NORMALIZING EFFECTS OF PLASMA ON ALTERED CATION TRANSPORT OF RED BLOOD CELLS FROM ESSENTIAL HYPERTENSIVE PATIENTS

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Net Na^+ and K^+ fluxes were measured in Na^+ -loaded red cells from 19 normotensive control subjects, 22 essential hypertensive patients, and 8 secondary hypertensive patients. The ratio of Na^+/K^+ net fluxes was significantly lower in essential hypertensive patients than in normotensive control subjects. However, by the addition of the patients' own plasma, the net Na^+ efflux rate was significantly increased in essential hypertensive patients, which caused the increment in the ratio of Na^+/K^+ net fluxes. This resulted in disappearance of the difference between normotensive and hypertensive subjects in the ratio of cation fluxes. It was possible that the abnormalities of cation transport in red cells from essential hypertensive patients might be compensated for by humoral factors in the plasma.

MANY experiments have already suggested an abnormality in cation concentration or cation transport in red cells from hypertensive patients.¹⁻¹⁰ The sodium (Na^+)-potassium (K^+) ATPase is the main system of cation transport in the red blood cell. However, some reports suggested abnormalities of other transport systems than the Na^+/K^+ ATPase in essential hypertension.^{5, 6, 10} Garay et al.⁵ investigated the cation flux of the red blood cell after increasing the Na^+ concentration, and found that the ratio of net Na^+ efflux to net K^+ influx rate was lower in essential hypertensive patients than in normotensive subjects. As a cause of this, they suggested a lower activity of the outward Na^+/K^+

cotransport system in essential hypertension.⁵ However, it has been reported recently that there might be a racial difference¹¹ or a considerable overlap^{7, 8} between essential hypertensive patients and normotensive subjects in this transport system. On the other hand, MacGregor et al.¹² have reported that inhibitors of Na^+/K^+ ATPase were present in plasma of essential hypertensive patients. The present study is aimed to find out whether there is any difference between normotensive and hypertensive patients of Japan in the cation transport of Na^+ -loaded red cell and whether the addition of the patient's own plasma exerts any effects on cation transport.

Key Words:

Essential Hypertension
Electrolytes
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Red Blood Cell

MATERIALS AND METHODS

The subjects were 19 normotensive control with no family histories of hypertension (5 men and 14 women, aged from 18 to 63), 22 essential hypertensive patients (7 men and 15 women,

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TABLE 1 CATION TRANSPORT OF RED CELLS IN NORMOTENSIVE CONTROLS AND HYPERTENSIVE PATIENTS

	Normotensive	Essential HT	Secondary HT
Number	19	22	8
Initial cell Na ⁺ (mmol/l cells)	80.9 ± 9.74	86.8 ± 14.80	87.5 ± 13.45
Initial cell K ⁺ (mmol/l cells)	29.7 ± 5.55	29.8 ± 5.86	25.9 ± 6.73
Net Na ⁺ efflux (mmol/l cells/h)	4.46 ± 1.16	4.00 ± 1.25	4.19 ± 1.59
Net K ⁺ influx (mmol/l cells/h)	2.26 ± 0.62	2.76 ± 0.88*	2.81 ± 0.99
Na ⁺ efflux/K ⁺ influx	2.05 ± 0.58	1.50 ± 0.40**	1.58 ± 0.52

* $p < 0.05$ ** $p < 0.01$ Mean ± SD (vs. Normotensives)

aged from 12 to 66) and 8 secondary hypertensive patients (3 men and 5 women, aged from 22 to 60). Secondary hypertensive patients consisted of six with renovascular hypertension, one with primary aldosteronism, and one with Cushing's syndrome. All subjects were examined during a period of normal salt intake (10–15g/day). Hypertensive patients had stopped all antihypertensive medication for at least 10 days before the examination.

Net Na⁺ efflux and net K⁺ influx rates were measured by Garay's method⁵ with slight modifications. Venous blood was drawn in heparinized tubes, and red blood cells were separated by centrifugation. Red cells were washed twice with approximately 10 volumes of 150 mmol/l sodium chloride. The washed and packed cells were suspended in the Na⁺-loading-medium to obtain a 5% value of packed red cells. The Na⁺-loading-medium contained (mmol/l) 150 sodium chloride, 1 magnesium chloride, 2.5 sodium phosphate (pH 7.4), and 0.02 2,5-p-chloromercuribenzenesulphonate (PCMBs). The cells were incubated for 20h at 4°C in the Na⁺-loading-medium which was renewed once after 4 to 6h incubation. The cells were resuspended in a sealing medium to reach a 8 to 10% volume of packed cells, and incubated for 1 hour at 37°C. The sealing medium contained (mmol/l) 145 sodium chloride, 5 potassium chloride, 1 magnesium chloride, 5.4 sodium phosphate (pH 7.4), 4 cysteine, 2 adenine, and 3 inosine. The cells were then resuspended in Ringer's medium containing (mmol/l) 145 sodium chloride, 5 potassium chloride, 1 magnesium chloride, 2.5 sodium phosphate (pH 7.4), and 10 glucose. The packed cell volume was about 1.3%. The cells were incubated at 37°C. After 0, 1, and 2 hours, incubation was stopped by sudden cooling to 4°C. The cells were then washed three times with a cold solution containing (mmol/l) 150 choline chloride, 2.5 Tris (pH 7.2),

NT: Normotension EH: Essential Hypertension SH: Secondary Hypertension

Fig. 1. Net Na⁺ efflux rate, net K⁺ influx rate, and the ratio of Na⁺/K⁺ net fluxes in red blood cells from 19 normotensive subjects, and 22 essential and 8 secondary hypertensive patients.

and 0.02 ouabain. Thereafter, they were hemolysed with distilled water. Sodium and potassium concentrations of the hemolysate were measured by a flame photometer (Hitachi-775), and hemoglobin was spectrophotometrically measured as oxyhemoglobin (541nm). The intracellular Na⁺ and K⁺ concentrations were adjusted by the value of hemoglobin concentration and expressed per liter of the original cells. The intracellular levels of sodium and potassium were plotted against time. Net flux values were obtained from the curve determined by linear-regression analysis and expressed in millimoles per liter of cells per hour. The curves were accepted only when the regression coefficient exceeded 0.98.

Subsequently, in 10 normotensive control subjects (4 men and 6 women, aged 20 to 63) and 10 essential hypertensive patients (2 men and 8 women, aged 12 to 66), the effects of plasma on cation transport were examined. The

Fig. 2a. Effects of the patient's own plasma on electrolyte transport of red blood cells from 10 normotensive control subjects. Mean $\pm$ SD.

Fig. 2b. Effects of the patient's own plasma on electrolyte transport of red blood cells from 10 essential hypertensive patients. Mean $\pm$ SD.

Na⁺-loaded red cells which had been treated in a sealing medium were incubated in Ringer's medium both with and without the patient's own plasma. Plasma was added to the Ringer's solution to 6% of the volume. Net flux values obtained by incubating in Ringer's medium in the presence and absence of plasma were compared.

RESULTS

There were no significant differences among the three groups in the initial cell Na⁺ and K⁺ concentrations (Table I), though secondary hypertensive patients tended to show higher Na⁺ and lower K⁺ concentrations. As seen in Table I and Fig. 1, the net K⁺ influx rate was significantly higher in essential hypertensive patients than in normotensive control subjects ($p < 0.05$). There were no significant differences among the three groups in the net Na⁺ efflux rate. The ratio of the Na⁺/K⁺ net fluxes was significantly lower in the group with essential hypertension than in the control group ($p < 0.01$). It also tended to be lower in secondary hypertensive patients, but was not significant compared with normotensive subjects.

By the addition of the patient's own plasma, cation transport in red cells of essential hypertensive patients was affected differently from that of normotensive controls. In normotensive control subjects, the net K⁺ influx rate was significantly increased from 2.34 ± 0.74 mmol/l/h (mean $\pm$ SD) to 3.03 ± 0.86 mmol/l/h by the addition of plasma ($p < 0.05$) (Fig. 2a). On the other hand, in essential hypertensive patients, the net Na⁺ efflux rate was significantly increased from 4.47 ± 1.21 mmol/l/h (mean $\pm$ SD) to 6.11 ± 1.03 mmol/l/h ($p < 0.01$), but there were no

significant changes in the net K⁺ influx rate. The ratio of Na⁺/K⁺ net fluxes also showed a tendency to increase upon the addition of plasma (from 1.58 ± 0.43 to 2.27 ± 0.79) (Fig 2b). There were no significant differences between normotensive (1.77 ± 0.57) and hypertensive subjects (2.27 ± 0.79) in the ratio of Na⁺/K⁺ net fluxes after the addition of the patient's own plasma.

DISCUSSION

Garay et al.⁵ measured the Na⁺ and K⁺ net flux rates of sodium-loaded red cells treated by PCMBs. They found an abnormally high K⁺ net flux rate and a decreased ratio of Na⁺/K⁺ net fluxes in the red blood cells from essential hypertensive patients. Moreover, they have proposed that this abnormality was due to decreased activity in the ouabain-resistant, furosemide-sensitive Na⁺, K⁺ cotransport system in essential hypertensive patients.⁶ However, it has been reported recently that there was no significant difference between normotensive and hypertensive subjects in the inhabitants of the Ivory Coast,¹¹ suggesting a racial difference in this cotransport system. On the other hand, Davidson et al.⁷ have reported that there was a considerable overlap in this transport system between normotensive and hypertensive subjects.

In the present study, we found a higher net K⁺ influx rate and a lower ratio of Na⁺/K⁺ net fluxes in essential hypertensive patients as compared to normotensive control subjects (Fig. 1). This suggests that the Na⁺, K⁺ cotransport system was also decreased in hypertensive patients of Japan. However, there was a large overlap between normotensive and hypertensive subjects, which was consistent with a few other reports.^{7, 9} In

contrast to Garay's study, the ratio of Na^+/K^+ net fluxes also tended to be lower in secondary hypertensive patients. This may be related to the family history of hypertension, which was recognized in 4 of the 8 secondary hypertensive patients, because it is known that the ratio of cation fluxes was also decreased in red cells from normotensive relatives of essential hypertensive patients⁵

In this study, effects of the patient's own plasma on cation transport were also investigated. In essential hypertensive patients, the net Na^+ efflux rate was significantly increased by the addition of plasma, whereas the net K^+ influx rate was increased in normotensive controls. By the addition of the patient's own plasma, the ratio of Na^+/K^+ net fluxes tended to be increased in hypertensive patients and decreased in normotensive subjects. Consequently, the significant difference in the ratio of Na^+/K^+ disappeared upon the addition of plasma. The dissociation between changes of the Na^+ and K^+ net flux rates makes it untenable that the effects of the plasma resulted from activation of the Na^+/K^+ ATPase transport system. It was possible that plasma might affect the ouabain-resistant, furosemide-sensitive Na^+ , K^+ cotransport system, though the effects on passive cation permeability could not be ruled out. The outward Na^+ , K^+ cotransport might be augmented in essential hypertensive patients by the addition of the patient's own plasma, since the ratio of Na^+/K^+ net fluxes as well as the net Na^+ efflux rate tended to be increased. At least, there was no significant difference between normotensive and hypertensive subjects in the ratio of Na^+/K^+ net fluxes when cation net fluxes were measured in Ringer's medium with plasma. This suggests that the lower Na^+ , K^+ cotransport in red cells which was genetically determined in essential hypertension might be compensated for by humoral factors in the plasma. The lower Na^+ , K^+ cotransport might not cause a rise of blood pressure in essential hypertension since it is compensated for by humoral factors, even if it could be a genetic

marker of essential hypertension.

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