Enhanced Red Cell Sodium Permeability in Patients with Borderline Hypertension

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SUMMARY

Red cell membrane sodium permeability was studied in 41 untreated patients with essential hypertension (20 borderline hypertensives and 21 established hypertensives) and 21 age matched normotensive subjects by means of the measurement of unidirectional passive influx of $^{22}\text{Na}^+$ into ouabain-treated erythrocytes. The mean value ($\pm$ SD) of $^{22}\text{Na}^+$ influx was greater in the hypertensives than in the normotensives (0.183 ± 0.047 vs 0.152 ± 0.047 mmol/l·cells/hr, respectively, $p < 0.02$). Among the patients with essential hypertension, the borderline hypertensives demonstrated a higher $^{22}\text{Na}^+$ influx than the established hypertensives (0.207 ± 0.043 vs 0.160 ± 0.038 mmol/l·cells/hr, respectively, $p < 0.001$), and $^{22}\text{Na}^+$ influx positively correlated with plasma renin activity ($r = 0.44$, $p < 0.005$). In 16 of 20 borderline hypertensives, 5 year blood pressure changes were examined retrospectively, and a positive correlation was observed between mean blood pressure increase and $^{22}\text{Na}^+$ influx value in these subjects ($r = 0.64$, $p < 0.01$). These results suggest that passive sodium influx may be altered in the course of the development of hypertension in relation to the changes in blood pressure level and that enhanced sodium permeability may be a characteristic of the early stage of essential hypertension.

Additional Indexing Words:

Sodium influx Erythrocyte Borderline hypertension

EPIDEMIOLOGICAL evidence indicates that the later occurrence of established hypertension is more common in individuals with borderline

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hypertension than in those with initially normal blood pressure.^{1),2)} Clinical studies also indicate that borderline hypertension has several physiological or hemodynamic characteristics and constitutes the first phase in the development of essential hypertension.^{2),3)}

Membrane sodium transport mechanisms are currently being studied in relation to the pathogenesis of essential hypertension. Previous studies have demonstrated an alteration of passive permeability of the erythrocyte membrane to sodium in patients with essential hypertension, showing an elevated Na-Li countertransport⁴⁾⁻⁸⁾ and an enhanced unidirectional passive Na influx.^{9),10)} The predictive value of these abnormalities for the later development of hypertensive disease has also been reported in normotensive offspring of hypertensive patients.^{11),12)} However, in order to evaluate the role of membrane sodium permeability in the development of essential hypertension, an attempt should be made to identify its characteristics in individuals with borderline hypertension, namely in those with an augmented risk of developing future hypertension. Therefore, the present study was designed to evaluate red cell sodium permeability and its relationship to other biochemical parameters in Japanese patients with borderline hypertension.

MATERIALS AND METHODS

Forty-one untreated patients with essential hypertension, 37 men and 4 women aged 22-62 years (mean age 45.9 ± 8.4 years) were studied. All patients had outpatient systolic pressure > 140 mmHg or diastolic pressure > 90 mmHg, measured on at least 3 different days in the sitting position. Of the 41 patients, 20 in whom at least one recorded blood pressure was below 160/95 mmHg were defined as borderline hypertensives and the other 21 in whom the blood pressure was consistently higher than 160/95 mmHg as established or sustained hypertensives. All patients had normal renal function and secondary causes of hypertension were ruled out by the usual screening examinations, including history, physical examination, urinalysis, blood chemistry, and when appropriate, radiologic evaluation. A family history of hypertension was checked for in each patient and 23 of 41 (10 borderline hypertensives and 13 established hypertensives) reported a positive family history in first degree relatives. All antihypertensive medications, if any, were discontinued for at least 2 weeks before the study.

Twenty-one healthy normotensive subjects, 18 men and 3 women aged 27-60 years (mean age 42.5 ± 9.9 years) were studied as an age matched control group. All had outpatient blood pressures below 140/90 mmHg and gave no personal or family history of hypertension.

To evaluate blood pressure changes in individuals, the data from periodic medical examinations for at least 5 and, if possible, 6 years were examined retrospectively. Blood pressure levels in the most recent 5 years were found to have been recorded yearly in 31 of the 41 hypertensive (16 borderline and 15 established hypertensive) patients and in 9 of the 21 normotensive subjects. Since the blood pressures of these borderline hypertensives and normotensive subjects had been recorded in the sitting position with no antihypertensive medication, 5 year blood pressure changes in each subject were calculated by subtracting the mean blood pressure over the previous 5 years from that of the present year. In 10 of the 16 borderline hypertensives, blood pressure of 6 years earlier could also be obtained, so that 6 year mean blood pressure changes were evaluated in the same manner. Blood pressure changes in the 15 established hypertensives could not be assessed and were excluded from this analysis because all of them had received antihypertensive treatment during the previous 5 years.

All subjects had been consuming their usual diets with no restriction of sodium intake prior to the study and were examined between 8 and 10 a.m. after an overnight fast. After 30 min rest in the supine position, blood pressure and pulse rate were determined and blood samples (10 ml) were taken by venipuncture into heparinized tubes for measurement of intraerythrocyte sodium content (RBC-Na) and $^{22}\text{Na}^+$ influx. Plasma renin activity (PRA), plasma aldosterone concentration (PAC), serum sodium, potassium and creatinine levels were also measured in all patients with essential hypertension.

$^{22}\text{Na}^+$ influx and RBC-Na

Assessment of the unidirectional passive $^{22}\text{Na}^+$ influx into erythrocytes was performed as previously reported by Eaton et al⁹⁾ with a slight modification. The cooled samples were centrifuged (1,750 g for 5 min) and the plasma and buffy coat were discarded. The packed erythrocytes were washed 3 times in a buffer containing 145 mM KCl, 5 mM NaCl, 10 mM Tris (hydroxymethyl) aminomethane, 10 mM morpholinopropanesulfonic acid (MOPS), 10 mM D-glucose and 0.1 mM ouabain, pH 7.4 at 37°C. After a part of the washed erythrocytes was set aside for the measurement of hematocrit and RBC-Na, 500 μl were admixed with 500 μl of the same buffer containing 0.1 μCi of $^{22}\text{Na}^+$. The cell suspension was incubated in a shaking water bath at 37°C for 60 min and then the cells were rapidly washed 3 times in 10 volumes of ice-cold non-radioactive buffer containing 75 mM MgCl_2 , 85 mM sucrose, 10 mM D-glucose, 10 mM Tris and 10 mM MOPS, pH 7.4 at 37°C. After the determination of the hematocrit, 300 μl of the washed erythrocytes were precipitated with 600 μl of 10% trichloroacetic acid. Five-hundreds μl of the clear supernatant were aspirated and its radioactivity

Fig. 1. $^{22}\text{Na}^+$ uptake by ouabain treated erythrocytes from a patient with essential hypertension. The accumulation of $^{22}\text{Na}^+$ was linear for 3 hours, although red cell sodium content (RBC-Na) did not show any change. The function was obtained by linear regression analysis.

counted. The $^{22}\text{Na}^+$ influx was calculated as described by Mahoney and co-workers⁹⁾ and was expressed as mmol Na^+ per liter cells per hour (mmol/l·cells/hr). A preliminary study indicated that $^{22}\text{Na}^+$ accumulation was linear for at least 3 hours as shown in Fig. 1.

RBC-Na was determined by the method of Kaya et al,¹³⁾ using capillary tubes as microhematocrits and was expressed as mmol Na^+ per liter cells (mmol/l·cells).

All measurements were done in duplicate. Within 6 pairs of the sample, the coefficients of variation for these assays were 7.9% for the $^{22}\text{Na}^+$ influx and 5.4% for the RBC-Na.

Statistics

The values were expressed as the mean $\pm$ standard deviation (SD). Differences between two means were analyzed using Student's unpaired t-test when the data was normally distributed. Regression coefficients were calculated using the method of least-squares. Significance was considered when the p value was less than 0.05.

RESULTS

A summary of the clinical characteristics and RBC-Na is shown in Table I. RBC-Na was significantly higher in hypertensives than in normotensives, whereas there were no significant differences related to age, sex, heart rate and body mass index between the 2 groups. Analysis of the patients with essential hypertension revealed that body mass index and PRA were significantly higher ($p < 0.05$) and blood pressure was lower ($p < 0.001$) in the borderline hypertensives than in the established hypertensives, while differences in the other variables were not statistically significant between the 2 subgroups of hypertensives.

Individual values for unidirectional passive $^{22}\text{Na}^+$ influx into ouabain treated erythrocytes are displayed in Fig. 2. The mean value of the $^{22}\text{Na}^+$ influx was significantly greater in the hypertensives than in the normotensives (0.183 ± 0.047 vs 0.152 ± 0.047 mmol/l·cells/hr, $p < 0.02$). This difference was due to the increased values of the $^{22}\text{Na}^+$ influx in the borderline hypertensives compared to those in the established hypertensives (0.207 ± 0.043 vs 0.160 ± 0.038 mmol/l·cells/hr, $p < 0.001$).

The data collected during periodic medical examination in the most

Table I. A Summary of Clinical Characteristics and Red Cell Sodium Content

	Normotensives (n=21)	All Hypertensives (n=41)	Hypertensives	
			Borderline (n=20)	Established (n=21)
Age (years)	42.5±9.9	45.9±8.4	44.0±9.4	47.8±7.0
Range	28-60	22-60	22-59	31-60
Sex (male/female)	17/3	37/4	20/0	17/4
Sitting blood pressure (mmHg)				
Systolic	117.3±9.1	154.2±15.7	145.3±12.3	162.8±14.0
Diastolic	74.7±9.2	98.1±5.8	93.6±4.5	102.3±3.2
Mean	88.9±9.2	116.8±7.7	110.9±3.9	122.6±5.4
Heart rate (bpm)	68.2±6.7	68.9±10.3	68.3±10.1	69.4±10.7
Body mass index (kg/m ²)	22.4±2.0	23.7±2.8	24.8±2.8*	22.8±2.5
Serum sodium (mEq/l)		140.5±2.2	140.1±2.2	140.8±2.2
Serum potassium (mEq/l)		4.1±0.4	4.2±0.4	4.0±0.4
Serum creatinine (mg/dl)		0.92±0.18	0.93±0.17	0.92±0.19
PRA (ng/ml/hr)		1.38±1.04	1.72±1.17*	1.06±0.81
PAC (pg/ml)		74.4±21.3	75.5±23.8	73.1±18.6
RBC-Na (mmol/l·cells)	9.48±1.29	10.32±0.93††	10.21±0.73†	10.43±1.10†

Values are mean±SD. PRA=plasma renin activity; PAC=plasma aldosterone concentration; RBC-Na=red cell sodium content.

† $p < 0.05$, †† $p < 0.01$ vs normotensives, * $p < 0.05$ vs established hypertensives.

Fig. 2. Values of unidirectional passive $^{22}\text{Na}^+$ influx in normotensives ($\circ$ NT: $n=21$) and all hypertensives ($\bullet$ HT: $n=41$) (left side). The difference in the average rate of $^{22}\text{Na}^+$ influx between the 2 groups was due to the higher values in patients with borderline hypertension (BHT: $n=20$) than in those with established hypertension (EHT: $n=21$) (right side). Bars represent mean $\pm$ standard deviation.

Fig. 3. $^{22}\text{Na}^+$ influx was positively correlated to 5 year mean blood pressure change (ΔMBP) in 16 borderline hypertensive patients (left side) and was also correlated to 6 year ΔMBP in 10 of these patients (right side).

recent 5 years revealed that the average values of mean blood pressure change were $+5.4 \pm 11.9$ mmHg for the 16 borderline hypertensives and -5.1 ± 7.9 mmHg for the 9 normotensive subjects. To evaluate the relationship between

the recent development of high blood pressure and red cell sodium permeability, regression analysis was performed. As shown in Fig. 3, there was a positive correlation between the 5 year mean blood pressure change and the value of $^{22}\text{Na}^+$ influx in the borderline hypertensives ($r=0.64$, $p<0.01$) but not in the normotensives ($r=0.12$, not significant). The positive relationship was also confirmed by a positive correlation between the 6 year mean blood pressure change and $^{22}\text{Na}^+$ influx in 10 of 16 patients ($r=0.69$, $p<0.05$) (Fig. 3), although the blood pressure levels 6 years before were not examined in the remainder of the patients.

Linear regression analysis also revealed a positive relationship between PRA and $^{22}\text{Na}^+$ influx in all the hypertensives ($r=0.44$, $p<0.005$) while PRA was significantly higher in the borderline hypertensives than in the established hypertensives and the correlation between PAC and $^{22}\text{Na}^+$ influx was not statistically significant. Although RBC-Na was significantly higher in each of the 2 subgroups of patients with essential hypertension than in the group of normotensives (Table I), it correlated weakly with the $^{22}\text{Na}^+$ influx only in the patients with borderline hypertension ($r=0.47$, $p<0.05$), but not in those with established hypertension or in the normotensive subjects. No significant correlation was found between the $^{22}\text{Na}^+$ influx and the other variables examined in the present study.

DISCUSSION

While several techniques have been employed for estimating sodium transport abnormalities across the red cell membrane, the method adopted in the present study has been reported to be a simpler and more direct one for the measurement of red cell sodium permeability.⁹⁾ The $^{22}\text{Na}^+$ influx value obtained by this method may reflect a combination of ouabain-insensitive sodium transport processes and it has been suggested that it correlates closely with the activity of the $\text{Na}^+\text{-Li}^+$ countertransport system.^{9),10)} A more recent study has also indicated that, under physiological conditions, the total $^{22}\text{Na}^+$ influx is closely correlated with the bicarbonate-independent phloretin-resistant part (Na^+ influx via Na^+ leak) as well as the phloretin-sensitive part (Na^+ influx via Na^+ countertransport) of the $^{22}\text{Na}^+$ influx.¹⁴⁾

The present results indicate that unidirectional passive $^{22}\text{Na}^+$ influx into ouabain treated erythrocytes was greater in the whole group of patients with essential hypertension than in the normotensive subjects, although a large area of overlap existed. This is consistent with the previous findings⁴⁾⁻⁷⁾ of increased values of the $\text{Na}^+\text{-Li}^+$ countertransport in which the excess levels had varied from 20% reported by Trevisan et al⁸⁾ to 130% by Canessa et al.⁴⁾

However, a contradictory finding has also been reported by Duhm et al.¹⁵⁾ Using the same technique as we have applied to Japanese subjects, $^{22}\text{Na}^+$ influx was found to be enhanced in white patients, but not in black¹⁰⁾ and Jewish patients.¹⁶⁾ Selection of participants and possibly varying severity of hypertension or different patient characteristics may have accounted for these differences. Therefore, in the present study, we divided the patients into 2 subgroups according to the levels of their blood pressure and found that the higher value of the sodium permeability in the hypertensives was due entirely to the increased $^{22}\text{Na}^+$ influx in the patients with borderline hypertension. This is comparable to the finding of Cooper et al.¹²⁾ that young adults with high-normal blood pressure had significantly increased levels of $\text{Na}^+\text{-Li}^+$ countertransport, although the participants were much younger than those in our study. A greater red cell Na/Na exchange has also been demonstrated in spontaneously hypertensive rats both at the prehypertensive stage and at the early hypertensive stage.¹⁷⁾ A positive relationship between the mean blood pressure increase over the past 5 to 6 years and the $^{22}\text{Na}^+$ influx value shown in the present study also provides evidence that the recent development of high blood pressure relates to the enhanced sodium permeability in the patients with borderline hypertension. From these observations, it is suggested that an enhanced sodium permeability is one of the pathophysiological characteristics in the early stage of the development of essential hypertension.

Factors other than hypertension which have been suggested as affecting red cell sodium permeability were also examined and a positive correlation between PRA and $^{22}\text{Na}^+$ influx was noted in our patients with essential hypertension. This seems to be consistent with previous findings,^{7),15)} but Tosteson et al.¹⁸⁾ demonstrated an inverse relationship showing that the mean value of Na-Li countertransport in patients with high PRA was much lower and equal to the values in normotensive subjects. In the present study, the renin status in each patient could not be precisely evaluated because a stimulus to provoke an increase in renin secretion was not applied and the sodium intake of the patients was ad libitum. Therefore, the biological significance of the positive correlation shown in this study is difficult to interpret at the moment, although there is a possibility that the higher PRA in the borderline hypertensives may have contributed to the enhanced $^{22}\text{Na}^+$ influx.

Racial differences in red cell sodium transport have also been emphasized in recent years. An increased $\text{Na}^+\text{-Li}^+$ countertransport⁶⁾ and an enhanced unidirectional passive $^{22}\text{Na}^+$ influx¹⁰⁾ were reported to be characteristic in white patients but not in black patients with essential hypertension. In the present study, even in the examination of a single ethnic group, a similar disparity in red cell sodium permeability was demonstrated between border-

line and established hypertensives. It has also been noted that the other pathophysiological differences between our borderline and established hypertensives were similar to the differences between white and black hypertensives, respectively. Namely, the black subjects were reported to have relatively lower PRA,¹⁹⁾ higher blood pressure,²⁰⁾ greater concentrations of red cell sodium²¹⁾ as well as the complete absence of abnormal red cell sodium permeability,^{9),10)} and these characteristics were also present in our patients with established hypertension. These findings do not deny the existence of genetic differences in the sodium transport system between races, but also strengthen the possibility that the difference in membrane sodium permeability is due to the varying severity of hypertension and the different pathophysiological characteristics of the patients.

With regard to the relationship between intraerythrocyte sodium content and membrane sodium transport, we have previously demonstrated that an increased red cell sodium content in Japanese patients with essential hypertension is due to a reduction of net sodium efflux from sodium-loaded erythrocytes.²²⁾ A suppression of $\text{Na}^+\text{-K}^+\text{-ATPase}$ and a consequent reduction of ouabain sensitive sodium efflux rate have also been proposed as causes of an increase in cell sodium content.²³⁾ In contrast to the findings of such unidirectional sodium efflux studies, it is debatable whether the increase in passive sodium permeability results in the elevation of intraerythrocyte sodium content. Trevisan et al⁸⁾ have observed no correlation between $\text{Na}^+\text{-Li}^+$ countertransport and red cell sodium content in the combined groups of normotensives and untreated hypertensives. On the other hand, others have demonstrated that the sodium content correlated positively with furosemide insensitive sodium influx²⁴⁾ and sodium-dependent lithium efflux⁵⁾ in patients with essential hypertension and in those with a positive family history of hypertension, respectively. In the present study, a raised sodium content was observed to the same extent in both the borderline and the established hypertensives, as we have previously confirmed in more than 2,000 rural Japanese,²⁵⁾ whereas a positive correlation between the red cell sodium content and the sodium permeability was demonstrated only in the group of borderline hypertensives but not in the group of established hypertensives. Limited *in vitro* study also indicated that the $^{22}\text{Na}^+$ accumulation into erythrocytes did not affect the cell sodium content for up to 3 hours, as shown in Fig. 1. These findings do not support the hypothesis that the permeability of the cell membrane has a role in determining sodium content and that it contributes to the etiologic mechanism in essential hypertension through an increase in cellular sodium.

From these observations of the present study, it is concluded that an

enhanced $^{22}\text{Na}^+$ influx is characteristic of Japanese patients with borderline hypertension in relation to the recent development of high blood pressure. The results also suggest that the membrane sodium permeability can be altered in the development of hypertension and may not only be governed by genetic factors but can also be influenced by exogeneous factors, although its precise etiologic role in human hypertension remains to be elucidated.

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