/96	5.7
9	28	F	*126/74	5.2

* Both parents hypertensives.

control samples (subjects 1–4) were obtained from the American Red Cross volunteers and family history of hypertension was not readily known. Subjects 5 and 6 both had a negative family history of hypertension. The last normotensive subject, no. 9, was different in that she was known to have two hypertensive parents. Both the hypertensive subjects (nos. 7 and 8) had a positive family history of hypertension.

2.2. ^{23}Na-NMR measurements

^{23}Na-NMR T_1 relaxation data were collected on a Jeol FX-100 and Nicolet NMC-300/WB spectrometer operating at 26.3 and 79.4 MHz, respectively, in the Fourier transform mode. The relaxation time T_1 was obtained with the inversion recovery technique [17]. Typical spectra showed a single peak with a linewidth of about 20 Hz, and the peak should represent almost all the Na^+ present in the sample [18]. A pulse sequence of ($P1$–$D1$–$P2$–$D2$–$D5$) was employed, where $P1$ and $P2$ were the 180 and 90° pulse widths, respectively. $D1$ was the variable waiting time for which 10 values had been selected ranging from 1 to 300 ms, D_2 the acquisition time for the free induction decay (FID), normally 0.5 s, for a resolution of 1 Hz/data point, and the sum of $D2$ and $D5$, 0.6 s, should be at least 3–5-times longer than the T_1 of interest. Typically, an accumulation of approx. 1000 FIDs for a sample of 1 mM Na^+ gave sufficient signal-to-noise ratio in the spectrum recorded on the NMC-300 spectrometer. Calculations of T_1 using peak heights were carried out with a three-parameter T1IR program. There was no apparent frequency dependence of T_1 between the two spectrometers operating at two different frequencies as also noted previously [11]. Each data point was determined usually by two runs. The experimental errors of T_1 obtained were, at most, ± 2 ms for low [Na^+] and ± 1 ms for high [Na^+] in the titration of NaCl. Approx. 5 and 10% of ^2H_2O were added to the ghost samples with final volumes of 1 and 3.5 ml, respectively, for the FX-100 and NMC-300 spectrometer to provide an internal NMR lock. The temperature of the probe was set at 30 ± 1°C. Several identical aliquots from each preparation were used in the titration to avoid sample degradation. All measurements were completed within a week after the blood was drawn. To assess the significance of differences in K_b values estimated for NBP and HBP, the p value was determined by unpaired t-test [19].

2.3. Other assays

Protein concentrations were determined by the method of Lowry et al. [20] using bovine serum albumin as the standard and by amino acid analysis on a Beckman 119 amino acid analyzer. All of the samples had protein concentrations in the range 3–7 mg/ml. Residual [Na^+] in the preparation was determined by atomic absorption spectroscopy on a Perkin Elmer 2380 model.

3. Results

Fig. 1 shows the James-Noggle plots for the first three normotensive subjects. All observed T_1 values fell within the range 30–70 ms. A value of $T_{1F} = 68$ ms for free Na^+ was used in eq. 2 as opposed to 57 ms measured in phosphate buffer [13]. T_1 measurements of $^{23}Na^+$ in the presence of egg yolk lecithin or lipids extracted from the ghost at 1 mg/ml did not show much variation from $T_{1F} = 68$ ms. Hence, we assumed that T_{1F} used in the calculation throughout the titration was the

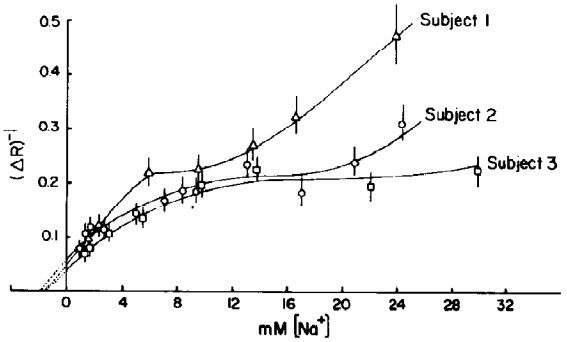

Fig. 1. James-Noggle plots for Na^+ titrations to the unsealed ghosts of subject 1 (△), 2 (○), and 3 (□) in 11.4 mM Tris-HCl at pH 7.4. [Na^+] has been normalized with respect to a protein concentration of 5 mg/ml, and is the same for all the following figures. Note the intercepts of the dotted lines with the negative [Na^+] axis.

same as that measured for NaCl (1–20 mM) alone in Tris buffer. Total [Na^+] was scaled according to the ratio of protein concentration in the sample to a protein concentration of 5 mg/ml. Data points with experimental error bars were connected by a smooth curve for each titration, except that the linear portion at low [Na^+] was determined using linear regression analysis with at least three data points ($r > 0.90$). Extrapolating the line onto the [Na^+] axis would give an estimate for apparent Na^+-binding affinity. There are several striking features in the plots. First, all three curves give apparent Na^+-binding constants in the range 500–1000 M^{-1}, i.e., 650, 500 and 660 M^{-1} for subjects 1, 2 and 3, respectively. Second, as the initial slope of the plot, $[C_s(R_{1B} - R_{1F})]^{-1}$, also gives a measure of the amount of Na^+ bound, the qualitatively similar slopes in fig. 1 indicate similar binding capacities in all three samples. Third, after the addition of 5–10 mM Na^+, the curves gradually rise up again beyond 10 and 20 mM Na^+ for subjects 1 and 2, respectively. Fig. 2 shows the James-Noggle plots for another normotensive subject, no. 4. To define the plot better,

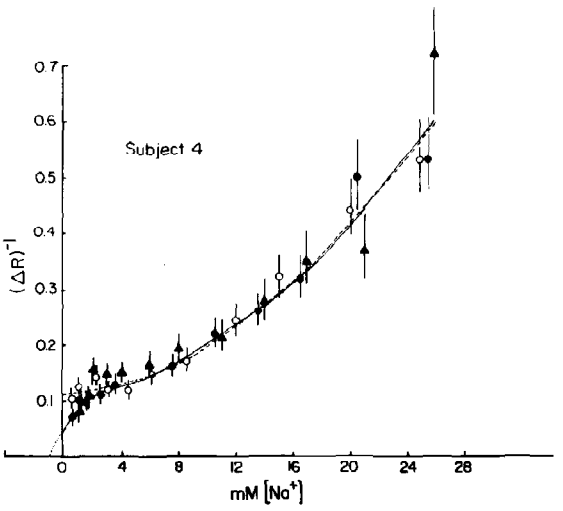

Fig. 2. James-Noggle plots for Na^+ titration to the unsealed ghosts, of subject 4 in the absence (●) and presence of 5 mM ouabain (○) in 11.4 mM Tris-HCl at pH 7.4. Note the intercept of the dotted line with the abscissa determined in the absence of ouabain. Data obtained in 5 mM phosphate buffer at pH 7.2 without ouabain (▲) are also sketched for comparison.

more titration points were chosen in the experiment. The apparent Na^+ affinity for the ghost membrane is estimated to be around 1000 M^{-1}. The shape of the curve resembles that of subject 1.

There are several possible Na^+ interaction mechanisms based on physiological experiments, and they have all been implicated in hypertension [21,22]. Among them is the Na^+,K^+ pump or ($Na^+ + K^+$)-ATPase. Ouabain, a specific inhibitor of the ($Na^+ + K^+$)-ATPase, is known to block the efflux of Na^+ and the influx of K^+ across the plasma membrane [14,15,23]. It is reasonable to assume that ouabain can affect the Na^+ interaction on the inside surface of the membrane [34]. However, ouabain was found not to alter the free $^{23}Na^+$ T_1 in NaCl solution, nor the T_{1F} values in the presence of hemoglobin/lecithin. This demonstrated the lack of effect of ouabain on T_{1F}. Hence, Na^+ titration of red cell membranes in the presence of ouabain should permit extraction of the ouabain-sensitive relaxation. This information is expected to reveal the dynamic behavior of membrane-bound ($Na^+ + K^+$)-ATPase in response to Na^+. The James-Noggle plot in fig. 2 also shows data obtained in the presence of 5 mM ouabain for subject 4. The ouabain effect occurred largely at low [Na^+] $\leqslant$ 4 mM, and diminished at higher [Na^+]. This indicates the existence of Na^+ binding to membrane ($Na^+ + K^+$)-ATPase at an early stage of the titration. With ouabain T_1 was 43 ms at [Na^+] = 0.5 mM as compared to 37 ms without ouabain at the same [Na^+]. Shown in fig. 3 are the reconstructed James-Noggle plots in which the reciprocal of the difference between experimentally measured relaxation rates in the absence and presence of ouabain was plotted vs. [Na^+]. The K_b for the ouabain-sensitive portion can be obtained from such plots by extrapolation. This parameter gives a measure of the specific Na^+ affinity to membrane ($Na^+ + K^+$)-ATPase. Thus, the line for subject 4 shows a very steep slope with an Na^+-binding constant ($\sim$ 2000 M^{-1}) which is substantially higher than that obtained in the absence of ouabain. This constant is in close agreement with the Na^+ affinity for ($Na^+ + K^+$)-ATPase determined by Kanike et al. [24] using other techniques.

Fig. 4 shows the James-Noggle plots for

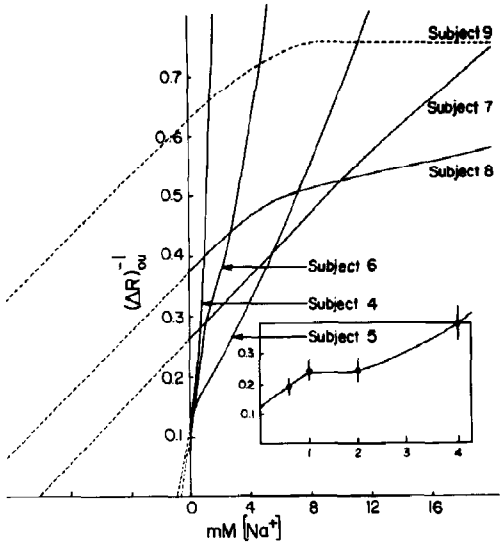

Fig. 3. Ouabain-sensitive James-Noggle plots for Na^+ titrations to the unsealed ghosts of subjects 4–9. These are difference curves defined as $(\Delta R)^{-1}_{ou} = (R_1 - R_{1,ou})^{-1}$, where R_1 and $R_{1,ou}$ are the experimental relaxation rates determined in the absence and presence of 5 mM ouabain, respectively. The individual $(\Delta R)^{-1}_{ou}$ data points for one sample (subject 5) are shown in the inset, and are omitted in the main plots for clarity. Smooth lines are drawn through those points. The vertical intercepts, $(\Delta R)^{-1}_{ou}$, are determined by the difference in the two R_1 values at $[Na^+] = 0$ mM shown in fig. 2 (for subject 4), fig. 4 (subject 5), fig. 5 (subject 6), fig. 6 (subjects 7 and 8), and fig. 7 (subject 9). All lines are extrapolated to negative values of $[Na^+]$.

normotensive subject 5 in the presence and absence of ouabain. The difference between the two curves is again striking. At $[Na^+] = 0.6$ mM, T_1 =

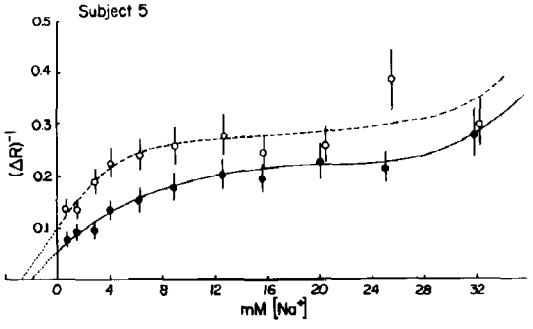

Fig. 4. James-Noggle plots for Na^+ titration to the unsealed ghosts of subject 5 in the absence (●) and presence of 5 mM ouabain (○) in 11.4 mM Tris-HCl at pH 7.4.

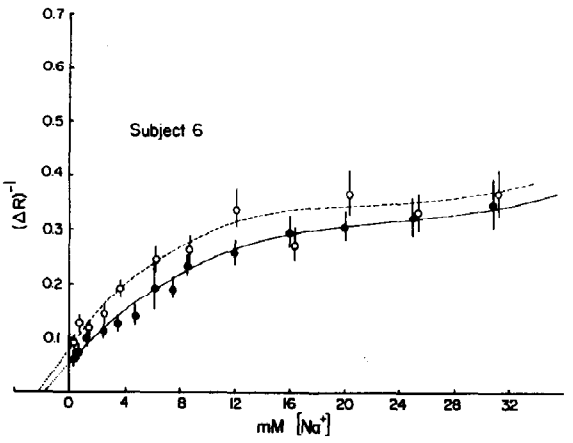

Fig. 5. James-Noggle plots for Na^+ titration to the unsealed ghosts of subject 6 in the absence (●) and presence of 5 mM ouabain (○) in 11.4 mM Tris-HCl at pH 7.4. The difference between the two plots beyond 12 mM Na^+ is not significant.

44.5 and 37 ms for samples with and without ouabain, respectively. The plot constructed for the ouabain-sensitive relaxation for this subject shown in fig. 3 exhibits an almost straight line except for a deflection around 1 mM Na^+. This may suggest an enzyme conformational change accompanying Na^+ activation. An apparent Na^+ affinity of approx. 500 M^{-1} falls within the range for normotensive blood sample mentioned above.

James-Noggle plots for normotensive subject 6 in the presence and absence of ouabain are shown

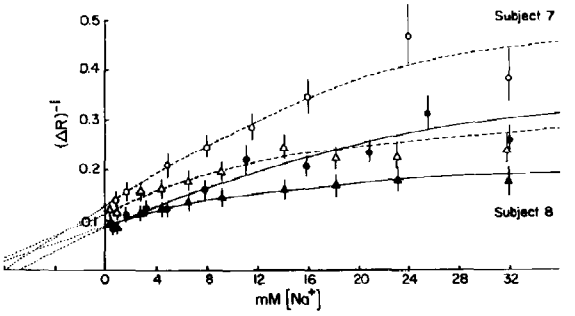

Fig. 6. James-Noggle plots for Na^+ titrations to the unsealed ghosts of subjects 7 and 8 in the absence (● and ▲, respectively), and presence of 5 mM ouabain (○ and △, respectively) in 11.4 mM Tris-HCl at pH 7.4. Note that the farther is the abscissa intercept, the smaller is the Na^+ affinity for the sample.

in fig. 5. The general appearance of the curve is similar to those for subjects 3 and 5, only with a higher plateau at $[Na^+] > 12$ mM. Na^+ affinity in fig. 5 appears very close to values presented above, approx. 600 M^{-1}. The plot for ouabain-sensitive relaxation in fig. 3, situated between those for subjects 4 and 5, again shows a deflection at 1 mM Na^+ along the almost straight line. It also extrapolates to a very high Na^+ affinity of ≥ 2000 M^{-1}.

Having studied the Na^+ binding in normotensive blood samples and presented some general features, we herein compare them with the following hypertensive samples. Fig. 6 shows typical James-Noggle plots for preparations from two HBP subjects, nos. 7 and 8, in the presence and absence of ouabain. In the case of subject 8, $T_1 = 45$ ms at $[Na^+] = 0.6$ mM with ouabain vs. 42 ms without ouabain. In general, this difference in T_1 is small for hypertensives, but the T_1 in the absence of ouabain starts with a higher value than that in NBP samples. It is noted that up to 20 mM Na^+ the shape of the curve appears more linear than that of the NBP curve. As a result, the HBP plots give a much lower apparent Na^+ affinity of 140 M^{-1} for subject 7 and 110 M^{-1} for subject 8 ($p < 0.025$ by unpaired t-test using 650 ± 150, mean $\pm$ S.D., and $n_1 = 6$ for NBP, $n_2 = 2$ for HBP). Thus, a 5-fold difference in Na^+ affinity is obtained between NBP and HBP samples. These results on the HBP samples are also supported by a previous study [13].

James-Noggle plots for the ouabain-sensitive relaxation in HBP samples are sketched in fig. 3. Subject 7 produces a nearly straight line up to 20 mM $[Na^+]$ with an apparent affinity of 100 M^{-1}, and the plot for subject 8 bends at $[Na^+] \sim 5$ mM and gives an apparent Na^+ affinity of less than 100 M^{-1} ($p < 0.05$ by unpaired t-test using $n_1 = 3$ for NBP, $n_2 = 2$ for HBP). As compared to those for NBP in fig. 3, both curves are flat and start with high values of $(\Delta R)^{-1}$ at low $[Na^+]$, indicating the slow relaxation rates and loose Na^+ binding to the membrane-bound $(Na^+ + K^+)$-ATPase. Comparison of the apparent Na^+ affinity to $(Na^+ + K^+)$-ATPase between NBP and HBP samples hereby yields an at least 10-fold difference.

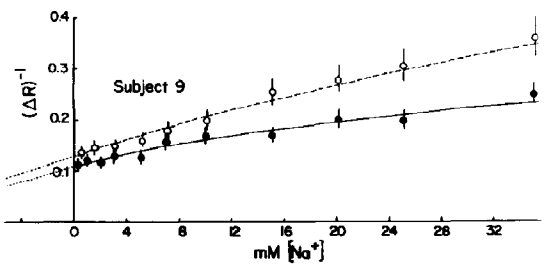

Fig. 7. James-Noggle plots for Na^+ titration to the unsealed ghosts of subject 9 in the absence (●) and presence of 5 mM ouabain (○) in 11.4 mM Tris-HCl at pH 7.4.

We also studied a preparation from subject 9 who appeared normotensive despite a positive familial history of hypertension. It is striking to find out that the Na^+ binding of this subject shown in fig. 7 appears more similar to the HBP curves in fig. 6 than to those of NBP. The ouabain-sensitive relaxation for the sample of this subject is also sketched in fig. 3. With large variation, the plot shows very high values for $(\Delta R)^{-1}$, and thus an extremely weak Na^+ affinity (< 70 M^{-1}) is obtained.

4. Discussion

Although several mechanisms in ghost membranes interact with Na^+, the apparently biphasic James-Noggle plot can be interpreted as the sum of two components: a steep region with a high Na^+ affinity and a flat portion with a very weak Na^+ affinity at $[Na^+] > 5$ mM. We have demonstrated that Na^+-binding affinity to membrane $(Na^+ + K^+)$-ATPase can be extracted from the steep component. The flat component, shared more or less in both normotensive and hypertensive samples, is probably due to Na^+ binding of other membrane proteins combined with lipids. The vertical scale, $(\Delta R)^{-1}$, is inversely correlated to the extent of Na^+ binding. Meanwhile, the slope of the component determines both the affinity and capacity of Na^+-binding mechanisms, indicated qualitatively by the curve prior to reaching approx. $(\Delta R)^{-1} = 0.6$. In reality, experimental errors limit meaningful determination of a curve beyond $(\Delta R)^{-1} = 0.5$, or $T_1 \geq 60$ ms. The major difference in Na^+ binding between normotensive and

hypertensive samples resides in the steep component in the James-Noggle plot, best displayed in low [Na^+] where the data allow extrapolation to yield a binding constant. We report in the present work that the normotensives have apparent Na^+ affinity in the range 500–1000 M^{-1}, while the hypertensives exhibit considerably lower affinity in the range 50–150 M^{-1} ($p < 0.025$).

There is a similarity of hypertensive samples to at least some normotensive samples. As shown in figs. 1 and 4–7, James-Noggle plots beyond 5 mM Na^+ display, within experimental errors, a similar shape for subjects 3, 5, 6 (normotensives), 7, 8 (hypertensives) and 9 (questionable normotensive). The origin of this similarity is not readily understood, but may reside in mechanisms other than ($Na^+ + K^+$)-ATPase. It would be interesting to study whether this is related to any tendency of developing hypertension in the future.

Unsealed ghost membranes allow studies of the Na^+ titration to the intracellular compartment. Moreover, the use of washed ghost membranes is also free of many interfering substances. Interaction of Na^+ observed in the absence of any added cofactors such as ATP would therefore be characteristic of the plasma membrane. Phosphorylation may or may not modify the Na^+ binding [34]; however, in the present study only the unsealed ghost preparation was used for simplicity and the ion gradient across the plasma membrane was absent. The choice of Tris buffer has several advantages: (i) there are no other cations in the sample for Na^+ titration, (ii) the absence of PO_4^{3-} facilitates subsequent ($Na^+ + K^+$)-ATPase activity assay, and (iii) $T_{1F} = 68$ ms in Tris buffer vs. 57 ms in phosphate buffer provides a wider range of observation. In applying the James-Noggle plot, the only assumption, $C_s \ll C_m$, is virtually valid, since the number of Na^+,K^+ pump in a red cell approximates 10^3 [25–27] and the sensitivity of ^{23}Na-NMR requires a few tenths of mM Na^+ ($C_m \sim 10^4 \times C_s$). A recent ^{23}Na-NMR study [11] using resealed red cells maintained that Na^+ binding to the cytoplasmic surface failed to enhance the intracellular Na^+ relaxation in the presence of shift reagent. It is possible that in their experiments the [Na^+]/[protein] ratios were relatively high, whereas our data have displayed the most variations of T_1 at relatively low [Na^+].

To investigate further and relate the difference in Na^+ affinity to an ion interaction mechanism, the membrane ($Na^+ + K^+$)-ATPase, we have measured the relaxation for the unsealed ghosts in the presence of ouabain. As relaxation data are presented in the usual James-Noggle plots, there may be a built-in systematic error in the calculation which arises from the choice of an R_{1F} value appropriate for a medium containing ghost membranes. This can be removed in the presentation of ouabain-sensitive relaxation depicted in fig. 3. These plots in fact reveal even greater differences in terms of Na^+ binding to membrane ($Na^+ + K^+$)-ATPase between normotensive and hypertensive samples. In the least favorable case, for instance, the slope of normotensive subject 5 is twice that of hypertensive subject 7. Furthermore, it is noted that in fig. 3 the binding of Na^+ to NBP ($Na^+ + K^+$)-ATPase appears to be high affinity but low capacity. Age seems to be related to the extents.

Swartz et al. [28] concluded, however, based on the equal ($Na^+ + K^+$)-ATPase activities they measured, that no differences in Na^+ affinity existed between normotensive and hypertensive samples. In contrast, a recent review by Parker and Berkowitz [29] listed all possible HBP ($Na^+ + K^+$)-ATPase activities with respect to NBP activities. The correlation of the ATPase activity to HBP has not been proved conclusively. Besides, we feel that the link is yet to be established between the affinity constant and the operationally defined Michaelis constant of the nonsubstrate Na^+ in a particular ATPase assay [30].

Our data have indicated an abnormality in Na^+ binding to membrane ($Na^+ + K^+$)-ATPase in hypertension, circumventing the dealings with ion transport and the enzyme activity that is subject to many variables [6,31,32]. This abnormality may be attributed either to alteration in the enzyme structure or to variation in the composition of surrounding lipids in the membrane. Both alterations may be genetically determined. Structural comparison of the purified enzyme between normotensives and hypertensives will help in delineating the various factors.

Since the Na^+ affinity of membrane ($Na^+ +$

K^+)-ATPase determines the ion distribution between the membrane and the cytosol, our data suggest that at a given total [Na^+] there will be more free intracellular Na^+ in the hypertensive red cells than in the normotensive. It is speculated that the abnormality of Na^+ interaction with membrane ($Na^+ + K^+$)-ATPase would be greatly manifest in smooth muscle cells which have an abundant amount of the enzyme (isozymes). In the present study it is not clear whether consequences of this phenomenon lead to hypertension [21], or if this abnormality serves only as a marker [33]. However, these results on a small number of both controls and hypertensive subjects are expected to provide a basis for further large-scale studies in the future.

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