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INFLUENCE OF SODIUM AND POTASSIUM BALANCES ON THE MAXIMUM BINDING OF OUABAIN TO ERYTHROCYTES IN NORMAL CHILDREN

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ABSTRACT

The maximum bindings of ouabain to erythrocytes (Bmax), serum sodium (Na) and potassium (K) concentrations, and urinary excretions of Na and K were measured in 72 normal children, on a normal diet, aged 10 to 15 years. Bmax did not show any significant correlation with serum Na and K, and urinary Na and K excretions.

These findings suggest that Bmax, which has been reported to be low in primary hypertension, may not be influenced by Na and K balances.

INTRODUCTION

In 1960, Losse et al reported increased intracellular sodium (Na) concentration in the erythrocytes of patients with essential or primary hypertension (1). Since then a number of studies on erythrocyte ion transport have been done in the cells of patients with primary hypertension (2, 3, 4). However, results have sometimes been contradictory, since there is a variety not only of research topics, but also of research techniques (5, 6).

A few studies have been done on children (4, 7), but available data remains scarce, and numerous facts are still unexplained. Considering the conflicting results obtained in adults, we surely need some fundamental information about erythrocyte Na transport before making conclusions about its role in childhood diseases. We therefore studied the influence of Na and K balances on Bmax in normal children.

SUBJECTS AND METHODS

72 normal 13 to 15 year-old children on a normal diet were studied. Twelve-hour

urine samples were collected, and aliquots were stored at −20°C until assay. Venous blood was taken and put into cold tubes containing lithium-heparin and then centrifuged at 3000 rpm for 15 minutes to separate plasma and red blood cells. Plasma and urinary Na, K and creatinine were measured by a routine autoanalyzer technique.

Red cells were washed three times with 10 volumes of 154 mmol/l sodium chloride, and recentrifuged for 10 minutes at 3000 rpm. All procedures were carried out at 4°C.

Measurement of Bmax

Measurement of Bmax was done according to Gardner and Conlon (8) with minor modifications, mainly in the volumes of cells and of washing media. 37.5μl of washed packed cells were pipetted into tubes containing 262.5μl of the following medium: NaCl 130 mmol/l, sucrose 20 mmol/l, and glucose 20 mmol/l buffered with HCl to a pH of 7.4. To this, ^{3}H-ouabain (Amersham) diluted with isotonic saline was added. To determine Bmax, the following five different ouabain concentrations were used: 2×10^{-7}M, 10^{-7}M, 5×10^{-8}M, 2.5×10^{-8}M, 1.25×10^{-8}M. The tubes were incubated at 37°C for 2 hours in a shaking water bath. Parallel incubations were performed with the addition of unlabelled ouabain (10^{-4} mol/l) to measure the non-saturable binding. The cells were then washed three times with isotonic saline. After the last wash, the cells were disrupted with 0.2 ml of 10% trichloro-acetic acid, agitated and centrifuged at 3000 rpm for 15 minutes. 0.1 ml of the supernatant was then pipetted into counting vials, and mixed with 3 ml of liquid scintillation fluid. All samples were measured in duplicate and counted for ^{3}H in a liquid scintillation counter. Specific binding (B_{max}) was taken as the difference between ^{3}H-ouabain bound in the absence and presence of unlabelled ouabain.

Children were divided into two groups according to the level of B_{max}: i. e., those with high B_{max} (top 20% tile of all; n=16) and those with low B_{max} (bottom 20% tile of all; n=16). Results were expressed as mean ±SD, and analyzed using unpaired t-tests. Simple correlation coefficients were calculated for B_{max} and plasma Na or K, and urinary Na or K excretion.

RESULTS

B_{max} showed no significant correlations with serum Na ($r=0.0172$, $P>0.05$), serum K ($r=0.0969$, $P>0.05$), urine Na excretion (Fig. 1), and urine K excretion (Fig. 2). There were no significant differences in serum Na and K, and urinary Na and K excretions between children with high B_{max} and those with low B_{max} (Table 1).

DISCUSSION

There are several pathways for Na transport in erythrocytes, as shown in Fig. 3 (5, 9). The first one is Na-Li countertransport, which is equivalent to Na-Na counter-transport in an actual state. In vivo, this channel is thought to exchange a single external Na ion for an internal one. This, therefore, cannot affect the overall distribution of Na across a cell membrane. The second one is furosemide sensitive Na-K

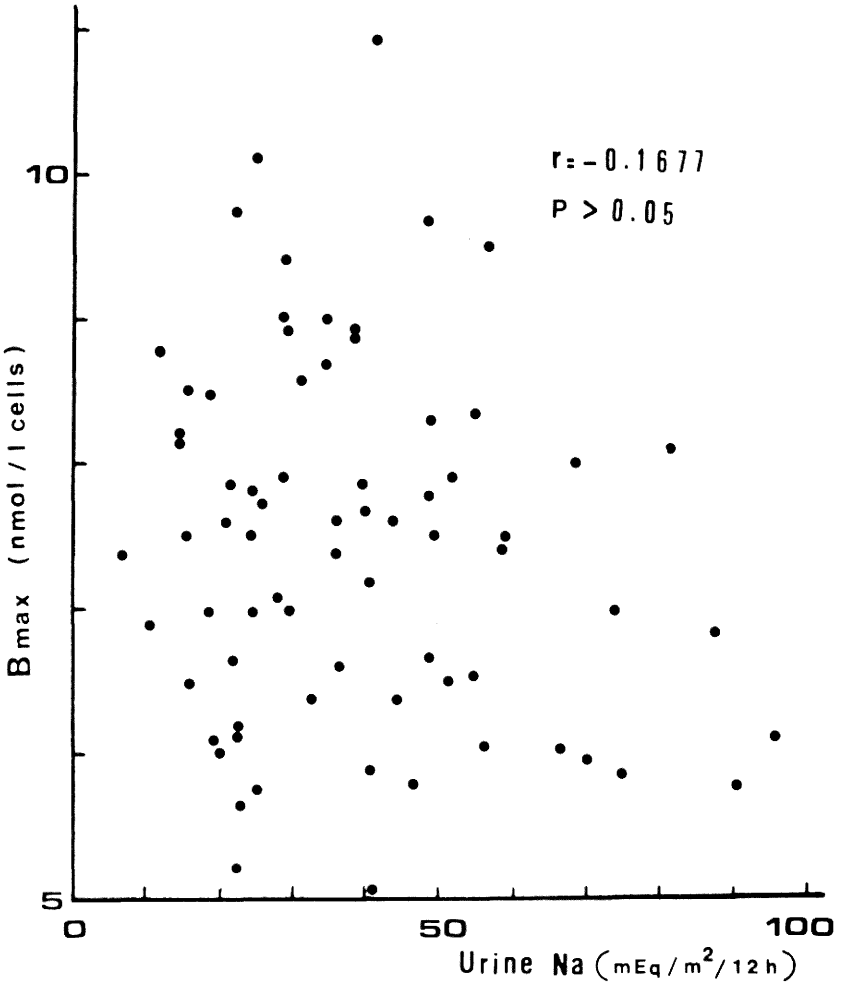

Fig. 1. Relationship between Bmax and urinary Na excretion

Table 1. Serum Na and K, and urinary excretion of Na and K in children with high B_{max} and children with low B_{max} (mean±SD)

	Children with high B_{max}	Children with low B_{max}	Statistical significance
serum Na (mEq/l)	140.4±1.7	140.5±1.5	N. S.
serum K (mEq/l)	4.3±0.4	4.4±0.5	N. S.
urine Na (mEq/m²/12h)	47.5±25.1	41.4±11.2	N. S.
urine K (mEq/m²/12h)	7.4±4.4	6.1±2.4	N. S.

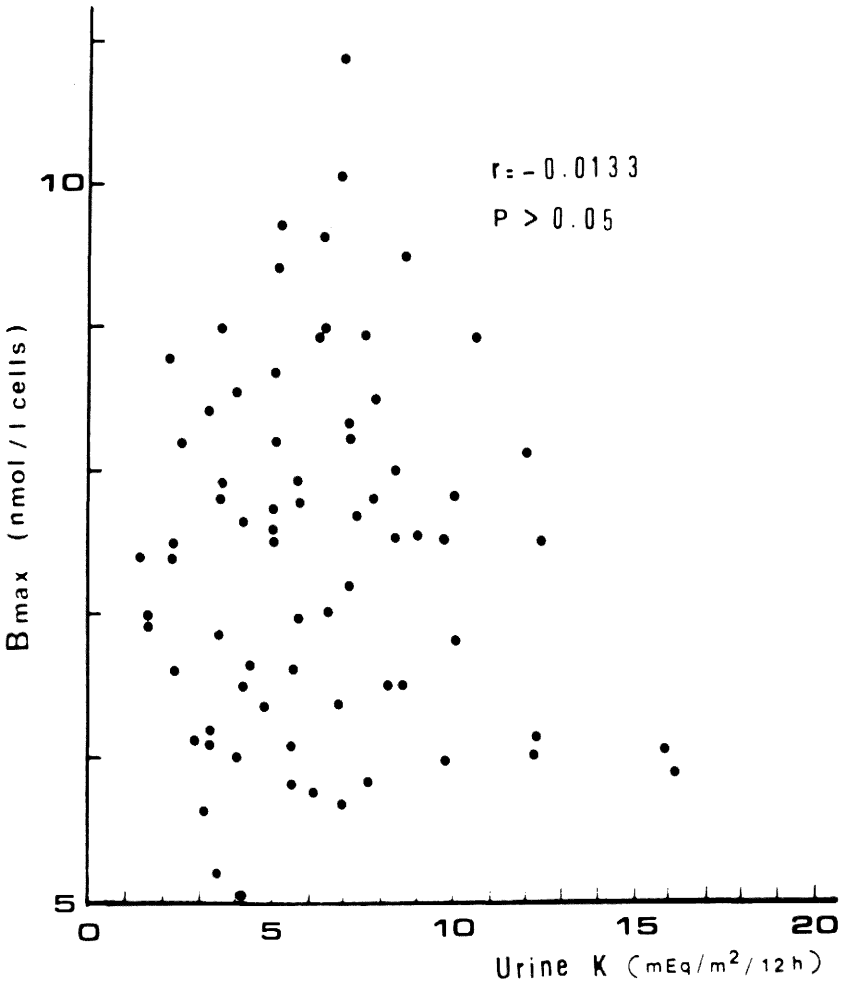

Fig. 2. Relationship between Bmax and urinary K excretion

cotransport, which lies dormant in the cell membrane unless intracellular Na levels rise above a critical level. This acts as a supporting mechanism for Na extrusion. The third one, the Na pump, is the best characterized and most widely studied pathway for cellular Na homeostasis (5, 9).

Since it has been repeatedly demonstrated that the number of ouabain-binding sites is directly related to the number of Na-K-ATPase units, maximum ouabain-binding capacity (B_{max}) can indicate the number of enzyme units per cell (10). This number has been shown to be low in both adults (11) and children (7) with primary hypertension; however, the abnormal B_{max} found in primary hypertension may only be caused by factors such as electrolyte balances. Assessment of B_{max}, therefore, seems difficult without a fundamental study of these factors.

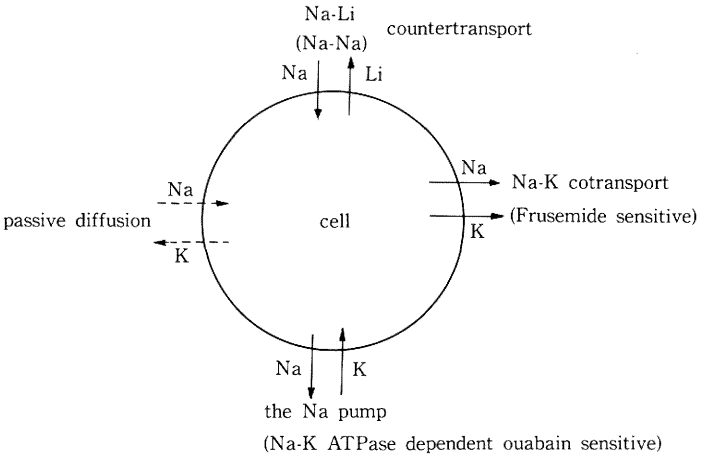

Fig. 3. Various pathways of Na transport in erythrocyte membrane

It has been reported that high salt intake may have some relevance to the onset of primary hypertension (12); however the relationship between salt intake and B_{max} has not been clarified yet, especially in children. In the present study, B_{max} did not correlate with serum Na and 12-hour Na excretion. In addition, there were no significant differences in serum Na and 12-hour Na excretion between children with low B_{max} and those with high B_{max}. These findings suggest that the low B_{max} previously reported in hypertensives may not be caused by high salt intake, since Na excretion reflects Na intake under general conditions.

Sufficient K intake has been observed to reduce blood pressure in hypertensives (13, 14). Its mechanism is unclear, although K has been reported to reduce blood pressure by improving Na transport in cell membranes (15). In the present study, however, either serum K level or urinary K excretion did not correlate with B_{max}. In addition, there were no significant differences in serum K and 12-hour K excretion between children with low B_{max} and those with high B_{max}. These findings suggest that K balance may not affect B_{max} on a normal diet.

We have already reported on the possibilities that adult hypertension starts in childhood (16), and that B_{max} and erthrocyte Na/K flux ratio can be genetic markers for essential hypertension (17). However we have also found that erthrocyte Na/K flux ratio is affected by serum K level, unlike B_{max} (18). B_{max} can, therefore, be a better genetic marker for hypertension as compared with erthrocyte Na/K flux ratio. Based on the present study, we need further investigations in the future to elucidate the mechanism of low B_{max} observed in hypertensive adults and children.

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REFERENCES

1) Losse, H., et al.: Wasser und elektrolytgehalt von erythrozyten bei arterieller hypertonie. *Klin. Wschr.,* **38**: 393-395, 1960.
2) Canessa, M., et al.: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *NEJM,* **302**: 772-776, 1980.
3) Garay, R. P., et al.: Laboratory distinction between essential and secondary hypertension by measurement of erythrocyte cation fluxes. *NEJM,* **302**: 769-771, 1980.
4) Mongeau, J. G.: Value of measurement of erythrocyte cation fluxes as a genetic marker in essential hypertension of adolescents and children. In: Brodehl, J., Ehrich, J. H. H. (eds), Paediatric Nephrology. Springer-Verlag, Berlin, 1984, pp 382-384.
5) Editorials: Cells, ions, and blood pressure. Lancet, ii: 965-967, 1982.
6) Tosteson, D. C., et al.: Membrane, ions and hypertension. Clinical Science, 61 (Suppl. 7): 5s-10s, 1981.
7) Uchiyama M, et al.: Sodium transport in erythrocytes: differences between normal children and children with primary and secondary hypertension. Clin. *Exp. Hypertension,* **A8**: 669-671, 1986.
8) Gardner, J. D., Conlon, T. P.: The effects of sodium and potassium on ouabain binding by human erythrocytes. *J. Gen. Physiol.,* **60**: 609-629, 1972.
9) Weissberg, P. L.: Ion transport studies-technical problems. *J. Hypertension,* 1 (Suppl. 2): 395-397, 1983.
10) Gelbart, A., Goldman, R. H.: Correlation between microsomal ($Na^+ + K^+$)-ATPase activity and [^{3}H] ouabain binding to heart tissue homogenates. *Biochem. Biophys. Acta,* **481**: 689-694, 1977.
11) Takayama, Y., et al.: Reduced number of erythrocyte sodium pump units in essential hypertension. *Tohoku J. Exp. Med.,* **143**: 383-384, 1984.
12) Meneely, G. R., Battarbee, H. D.: High sodium-low potassium environment and hypertension. *Am. J. Cardiol.,* **38**: 768-784, 1978.
13) MacGregor, G. A., et al.: A double blind randomised crossover trial of moderate potassium supplementation in essential hypertension. *Lancet,* ii: 567-570, 1982.
14) Iimura, O., et al.: Studies on the hypotensive effect of high potassium intake in patients with essential hypertension. *Clin. Sci.,* **61**: 77s-80s, 1981.
15) Smith, S. J., et al.: Does potassium lower blood pressure by increasing sodium excretion? A metabolic study in patients with mild to moderate essential hypertension. *J. Hypertension,* 1 (Suppl 2): 27-30, 1983.
16) Uchiyama, M., et al.: Is childhood hypertension a predictor of adult hypertension? Lancet, i: 1247, 1984.
17) Uchiyama, M., et al.: Membrane electrolyte transport in children with primary hypertension. In: Recent advances in pediatric nephrology, Excerpta Medica, Amsterdam, 1987, pp. 133-138.
18) Uchiyama, M., et al.: Erythrocyte Na/K flux ratio in relation to sodium and potassium balances in normal children. *Acta Medica et Biologica,* **35**: 69-76, 1987.