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Raised Sodium Pump Activity and a Circulating Sodium Transport Inhibitor Demonstrated on Red Blood Cells of Patients with Untreated Essential Hypertension: Correlation of Pump Activity with Potassium Permeability

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Summary. We have studied sodium potassium ATPase activity, the effect of endogenous plasma on sodium pump activity, potassium permeability and intracellular sodium and potassium concentrations in normotensive subjects without ($n=36$) and with ($n=33$) a positive family history of hypertension, and in patients with untreated essential hypertension ($n=52$). Sodium pump activity was studied as ouabain sensitive uptake of rubidium 86 in washed red blood cells, incubated in an artificial medium closely resembling the anorganic constituents of plasma. Any influence of endogenous plasma on sodium pump activity was investigated by re-incubating the washed red blood cells in their own plasma and comparing ouabain sensitive rubidium uptake in the two media. To correct for any possible differences in external potassium concentration, a function for the relation between extracellular potassium concentration and absolute transport rates was derived experimentally. From this, actual transport rates in plasma were corrected by computer to an extracellular potassium concentration of 4.0 mmol/l. Sodium pump activity, concentration of circulating sodium transport inhibitor, potassium permeability and intracellular electrolytes were not statistically different in subjects with and without a positive family history of hypertension. Hypertensives had significantly raised sodium pump activity in artificial medium, but not when red cells were re-incubated in their own plasma. Thus, endogenous plasma inhibited the sodium pump by between 12% and 15%. Hypertensives also had a significantly raised potassium permeability. Potassium permeability and sodium pump activity were correlated significantly. Intracellular sodium concentrations were similar in normotensives and hypertensives, but the later showed a significantly lower intracellular potassium concentration. We conclude that sodium pump activity and potassium permeability as well as intracellular electrolytes are comparable in subjects with and without a positive family history of hypertension and thus play no role in the initiation of essential hypertension. In the course of essential hypertension apparently a membrane defect develops, leading to enhanced permeability of the cell membrane, which is compensated by an increased activity of the sodium pump. Our results also demonstrate that endogenous plasma may inhibit ouabain sensitive potassium inward transport in erythrocytes of patients with essential hypertension.

Key words: Natrium Kalium ATPase activity – Potassium permeability – Intracellular sodium – Normal subjects – Essential hypertension – Sodium transport inhibitor

Introduction

For anybody interested in the pathogenesis of essential hypertension it is difficult to understand that simple questions such as whether in human hypertension or in subjects with heredity of hypertension intracellular electrolytes are altered or not, and whether membrane electrolyte transport systems are altered or not, are apparently so difficult to solve even for the comparatively simple model of the red blood cell [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. Apparently important methodological

differences exist between investigators. Additionally, some of the discrepancies could be explained by previous drug therapy which might have influenced intracellular electrolytes and transport rates. We have studied a large number of normotensive subjects, with and without a heredity of hypertension and a large number of patients with essential hypertension, observing two principles, which appeared important to us: (1) to use an in vitro method which simulates as closely as possible in vivo conditions, and (2) to investigate only subjects who had never had antihypertensive treatment in the past.

Material and Methods

We studied 69 normotensive volunteers, age range 20–30 years, and 52 patients with essential hypertension, WHO I–II, age range 20–40 years, who had never been given anti-hypertensive treatment before the investigation. Secondary hypertension was excluded by the usual tests, including sonography of the kidney, urinary analysis, blood chemistry, plasma renin and aldosterone measurements and urinary catecholamine determinations. All subjects were studied on their usual sodium intake of between 100–250 mmol per day.

Study Protocol

After a 24-h urine collection for determination of urinary sodium and potassium and after an overnight fast, 30 ml heparinized blood was taken after 90 min supine bedrest for red cell transport studies. During the time of supine bedrest, blood pressure was measured continuously with an oscillometric method and blood pressure during this time was averaged by an on-line computer.

Methods

Methods for red cell transport studies and intracellular electrolyte measurements were same as reported in our previous study [14]: 10 ml red cells separated from the plasma at 4° C were washed twice with an equal volume of cold isotonic saline; then 250 µl washed erythrocytes were incubated in triplicate in 500 µl artificial medium closely resembling that of the plasma (sodium, potassium, calcium, magnesium, phosphate and glucose in concentrations of 145, 4.0, 1.5, 0.5 and 1.36 mmol/l, respectively, pH adjusted to 7.4 with Tris buffer), in the presence or absence of ouabain 1.0×10^{-4} Mol and in the presence of ouabain 1.0×10^{-4} M and 1.07 mmol furosemide. To each sample 15,000 CPM rubidium-86 were added and the samples were incubated for 1 h at 37° C in a shaking water bath. Then the samples were cooled rapidly to 4° C, washed twice with cold isotonic sodium chloride solution and counted in a gamma counter for the uptake of rubidium. For the calculation of ATPase activity the ouabain sensitive uptake rate of rubidium was multiplied by the amount of extracellular potassium present in the incubation medium. For the calculation of potassium permeability the ouabain and furosemide resistant uptake rate of rubidium was multipled by the amount of extracellular potassium in the incubation medium. The results are expressed as µmol potassium/l red cells/h. For the measurement of circulating inhibitors of the sodium pump in plasma, the washed erythrocytes were additionally resuspended and incubated for 1 h

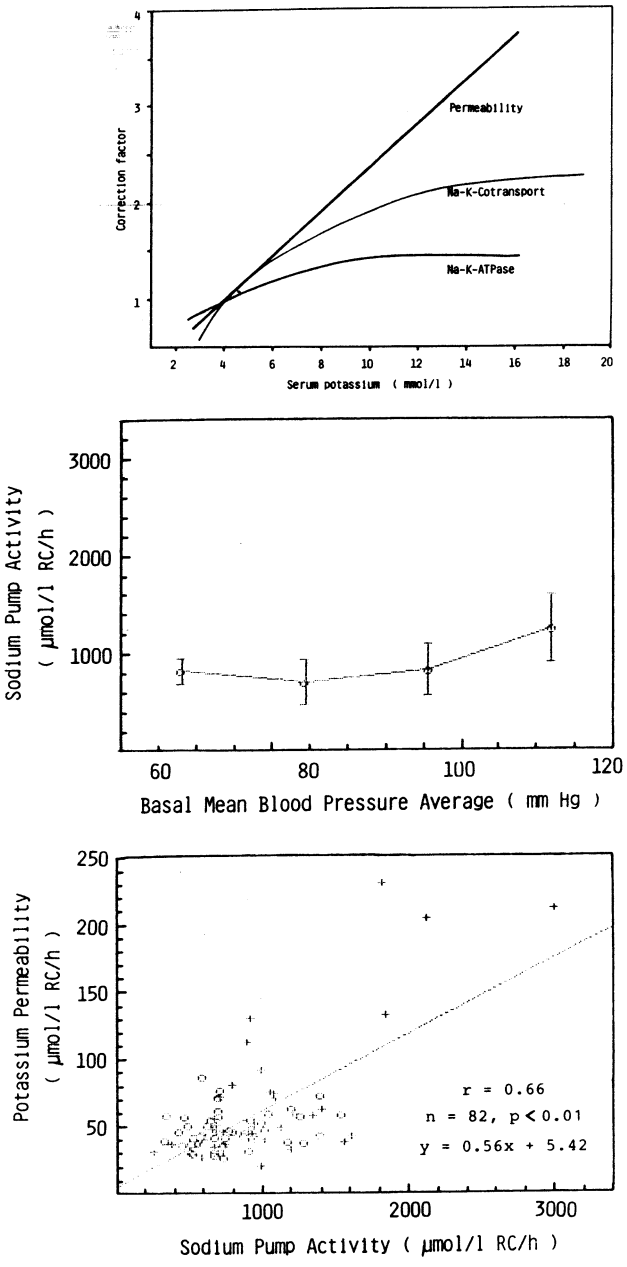

Fig. 1. *Top:* mean curve of the relation of sodium pump activity, sodium potassium co-transport (not reported in the present study) and potassium permeability to external potassium concentration. A computer program was used to correct the transport rates in plasma to potassium concentration of 4.0 mmol/l. The correction factor is shown on the y axis. The experimentally derived curve of potassium permeability to external potassium, which was found to be a straight line, emphasizes that all important active transport rates were effectively blocked by ouabain and furosemide. *Middle:* relation of sodium pump activity to basal mean blood pressure average in normotensive and hypertensive subjects. As can be seen sodium pump activity rises in the later stages of essential hypertension, simultaneously the variability of pump activity increases. *Bottom:* Significant positive correlation between potassium permeability and sodium pump activity found in patients with essential hypertension, which confirms the relation of these variables reported in the transporting epithelia of many experimental animals

Table 1. Transport rates, intracellular electrolytes and clinical data in normotensive and hypertensive subjects

	Normotensives		Hypertensives
	neg. FH ($n=36$)	pos. FH ($n=33$)	($n=52$)
Na-pump activity in artificial medium (μmol/l/h)	796.8 ± 57.2	851.9 ± 41.9	1,070.6 ± 62.7
Change of sodium pump activity by endogenous plasma (% of activity in an artificial medium)	107.5 ± 7.0	105.0 ± 5.3	93.2 ± 3.8
K-permeability (μmol/l/h)	44.1 ± 2.5	49.4 ± 2.8	69.6 ± 8.7
Intracellular sodium (mmol/l)	10.1 ± 0.6	11.5 ± 0.7	10.2 ± 0.5
Intracellular potassium (mmol/l)	86.3 ± 2.2	86.6 ± 3.1	78.6 ± 1.5
1-h mean blood pressure average (mm Hg)	81.9 ± 1.1	84.4 ± 1.2	92.8 ± 1.7
24-h urinary sodium (mmol)	189.3 ± 13.7	198.9 ± 13.3	187.3 ± 15.1

Mean ± SEM, [a] $P<0.05$ [b] $P<0.01$ [c] $P<0.001$ (U test of Mann Whitney)

at 37° C in their own plasma, using exactly the same protocol as was used for the incubation in artificial medium. The rubidium uptake rates of erythrocytes in plasma were expressed as a percentage of the uptake rates in the artifical medium after correcting by a computer program for any possible differences in the concentration of external potassium. For that purpose calibration curves for the relation between external potassium concentration to sodium pump activity were derived experimentally in a number of normal subjects, the mean curves derived (shown in Fig. 1, *top*) were used to correct sodium pump activity in plasma to a serum potassium of 4.0 mmol/l. The sodium pump rate in plasma was calculated as a percentage of the sodium pump rate in the artificial medium. For the determination of intracellular electrolytes the red cells were washed twice in cold isotonic magnesium chloride solution, were haemolysed in an equal volume of distilled water and homogenized on an ultraturax,; sodium and potassium were measured after centrifugation of the cell detritus by flame photometry.

Statistical methods were student's unpaired t test and the U test of Mann-Whitney for comparison of groups.

Results

The results are shown in Table 1. As can be seen there were no significant differences in 24-h urinary sodium excretion, sodium pump activity, effect of endogenous plasma on pump activity, potassium permeability, intracellular sodium and potassium concentration between normotensive subjects with and without a heredity of hypertension. There was, however, a significantly higher mean blood pressure average in subjects with a positive family history of hypertension. Hypertensives had a significantly raised sodium pump activity in artificial medium, which was significantly lower when the washed red cells were re-incubated in their own plasma as compared to normotensive subjects. Hypertensives also had a significantly enhanced potassium permeability as compared with normotensive subjects. Intracellular sodium concentration was not significantly different in the three groups; intracellular potassium, however, was significantly lower in hypertensive subjects. As can be seen from Fig. 1 (*middle section*) sodium pump activity was related to the basal blood pressure average, the highest values being measured in the subjects with the highest blood pressure. As is shown in Fig. 1 (*bottom*) sodium pump activity correlated significantly with potassium permeability in hypertensive subjects.

Discussion

We are unable to confirm any difference in sodium pump activity, effect of endogenous plasma on pump activity, potassium permeability, intracellular sodium and potassium concentration in normotensive subjects with and without a heredity of hypertension. The subjects studied were all medical students and medical doctors and were probably better suited to obtaining the family history of hypertension (defined as established hypertension in parents or grandparents before the age of 65) than

the general population. The fact that subjects with a positive family history of hypertension had a significantly higher basal blood pressure average than normotensive subjects with negative family history of hypertension gives further evidence that our classification was correct. We, therefore, believe that primary defects of the sodium pump, or an alteration of a circulating sodium transport inhibitor or of cell membrane permeability are not primary events in the initiation of essential hypertension.

We were, however, able to demonstrate that subjects with established hypertension have a raised sodium pump activity, which confirms the results of those workers [10, 13] who have used rubidium uptake as a measure of sodium pump activity. We are unable to explain why some workers who have measured ouabain-sensitive outward transport of sodium as a measure of sodium pump activity find a reduced sodium pump activity in essential hypertension [12]. These contrasting results could suggest an alteration of the stoichiometry of the sodium pump in essential hypertension. As is shown in Fig. 1, *middle section*, this alteration of the pump mainly occurs in the more severe and, therefore probably the later stages of hypertension. This would suggest that the abnormality of the sodium pump occurs as a consequence rather than a cause of essential hypertension. Our results, to our knowledge, demonstrate for the first time in red cells that the sodium pump might be inhibited by the endogenous plasma in essential hypertension [15]. We are currently investigating further which constituents of plasma could be responsible for this effect.

Using the passive uptake of radioactive rubidium as tracer for potassium we were also able to confirm that cell membrane permeability may indeed be raised in essential hypertension as has been suggested by other workers [2, 3]. Interestingly, potassium permeability correlates positively with the activity of the sodium pump (Fig. 1, *bottom*) and enhanced permability is therefore also found in the later stages of essential hypertension. It is interesting to note that a linkage of potassium permeability and sodium pump activity has been reported in transporting epithelia of many species [16]. From our results it appears that in the course of essential hypertension a membrane defect develops, leading to enhanced potassium (and probably sodium) permeability of the cell membrane, which is compensated by an enhanced activity of the sodium pump. This is in perfect agreement with the findings obtained in the vessels of spontaneously hypertensive rats (for a recent review see [17]). Our results suggest that these are secondary phenomena developing in the course of essential hypertension, but do not play a primary role in the initiation of essential hypertension. This would also explain why drugs that affect cell membrane transport such as calcium entry blockers have the most pronounced effect in severe hypertension, but do not alter blood pressure in normotensives.

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