ABNORMAL RED CELL SODIUM CONTENT AND EFFLUX IN JAPANESE PATIENTS WITH ESSENTIAL HYPERTENSION

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Net sodium (Na) efflux and potassium (K) influx were determined in Na-loaded/K-depleted erythrocytes derived from 37 patients with essential hypertension and 25 age-matched normotensive subjects with no family history of hypertension, together with the measurement of basal red cell sodium and potassium contents. Intraerythrocyte sodium content was significantly higher in the essential hypertensives than in the normotensives (10.9 ± 1.4 vs 10.0 ± 1.2 mmol/L·cells, mean ± SD, p < 0.02), but potassium content was nearly equal between the two groups. Net Na efflux in the hypertensives was significantly reduced compared with that in the normotensives (4.57 ± 0.70 vs 5.18 ± 1.02 mmol/L·cells·hr, p < 0.01), but both net K influx and net Na/K flux ratio were not significantly different between the two groups. Net Na efflux and K influx showed a significant inverse correlation with red cell sodium content (r = −0.64 and r = −0.56, respectively, p < 0.001). These results suggest that the reduced net Na efflux with the increase of red cell sodium content may be related to the pathogenesis of essential hypertension. However, it is impossible to determine the genetic marker of essential hypertension by using the net Na/K flux ratio of Japanese subjects, although Garay et al. have reported that this index was abnormally low in the case of Europeans.

Essential hypertension has long been attributed to changes in the balance or distribution of sodium but its exact mechanism remains unclear. In 1952, Tobian proposed that increased sodium and water in arterial tissue caused increased peripheral vascular resistance and Losse also reported higher sodium content in erythrocytes derived from patients with essential hypertension. Recently, some studies on sodium handling in cells have provided a new insight into the possible etiological mechanism of essential hypertension. In 1979, Garay and Meyer demonstrated that the net sodium (Na) efflux of erythrocytes decreased in advanced hypertensive patients and that the net potassium (K) influx was increased in mild cases, and they suggested that the consequent reduction of net sodium to potassium (Na/K) flux ratio could distinguish the patients with essential hypertension from the secondary hypertensive or normotensive individuals and it might be a genetic marker of essential hypertension. Since then, many investigators have examined several kinds of sodium transport systems across the red cell membrane, i.e. ouabain sensitive Na-K transport, furosemide sensitive Na-K co-transport, Na-Li countertransport. But most of these transport studies failed to establish a good

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indicator of essential hypertension because of large overlap between hypertensive patients and normotensive individuals. In addition, some reported racial or geographical differences in red cell sodium transport between blacks and whites or between subjects in France and the Ivory Coast. On Japanese, there are only a few reports concerning with these flux abnormalities. Thus we examined net Na and K flux across the red cell membrane and its relationship to the red cell sodium content and also attempted to clarify whether such flux study can detect a genetic indicator of essential hypertension in Japanese or not.

MATERIALS AND METHODS

Thirty-seven patients with essential hypertension (all of them Japanese males) aged 32 to 60 (44 ± 8 years old; mean ± SD) were studied. Essential hypertension was defined as blood pressure above 160/95 mmHg on at least two measurements on different days in the sitting position. Blood pressure was measured again after at least 30 minutes rest in the supine position and blood sampling was done for the determination of plasma sodium and potassium concentrations, BUN, serum creatinine and plasma renin activity. In some of these patients (32 out of 37 patients), plasma aldosterone concentrations were also measured. None of these patients showed abnormal values of plasma sodium and potassium and no one had renal impairment or papilledema and secondary causes of hypertension which were ruled out by the usual screening examinations in our laboratory. All hypertensive patients were on a free diet with no restriction of sodium intake and none had received antihypertensive drugs for at least two weeks before blood sampling. Each patient was asked about his family history of hypertension and 22 out of 37 patients had a history of stroke and/or hypertension in their first relatives.

Twenty-five healthy normotensive male subjects aged 26 to 64 (41 ± 11 years old) were studied as an age-matched control group. After repeated measurements on different days, their blood pressure in the sitting position never exceeded 140/90 mmHg and all of them had no known history of deaths due to stroke and no known history of hypertension in their first and second relatives. In addition, their body weight and height were not significantly different from that of the hypertensive group and also their plasma sodium, potassium, BUN, serum creatinine levels were within the normal range.

Intracellular Sodium and Potassium Contents

Intraerythrocyte sodium and potassium contents were determined by the method of Kaya et al. with slight modification. Two ml of freshly drawn venous blood, collected in heparinized tube was centrifuged at room temperature. Forty µl of packed cells were aspirated and injected into capillary tubes for microhematocrits (75 mm length) in duplicate and centrifuged at 11,000 rpm for 5 min. The final hematocrit values of the packed cells were approximately 80–90%.

The capillary tubes were then cut at the 70% point (28 µl) and 50% (20 µl) of the total blood volume and were transferred into 3 ml and 10 ml of “Diluted Lithium Solution” for the measurement of Na and K concentration, respectively. Red cell sodium (RBC-Na) and potassium (RBC-K) contents were determined by a flame photometer (Hitachi 205) and approximated by the following functions:

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\text{RBC-Na} = \left(\frac{(A-Na \times \frac{15}{28} \times 100)}{(P-Na \times \text{Ptr})}\right) / (100 - \text{Ptr})
\]

\[
\text{RBC-K} = \left(\frac{(A-K \times \frac{50}{20} \times 100)}{(P-K \times \text{Ptr})}\right) / (100 - \text{Ptr})
\]

A-Na, A-K : Na and K readings of the hemolysate
P-Na, P-K : plasma Na and K concentrations
Ptr : percentage of trapped plasma
15 (µl) : the volume of the Na standard solution (Na=50 mEq/L) in 3 ml of the “Diluted Lithium Solution”
50 (µl) : the volume of the K standard solution (K=50 mEq/L) in 10ml of the “Diluted Lithium Solution”

RBC-Na and RBC-K were expressed as millimoles per liter of the original cells and corrected by plasma Na and K concentrations of which 3% (v/v) was trapped in capillary tubes detected by 131I-radioactive human serum albumin.

Net Na and K Fluxes

Net Na and K fluxes were measured in Na-loaded/K-depleted erythrocytes by the method

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Fig. 1. Net Na (●) and K (○) fluxes in Na-loaded/K-depleted erythrocytes from a normotensive individual.

Fig. 2. Basal red cell sodium (RBC-Na), potassium (RBC-K) and sodium to potassium ratio (RBC-Na/K) in essential hypertensives (EHT: n = 37) and normotensive controls (NT: n = 25). Bars represent mean ± standard deviation.

used by Garay and Meyer with slight modification. Six ml of freshly drawn venous blood was collected in a heparinized tube and centrifuged at 3000 rpm for 10 min and plasma anduffy coat were aspirated. The red cells were then washed twice with approximately 10 volumes of 150 mmol/L choline chloride. All procedures were carried out at 4°C. One ml of washed

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FIG. 3. Net Na efflux, K influx and Na/K flux ratio of Na-loaded/K-depleted erythrocytes in essential hypertensives (EHT: n = 37) and normotensive controls (NT: n = 25). Bars represent mean ± standard deviation.

FIG. 4. Correlation between age and net Na efflux, net K influx in essential hypertensives (○: n = 37) and normotensive controls (●: n = 25).

Erythrocytes was then suspended in an Na-loading medium containing (mmol/L) 150 sodium chloride, 1 magnesium chloride, 2.5 sodium phosphate (pH 7.4 at 4°C) and 0.02 p-chloromercuriphenylsulfonic acid (PCMP) to give a final hematocrit of 10%, and incubated for 20
hours at 4°C. After that, the red cells were incubated for 1 hour at 37°C in an Na-K medium containing (mmol/L) 145 sodium chloride, 5 potassium chloride, 1 magnesium chloride, 10 glucose, 2.5 sodium phosphate (pH 7.4 at 4°C), 4 cysteine, 2 adenine and 3 inosine. The cells were then spun down at 4°C and the exact hematocrit was measured after the aspiration of supernatant. One half ml of the packed cells was then resuspended in 19.5 ml of Na-K medium without cysteine to give a final hematocrit of approximately 5% and the resulting suspension was distributed into 8 tubes (2 ml per tube) at 4°C. The tubes were then incubated at 37°C with
gentle shaking. Immediately before incubation and after incubation at 1, 2 and 3 hour, two tubes were transferred to 4°C and cells were washed twice with 150 mmol/L choline chloride. After the last wash, cells were lysed with distilled water to reach a final volume of exactly 5 ml. Sodium and potassium concentrations of hemolyzate in each tube were determined by a flame photometer and corrected by the packed cell volume value. The intracellular sodium and potassium levels were plotted as a function of time and the net flux values were obtained from the slope of these functions by linear regression analysis and expressed in millimole per liter of cells per hour (Fig. 1). The regression coefficients in all cases were between 0.984 and 0.999 for net Na efflux and between 0.883 and 0.999 for net K influx. Net Na efflux and net K influx correlated highly significantly with each other (r = 0.87, p < 0.001).

Statistics

The differences of the average values were tested for significance by means of student’s unpaired t-test. All results were expressed as mean ± standard deviation.

RESULTS

Intracellular Cation Content (Fig. 2)

The basal erythrocyte sodium content was significantly higher in hypertensive patients than in normotensive subjects (10.9 ± 1.4 vs 10.0 ± 1.2 mmol/L-cells, p < 0.02) but there was no significant difference in potassium content between the two groups. The ratio of intracellular sodium and potassium content in the hypertensives was significantly higher than that in the normotensives (0.109 ± 0.015 vs 0.099 ± 0.013, p < 0.02). Sodium and potassium contents in the red cells were related neither to plasma renin activity nor to plasma aldosterone concentration both in the hypertensives and in the normotensives, but a weak positive relationship was noted between age and intracellular sodium content (r = 0.25, p < 0.05).

Erythrocyte Cation Fluxes

Net Na and K flux values of Na-loaded/K-depleted erythrocytes are shown in Fig. 3. The initial values of sodium and potassium contents after PCMPS treatment were similar in the two groups — (mmol/L-cells) 47.2 ± 7.3 and 52.6 ± 7.6 for the normotensives, 44.7 ± 6.6 and 50.6 ± 6.1 for the hypertensives, respectively. The mean value of the net Na efflux of the hypertensive patients was significantly reduced compared to that of the normotensive subjects (4.57 ± 0.70 vs 5.18 ± 1.02 mmol/L-cells hr, p < 0.01) but the mean values of the net K influx and the net Na/K flux ratio were not different between the two groups.

There was no relation between body weight and the net cation fluxes but an inverse correlation was noted between age and net Na efflux (r = −0.41, p < 0.001) and between age and net K influx (r = −0.36, p < 0.005) (Fig. 4). Neither plasma renin activity nor aldosterone concentration nor the mean blood pressure in the sitting or the supine position correlated with the net Na efflux or net K influx in the hypertensive patients.

The relation between the red cell sodium content and the net flux value is shown in Fig. 5. Both net Na efflux and K influx were inversely correlated with erythrocyte sodium content (r = −0.64 and r = −0.56, respectively, p < 0.001) and significant relationships were also found when observed separately in the hypertensive subjects (r = −0.54 and r = −0.43, respectively, p < 0.01) or in the normotensive subjects (r = −0.72 and r = −0.73, respectively, p < 0.001).

DISCUSSION

In this study, we selected the study population and the control subjects carefully in order to clarify how essential hypertension related to the red cell sodium content and sodium transport of Japanese patients. Bellin et al.13 have determined the values of erythrocyte sodium content in 142 healthy subjects and reported that these were lower in young women than in men, and others6 found higher cell sodium content in black subjects than in white. It has also been noted that some disorders, such as hypokalemia, acute digitalis administration, hyperthyroidism and chronic renal failure may alter erythrocyte sodium content.14 As for sodium transport studies, a higher ouabain-sensitive sodium efflux was observed in erythrocytes of obese subjects15 and a sexual difference of sodium handling may exist.9 It has also been reported that patients with hyperthyroidism or chronic renal failure have a lower activity of the sodium pump.14 From such points of view, we selected only Japanese male patients with essential hypertension who had.
none of the complications or disorders mentioned above. As control subjects, we also carefully selected healthy male subjects with no family history of hypertension whose age and body weight were matched to the hypertensive patients.

The results of the present study showed that the erythrocyte sodium content of the hypertensive patients was significantly higher than that of the normotensive controls, although a considerably large overlap was found between the two groups. This is compatible with the data of Losse\(^2\) who first reported the increased sodium content in erythrocytes. Also, our finding of decreased ability to transport sodium out of the red cells in essential hypertension is consistent with the findings of several investigators, although they used different methods. Aderounmu\(^6\) has shown that there is a reduction of active sodium efflux and its rate constant using sodium loaded erythrocytes which were stored for 10 days in a high sodium medium. Others\(^7,16\) have reported a decreased net and ouabain sensitive sodium efflux rate constant in hypertensives using \(^{22}\)Na. Such a membrane defect of the sodium pump has also been found in the leucocytes\(^16,17\) or lymphocytes\(^18\) of hypertensive patients and in the erythrocytes of spontaneously hypertensive rats\(^19\). However, these data failed to demonstrate the exact relationship between such membrane defects and the increased intracellular sodium content. We studied the net cation flux value simultaneously with the intracellular cation content in all subjects and found that the red cell sodium content correlated inversely with the net sodium efflux value. This inverse correlation was also noted when observed separately in the hypertensive patients or in the normotensive subjects. Therefore, we postulated that the reduced net sodium efflux caused the high intracellular sodium content.

Recently, Garay and Meyer\(^3\) described abnormal low net Na/K flux ratio in Na-loaded/K-depleted erythrocytes of patients with essential hypertension and normotensives with family history of hypertension. They suggested that this flux abnormality may be a genetically transmitted defect. On the other hand, Swarts et al\(^5\) performed the same examination as Garay’s and found no significant difference of net Na efflux, K influx and Na/K flux ratio. In the present study, we found a significant reduction of net Na efflux in Japanese patients and it is compatible with Garay’s result of a reduced net Na efflux in cases of advanced hypertension, but we did not detect an increased net K influx and a consequent reduction of net Na/K flux ratio. Therefore, unlike Garay’s data, the net Na/K flux ratio did not clearly separate the patients from the normotensive controls and this index does not seem to be useful for diagnosis of essential hypertension or of a genetic predisposition to hypertension in Japanese.

Such differences of flux abnormality between French and Japanese may reflect not only the difference of a genetic predisposition to hypertension between the two races but also environmental differences. In fact, more recently Garay et al\(^11\) have found a higher incidence of abnormally low Na-K co-transport activity even in normotensive subjects living in the Ivory Coast and they suggested that a geographical variation from one population to another may exist. Also Woods\(^10\) has reported that red cell rubidium uptake was significantly lower in normotensive blacks than in normotensive whites and others reported a higher prevalence of hypertension in blacks than in whites. Besides such genetic differences between races, Morgan et al\(^20\) proposed that environmental differences such as the difference of dietary salt intake may affect the red cell sodium transport showing that an increased sodium intake actually causes a fall in the red cell \(^{22}\)Na efflux rate constant. Thus, there is a possibility that the higher salt intake of Japanese compared with other industrialized countries alters the membrane sodium transport and suppresses the erythrocyte sodium efflux, although the dietary salt intake was not examined in the present study. The mechanism how such environmental factors regulates the membrane sodium transport has not yet been understood in Japanese subjects and this hypothesis still remains uncertain.

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REFERENCES

3. GARAY RP, MEYER P: A new test showing ab-

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normal net Na\(^+\) and K\(^+\) fluxes in erythrocytes of essential hypertensive patients. *Lancet* 1: 349, 1979


