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Examination of the Ouabain-Sensitive Na-K Pump in Essential Hypertensive Children of Normal Body Weight

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The ouabain-sensitive Na-K pump of erythrocytes was examined in 17 normotensive and 15 essential hypertensive children aged 6—16 years. Children who proved to be fat according to skinfold measurements were excluded from the examinations. The activity of the ouabain-sensitive Na-K pump was assessed by measuring the ratio of Na-ion efflux and K-ion influx through the erythrocyte membrane previously treated with para-chloro-mercury benzol sulphonate. In essential hypertensive children the ratio of the Na/K fluxes was found to be characteristically 5.8 ± 2.0, showing a mathematically significantly more active ($p < 0.05$) Na-K pump function than in the control group 9.6 ± 5.8. According to the results, however, the method is suitable only for the separation of groups and not of individuals.

In the developed countries some 50% of the mortality is caused by diseases of the circulatory system. During the last decade this high mortality ratio started to decrease in several countries, so in Finland [11], Belgium [12] and the United Kingdom [9], among others. According to WHO [23] the mortality of cardiovascular diseases has considerably been reduced in a large part of the world, while in other countries e.g. in Hungary, it still increases moderately. In 1960 out of 10 000 Hungarian inhabitants 45 had died from cardiovascular disease while in 1982 this number was 73.

In countries where the mortality has decreased, the health organizations carried out an effective prevention programme, trying to recognize the earliest possible the risk factors of the cardiovascular system and to eliminate them. One of the best known risk factors present in 10—20% of the grown-up population, is essential hypertension.

According to Rossi and König [20], the frequency of hypertension in childhood is only 1—3%. According to Bühlmeyer [2] hypertension in childhood is of renal origin in 79% and of essential character only in 5%; other workers have observed essential hypertension in children more frequently. All data state, however, that essential hypertension begins in childhood, and that its rate increases with age.

According to present knowledge, essential hypertension is the consequence of multifactorial causes [4]. It seems to manifest itself under the effect of environmental factors in

genetically determined persons. If this hypothesis would agree with the facts, early recognition of the endangered persons were possible with the help of genetical markers, and thus the onset of the disease could be prevented.

In the last 15 years, several selective and non-selective population studies have dealt with the cardiovascular risk factors. Specific investigations were carried out primarily in children whose parents had had cardiac infarction in their young days. In their case it is namely likely that under similar aetiologic and familial circumstances a metabolic disturbance of the lipoproteins would occur more frequently than in the healthy population [10]. At the same time, other authors examined the erythrocyte membrane. Its ion content and transport mechanism were compared in a normotonic population and in a population with essential hypertension and their direct relatives.

Some of the main steps in research were as follows. In 1960 Losse et al observed a different Na content [14] and in 1977 Poston et al [19] a different Na permeability and Ca-binding power of the red blood cell membrane and an increased Na-K ATP-activity in hypertonics compared to the control group. In 1978 new data were reported on the lithium ion transport of erythrocytes [16] and on their Na-K pump [21]. Then Garay and Meyer [6] described a new test which is able to detect the changed activity of Na^+ and K^+ flux in the erythrocytes of patients with essential hypertension. This was soon followed by the observation of Meyer et al [15] of the activity decrease of Na-K cotransport in ouabain treated erythrocytes in patients with essential hypertension and in their direct descendants. These results have been confirmed by Davidson et al [4].

Na-Li exchange can similarly be examined in the membranes of ouabain inhibited erythrocytes; it was found to be increased in the erythrocytes of patients with hypertension [3, 5, 22].

From among the above-mentioned observations the most important ones from the point of view of essential hypertension are shown in Table I. The present work deals with the ouabain sensitive Na-K pump (Fig. 1) in normotensive and essentially hypertensive children.

TABLE I

Transport processes in the erythrocytes of essentially hypertensive (EH) and normotensive patients

Transport process	Ions	Inhibitor	Transport protein	Alteration in E.H.
Na-K pump	**Na(Li), K(Li)**	**ouabain**	**Na-K ATP-ase**	increase
Na-Li countertransport	**Na, Li**	**phloretin**	**undefined**	increase
Na-K cotransport	**Na, K**	**furosemide**	**nndefined**	decrease

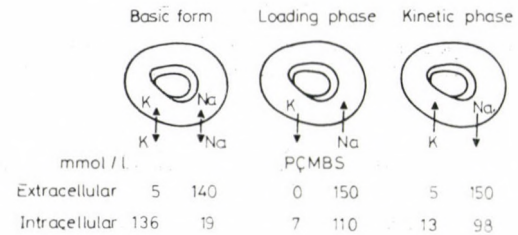

FIG. 1. Simplified scheme of method used for examination of the ouabain sensitive Na-K pump

PATIENTS AND METHODS

Data of 17 healthy normotensive children whose familial history did not reveal hypertensive relatives were compared with those of 15 earlier examined children who were treated for hypertension. The diagnosis of essential hypertension was qualified on the basis of routine examinations [20]. The age of the children ranged from 6 to 16 years. Their skinfolds including the calf skinfold were measured with Holtain caliper according to the International Biological Program (IBP) on the left side of their body. Children who proved to be fat on basis of the Parizková and Roth [17] formula were excluded from the examinations.

Blood pressure of the children was measured on the right upper arm in sitting position, after 5 minutes of relaxation, with a mercury tonometer using a cuff suited to the width of the upper arm. To the patients subjected to examination no drugs have been administered for one week prior to blood analysis which after 12 hours of starvation was done at 8 o'clock a.m.

The ouabain sensitive Na-K pump of the erythrocytes was examined by Garay and Meyer's method [6] with some modifications. Freshly collected heparinized venous blood was centrifuged at 1750 g, at a temperature of 4 °C for 5 min. The red blood cells obtained were washed twice in 20 vol per cent concentrated solution containing 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; pH = 7.4±0.02, and then they were placed into a Na-loading solution of the following composition; 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 0.1 mmol/L PCMBS (parachloromercury benzolsulphonate); 6 mmol/L glucose; pH 7.4±0.02. The red blood cells were incubated in this solution for 20 hours and 4 hours later the solution was exchanged for a fresh one, centrifuged and the supernatant was weighed and incubated in a solution containing 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 6 mmol/L glucose and 4 mmol/L cysteine at a temperature of 37 °C for 1 hour, in order to wash out the PCMBS.

One ml of the suspension obtained by centrifugation at 1750 g at 4 °C for 5 min was mixed in Ringer's solution composed of 145 mmol/L NaCl; 5 mmol/L KCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 10 mmol/L glucose; pH 7.4±0.02. Two ml of this were then incubated for 0, 1, 2, 3, 4 and 5 hours at 37 °C, centrifuged as described above, then washed twice with 150 mmol/L choline chloride. To each incubation time two parallel samples belonged.

The samples obtained were haemolysed in 4 ml distilled water and their Na^+ and K^+ content was measured by flame photometry. In this way the original volume could be calculated. The volume loss occurring between the consecutive steps was checked by haemoglobin and haematocrit determination and then corrected.

The ratio of Na-K fluxes was calculated with the least squares method.

TABLE II

Na^+ and K^+ ion-concentrations of erythrocytes of normotensive and hypertensive children after PCMBS treatment. Ion-transport in mmol/l in erythrocytes, measured hourly

	Na_{effl}/K_{infl}	Na_{start} mmol/l	Na_{effl} mmol/l/h	K_{start} mmol/l	K_{infl} mmol/l/h
Control	9.6	104	2.6	6.7	0.42
n = 17	±5.8	±23	±1.1	±5.3	±0.34
Hypertensive	5.8	111	3.4	9.6	0.63
n = 15	±2.0	±18	±1.5	±7.0	±0.29
p <	0.05	0.35	0.15	0.2	0.1

Abbreviations:

Na_{start}/K_{start} = ion concentrations after PCMBS treatment
Na_{effl}/K_{infl} = ion concentrations
Na_{effl}/K_{infl} = quotient of ions and out of the cell; ratio

RESULTS

In conformity with data in the literature, the intracellular Na^+ concentration of erythrocytes treated with PCMBS of hypertensive patients was found to be high, 111±18 mmol/L, while the value in the control group was 104±23 mmol/L. A similar non-significant increase was observed in the original intracellular K^+ concentration, 9.6±7.0 mmol/L versus 6.7±5.3 mmol/L in the controls. Examining the Na-K pump of erythrocytes in hypertensive patients, there was an increased intracellular accumulation of K^+ (K_{infl}) and also an increased Na^+ efflux (Na_{effl}) as compared to the controls. To judge the operation of the pump, the ratio

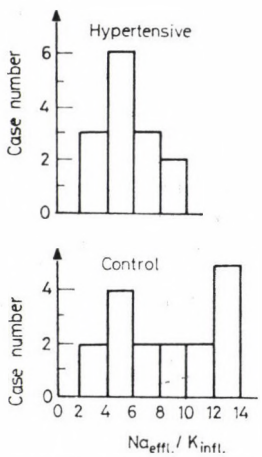

FIG. 2. Number of essentially hypertensive and normotensive children plotted against Na_{effl}/K_{infl}

Na_{effl}/K_{infl} was examined. In the control group it amounted to 9.6 ± 5.8, while in the essentially hypertensives to 5.8 ± 2.0; the difference was significant ($p < 0.05$) (Table II). Plotting the case numbers against Na_{effl}/K_{infl} (Fig. 2), the majority of Na_{effl}/K_{infl} values of hypertensive patients lay under 6, while those of the controls were usually higher. It was also remarkable that while the values of the hypertensives were evenly distributed along an incidence maximum, those of normotensives were either above 12, or near to the values of hypertensives.

Discussion

The purpose of this work was to find a method which would allow to select from among the total population those children who at the time of the examination were still normotensive, but were inclined to develop essential hypertension. Since for technical reasons the effectiveness of the various methods cannot be measured directly, therefore an inverse situation was created wherein children diagnosed as essentially hypertensive were separated from the normotensives.

The results obtained permit to outline a hypothesis. In the erythrocytes of patients, the K-Na cotransport decreases and consequently the Na^+ content of cells is elevated, causing a volume increase in the erythrocytes. The ouabain sensitive Na-K pump is not always able to compensate this pathological situation. According to Blaustein's hypothesis [1] the mechanism takes place not only in erythrocytes, but also in all kinds of cells, thus also in the smooth muscle cells. The increased Na^+ content of the smooth muscle cells may inhibit the Na-Ca exchange. The increased Ca^{2+} concentration would then lead to a constriction of smooth muscle cells, to vasoconstriction and hypertension.

Some other authors assumed that essential hypertension was caused by the increased activity of a Na-transport inhibitor circulating in the blood [13], but other researchers refused this concept [22].

Postnov et al [19] also assumed the existence of a hypothetic substance. They observed a reduced Na^+ efflux when incubating the leukocytes of normotensive subjects with the plasma of hypertensive patients.

According to various hypotheses, hypertension is presumably a multifactorial disease. However, the ion content, as well as the ion exchange through the erythrocyte cell membranes of essentially hypertensives and of their relatives differ from that of normotensives. This observation would allow the following considerations. The symptom-free risk population could be recognized in due time and, by changing the environmental factors, the prevention of hypertension could be possible. The distinction between primary and secondary hypertension could be done with a single examination [7] and so the environmental and genetic causes of essential hypertension could be

differentiated. These possibilities could, however, be only realized if the techniques are further improved. The results obtained by the currently used methods including those obtained with the ouabain sensitive Na-K pump, are suitable only for the separation of groups but because of the wide scatterings they are inadequate for the diagnosis of individual cases. Besides, due to diagnostic difficulties some secondary hypertensions are misdiagnosed for essential hypertension, and the control group may contain such normotensives who on the basis of their genetic characteristics are inclined to hypertension. To this last group may have belonged the four normotensive children included in our examination, whose Na_{effl}/K_{infl} values were similar to those of hypertensive patients.

References

1. Blaustein MP: Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. Am J Physiol 232:165, 1977
2. Bühlmeyer K; Differentialdiagnose des Hypertoniebefundes im Kindesalter. Münch Med Wochenschr 116:711, 1974
3. Canessa M, Adragna N, Solomon HS, Conolly TM, Tosteson DC: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. N Engl J Med 302:772, 1980
4. Davidson JS, Opie LH, Keding B: Sodium-potassium cotransport activity as genetic marker in essential hypertension. Br Med J 284:539, 1982
5. Duhm J, Göbel BO; Sodium-lithium exchange and sodium-potassium cotransport in human erythrocytes. Hypertension 4:477, 1982
6. Garay RP, Meyer P: A new test showing abnormal net Na^+ and K^+ fluxes in erythrocytes of essential hypertensive patients. Lancet 1:349, 1979
7. Garay RP, Elghosi JL, Dagher G, Meyer P: Laboratory distinction between essential and secondary hypertension by measurement of erythrocyte cation fluxes. N Engl J Med 302:769, 1980
8. Heagerty AM, Thurston H, Bing RF, Swales JD: Sodium transport and hypertension. Lancet 1:591, 1983
9. Heller RF, Hayward D, Hobbs MST: Decline in rate of death of ischaemic heart disease in United Kingdom. Br Med J 286:260, 1983
10. Ibsen KK, Lous P, Andersen GE: Coronary heart risk factors in 177 children and young adults whose fathers died from ischaemic heart disease before age 45. Acta Pediatr Scand 71:609, 1982
11. Salonen JT, Puska P, Kottke TE, Tuomilehto J, Nissinen A: Decline in mortality from coronary heart disease in Finland from 1969 to 1979. Br Med J 286:1857, 1983
12. Kornitzer M, De Backer G, Draimaix M, Kittel F, Thilly C, Graffar M: Belgian heart disease prevention project: Incidence and mortality results. Lancet 1:1066, 1983
13. Mac Gregor G, Fenton S, Alaghband ZJ, Markandu N, Roulston J, De Wardener H: Evidence for a raised concentration of a circulating sodium transport inhibitor in essential hypertension. Br Med J 283:1355, 1981
14. Losse H, Wehmayer H, Wessels F: Der Wasser- und Elektrolytgehalt bei arterieller Hypertonie. Klin Wochenschr 38:393, 1960
15. Meyer P, Garay RP, Nazaret C, Dagher G, Bellet M, Broyer M, Feingold J: Inheritance of abnormal erythrocyte cation transport in essential hypertension. Br Med J 282:1114, 1981
16. Pandey GN, Sarkadi B, Haas M, Gunn RB, Davis JM, Tosteson DC: Lithium transport pathways in human red blood cells. J Gen Physiol 72:233, 1978
17. Parizková J, Roth Z: The assessment of depot fat in children from skinfold thickness measurement by Holtain caliper. Human Biol 44:613, 1972
18. Poston L, Sewell RB, Wilkinson SP, Richardson PJ, Williams R, Clarkson EM, Mac Gregor GA, de Wardener HE: Evidence for a circulating sodium transport inhibitor in essential hypertension. Br Med J 282:847, 1981
19. Postonov YU, Orlov SN, Shevchenko A, Adler AM: Altered sodium permeability, calcium binding and Na-K-ATPase activity in the red blood cell membrane essential hypertension. Pflügers Arch 371:263, 1977

20. Rossi E, König R: Hypertension in children: clinical aspects. In: Giovannelli G ed, Hypertension in children and adolescents. Raven Press, New York 1981 p. 195.
21. Tosteson DC, Sarkadi B; The Na-K pump in red cells. In: Tosteson DC ed, Membrane transport in biology. Springer Verlag, Heidelberg 1979
22. Woods JW, Falk RJ, Pittman AW, Klemmer PJ, Watson BS, Namboodiri K: Increased red cell sodium-lithium countertransport in normotensive sons of hypertensive parents. N Engl J Med 306:593, 1982
23. World Health Organisation: Technical Report series 678: Prevention of coronary heart disease. Geneva 1982

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