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ERYTHROCYTE NA/K FLUX RATIO IN RELATION TO SODIUM AND POTASSIUM BALANCES IN NORMAL CHILDREN

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ABSTRACT

Erythrocyte Na/K flux ratio, serum sodium (Na) and potassium (K), and urinary excretions of Na and K were measured in 81 normal children on a normal diet, aged 13 to 15 years. Erythrocyte Na/K flux ratio was negatively correlated with serum K level, although it showed no significant correlation with either serum Na, urinary Na and K excretions or fractional excretions of filtered Na and K. These findings suggest that erythrocyte Na/K flux ratio, which has been reported to be abnormal under some pathological conditions including essential hypertension, may depend on serum K level, and that the flux ratio may not have a role in the renal Na or K control mechanism.

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, 5). However, results of these studies have sometimes been contradictory, since there is a variety not only of research topics, but also of research techniques.

A few studies have been reported in children (5, 6). The data which are available remain, however, scarce, and numerous facts are still unexplained. Considering the conflicting results obtained in adults, we surely need to have some fundamental knowledge concerning erythrocyte Na transport before we can reach conclusions about its abnormality in childhood diseases. We therefore studied erythrocyte sodium transport in normal children to elucidate its relation to Na and potassium (K) balances.

SUBJECTS and METHODS

81 normal children on a normal diet, aged 13 to 15 years, were studied. Twelve-hour urine samples were collected, and aliquots were stored at −20℃ until assay. Venous blood was taken into cold tubes containing lithium-heparin and centrifuged at 3000 rpm for 15 minutes to separate plasma and red blood cells.

Plasma and urinary Na, K and creatinine (Cr) were measured by a routine autoanalyser technique. Fractional excretion of filtered Na (FENa) or potassium (FEK) was calculated as follows: (urine Na x plasma Cr) x 100/(plasma Na x urine Cr)%.

Red cells were washed twice with 10 volumes of 150 mmol/l sodium chloride, and recentrifuged for 10 minutes at 3000 rpm. All procedures were carried out at 4℃. The internal Na content of the red cells was increased and the K content reduced using a procedure similar to that described by Garrahan and Rega (6). 0.75 ml of washed and packed cells were suspended in a "sodium-loading-medium", the packed red cells finally constituting 5% of the volume. The Na-loading-medium contained (mmol/l) 150 sodium chloride, 1 magnesium chloride, 2.5 sodium phosphate (pH 7.4 at 4°C), and 0.1 2,5-p-chlormercuribenzenesulphonate (P. C. M. B. S.). Cells were incubated for 20 hours at 4° C in Na-loading-medium which was renewed once after a 6-hour incubation period. Cells were then centrifuged at 3000 rpm at 4°C for 10 minutes and the supernatant was discarded. Cells were afterwards resuspended in Na-K Ringer medium containing (mmol/l) 145 sodium chloride, 5 potassium chloride, 1 magnesium chloride, 2.5 sodium phosphate (pH 7.4 at 4°C) and 10 glucose to reach a volume of packed cells of 10%. Neutralised cysteine was added to the medium for a final concentration of 4 mmol/l. Cell suspensions were incubated at 37°C for 1 hour. Cells were then spun down at 4°C for 10 minutes at 3000 rpm and resuspended in the Na-K Ringer medium without cysteine until packed cells constituted approximately 50% of the volume. An aliquot of this suspension, together with one of P. C. M. B. S. untreated cells, was set aside to measure intracellular Na, K, haemoglobin, and the volume of packed cells.

Measurement of cation movements (3)

0.6 ml of cell suspension was added to 22 ml of cold Na-K Ringer medium and the resulting suspension distributed in 6 tubes (2 ml per tube) at 0°C. The final packed cell volume was approximately 1.3%. Tubes were incubated at 37°C. At hour 1, 2, 3 two tubes were transferred to 0°C and the cells were washed three times with 150 mmol/l choline chloride at 4°C. After the last wash the cells were spun down at 4°C for 10 minutes at 3000 rpm. Supernatant was discarded and the cells were lysed with 1 ml of distilled water. 0.9 ml of the haemolysate was added to 4 ml of distilled water to measure K, and 1 ml of this solution was added to 4 ml of distilled water to measure Na and haemoglobin.

Intracellular Na and K concentrations were estimated by flame photometry. To avoid error, these concentrations were always expressed per litre of original cells and

corrected by the value of the haemoglobin. Haemoglobin was estimated as oxyhaemoglobin by measuring light absorption at 541 nm.

When high Na/low K human erythrocytes were incubated in physiological Na-K Ringer medium, they tended to recover their stationary low Na/high K content. During the 3 hours of flux measurement, the net fluxes of Na and K obtained were linear in time for all the erythrocyte samples studied. The values were obtained by linear regression analysis. The values were available for assessment when the regression coefficients were over 0.90 for the net K influx and the net Na efflux. Finally, Na/K net flux ratio was calculated for this study.

Subjects were divided into two groups, those with a high erythrocyte Na/K flux ratio belonging to the top 20th percentile (n=16) and those with a low ratio belonging to the bottom 20th percentile (n=16) in respect to the whole group. Results were expressed as the mean±SD and analysed using unpaired t-tests. Simple correlation coefficients were calculated for erythrocyte Na/K flux ratio and plasma Na or K, urinary Na or K excretion, FENa and FEK in all the subjects (n=81).

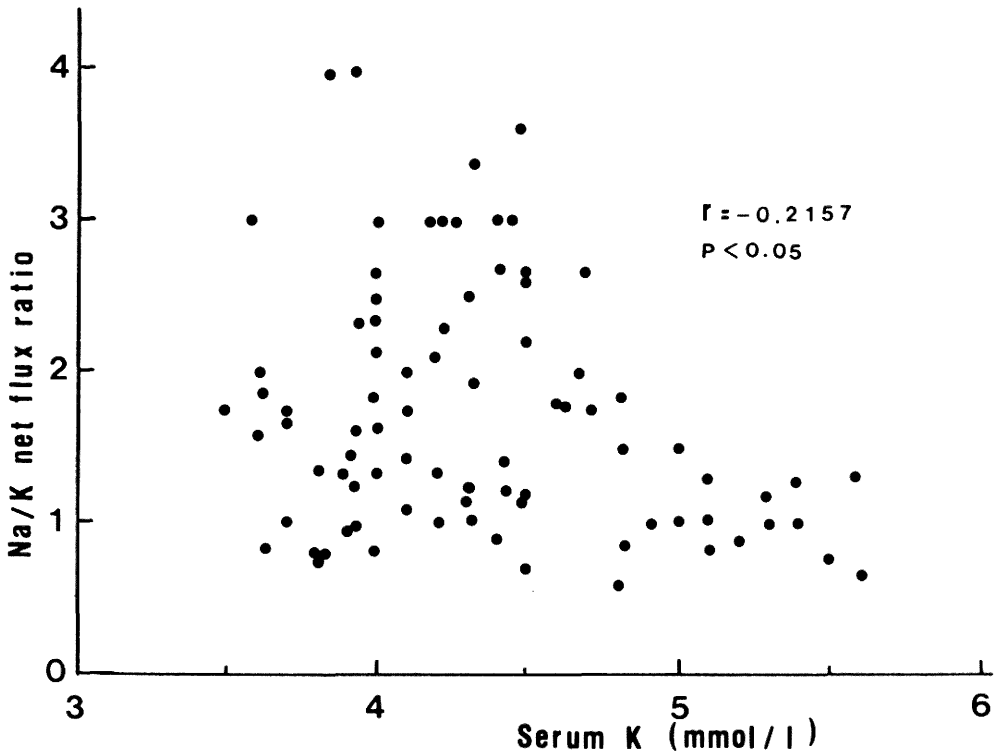

Fig. 1 Relationship between erythrocyte Na/K flux ratio and serum K

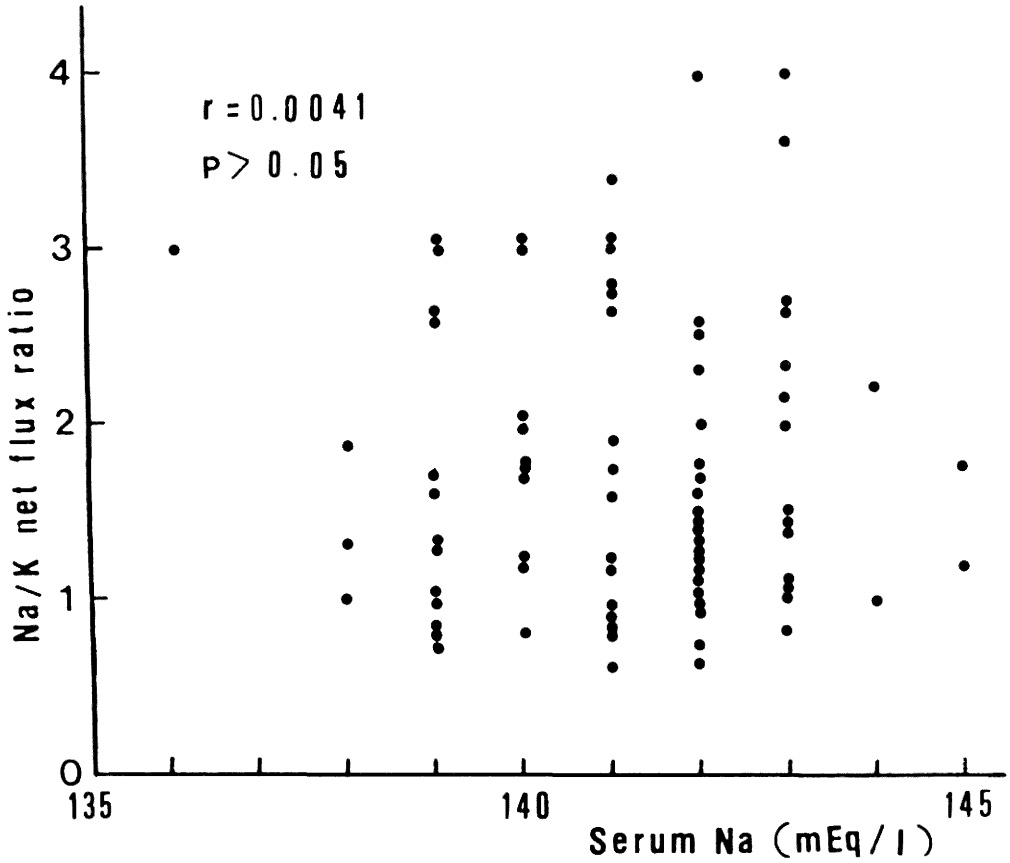

Fig. 2 Relationship between erythrocyte Na/K flux ratio and serum Na

RESULTS

The ratio of Na/K net fluxes showed a significant negative correlation with serum K (Fig. 1), but no significannt correlations with plasma Na (Fig. 2), urinary Na or K excretion (Figs. 3, 4), FENa (Fig. 5) and FEK ($r=-0.1019$, $P>0.05$). There were no significant differences in plasma Na or K, urinary Na or K excretion, FENa and FEK between children with high and low erythrocyte Na/K flux ratio (Table 1).

DISCUSSION

The ratio of Na/K net fluxes has been studied primarily in adult patients with essential hypertension (2, 3, 4), however the results are conflicting in that this ratio is low in French and Japanese hypertensives (3, 4, 7), but normal in South African and American hypertensives (8). This may simply depend on racial differences, although some factors, including electrolyte balances, may possibly influence this ratio. Assessment of this ratio

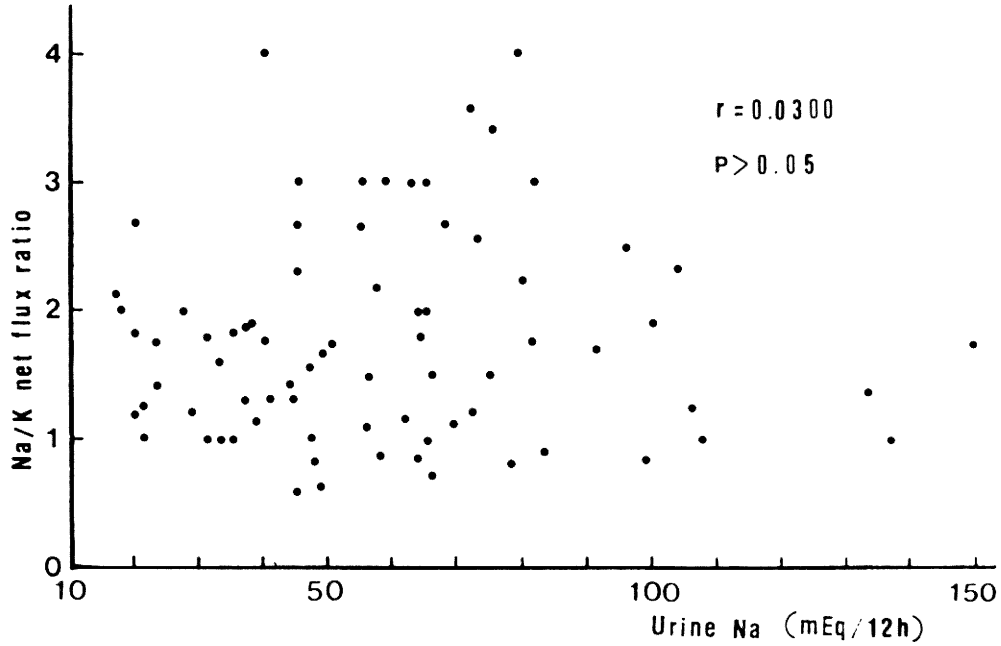

Fig. 3 Relationship between erythrocyte Na/K flux ratio and 12-hour urinary Na excretion

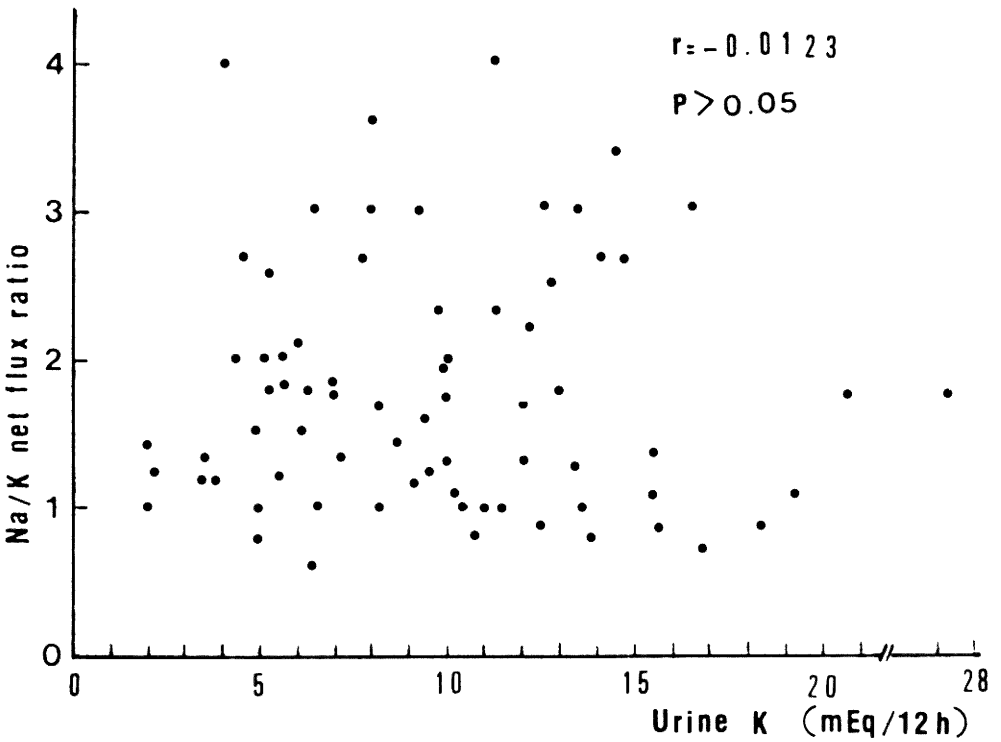

Fig. 4 Relationship between erythrocyte Na/K flux ratio and 12-hour urinary K excretion

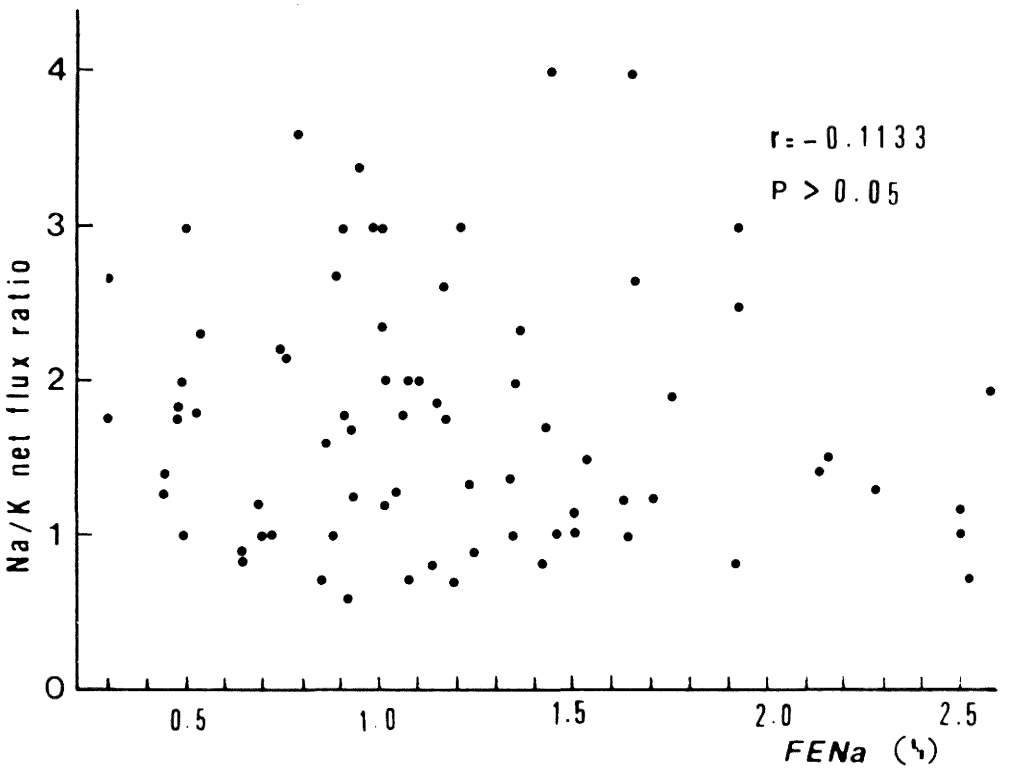

Fig. 5 Relationship between erythrocyte Na/K flux ratio and FENa

Table. 1 Differences in serum Na or K, urinary Na or K excretion and FENa between children with high erythrocyte Na/K flux ratio (high ratio group) and those with low erythrocyte Na/K flux ratio (low ratio group)

	high ratio group	low ratio group	statistical significance
serum Na (mEq/l)	140.8±1.9	140.6±1.5	N. S.
serum K (mEq/l)	4.2±0.3	4.6±0.6	N. S.
urine Na (mEq/12h)	59.6±16.8	61.3±24.9	N. S.
urine K (mEq/12h)	10.1±4.1	10.8±4.5	N. S.
FENa (%)	1.07±0.45	1.43±1.12	N. S.

seems difficult without a fundamental study of these factors.

In the present study, this ratio did not correlate with serum Na and 12-hour Na excretion, suggesting that Na balance may not affect this ratio. Low erythrocyte Na/K flux ratio previously reported in Japanese hypertensives may not be caused by the custom of high salt intake in Japan, since urinary Na excretion reflects Na intake under general conditions.

Sufficient K intake has been known to reduce blood pressure in hypertensives (9); this

is thought to be the effect of improved Na transport in cell membranes (10, 11, 12). In the present study, though, low serum K level was, on the contrary, associated with high erythrocyte Na/K flux ratio. These findings suggest that erythrocyte Na/K flux ratio may change to compensate for the low K level which elevates blood pressure by some other mechanisms. There is still a possibility that an excessively low level of serum K over a long period of time may decrease erythrocyte Na/K flux ratio, but this has not been confirmed by any studies, including the present one.

There have been no reports on the relation of erythrocyte Na/K flux ratio to the flux ratio in renal tubules or to tubular Na reabsorption. In the present study, erythrocyte Na/K flux ratio did not show any correlation with FENa and FEK, suggesting that this ratio may not be of significant relevance to Na or K absorption in renal tubules.

We have already reported the possibilities that adult hypertension starts in childhood (13), and that erythrocyte Na transport can be a genetic marker for essential hypertension (14). However careful consideration might be needed to evaluate erytrhrocyte Na/K flux ratio, since it may be affected by serum potassium.

ACKNOWLEDGEMENTS

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