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EFFECT OF ACUTE SALINE LOADING ON THE ERYTHROCYTE SODIUM TRANSPORT IN CHILDREN

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ABSTRACT

Sixteen children with labile hypertension were infused with physiological saline solution (15 ml/kg/h) for 1 h after they had been supine for 90 min. Blood was taken twice before and after infusion, and the effect of each plasma (25%) on both the maximum binding of ouabain to erythrocytes (Bmax) and the affinity of the sodium pump (Kd) for its specific inhibitor 'ouabain' were examined.

Neither Bmax nor Kd was influenced by the plasma after saline infusion, suggesting that a ciculating sodium pump inhibitor may not appear by acute saline infusion in hypertensive children unlike in hypertensive adults.

INTRODUCTION

The aetiology of essential hypertension is unknown. However, much evidence suggests that an abnormal sodium metabolism plays a critical role. Especially, have salt intake[1-4] and cell sodium transport[5-8] been implicated as factors causing hypertension, and a connection between the erythrocyte total ^{22}Na efflux rate constant and sodium ingestion has been found in hypertensive patients[9].

As they have previously reported in hypertensive adults[10,11] the authors have already found that erythrocyte sodium transport is abnormal in normotensive children with a family history of essential hypertension[12]. Recently, an acute salt load has been reported to have some effects on cell membrane sodium transport by producing a circulating sodium transport inhibitor in hypertensive adults[13,14]. As yet, it is not known whether by using the same protocol this circulating sodium transport inhibitor also appears in the plasma of children.

Subjects and Methods

Sixteen male children with labile hypertension, aged 12 to 15 years were examined. All of them were normotensive on the day of examination. They were fasted overnight and infused with physiological saline solution (15 ml/kg/h) for 1 h after they had been supine for 90 min in the morning. Before and after the infusion, venous blood was taken and put into cold tubes containing lithium-heparin which were then centrifuged at 3000 rpm for 15 min to separate the plasma and erythrocytes. The erythrocytes were washed three times with 10 volumes of 154 mmol/l sodium chloride and recentrifuged for 10 min at 3000 rpm.

The effect of plasma on both maximum binding of ouabain to erythrocytes (Bmax) and the affinity of the sodium pump (Kd) for its specific inhibitor 'ouabain' were studied in various ways as shown below. All procedures were carried out at 4℃.

Materials

1) Erythrocytes obtained before the infusion.
2) 25% plasma (diluted with phosphate buffer) obtained before the infusion.
3) Erythrocytes obtained after the infusion.
4) 25% plasma (biluted with phosphate buffer) obtained after the infusion.

Combination for Bmax measurements

Plan A: 1)+2)
Plan B: 1)+4)
Plan C: 3)+2)
Plan D: 3)+4)

Final concentration of potassium was adjusted to 1.25 mmol/l in each tube. This concentration of potassium does not influence Kd[15], although potassium generally competes with ouabain binding to erythrocytes.

Measurement of Bmax and Kd

Measurement of Bmax was done according to Gardner and Conlon[16] with minor modifications. 37.5 μl of washed packed cells were pipetted into tubes containing 65.6 μl of subject's plasma (25%) and 196.9 μl 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, was added ^{3}H-ouabain (Amersham) diluted with isotonic saline. 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℃ for 2 h 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 min. The supernatant, 0.1 ml 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 (Bmax) was taken as the difference between ^{3}H-ouabain bound in the absence and presence of unlabelled ouabain. Kd was calculated as well as Bmax by using a microcomputer and a curve-fitting program.

Results were expressed as means ± SD. Statistical analysis was carried out by using t-tests.

RESULTS

Bmax and Kd did not change significantly in every combination of erythrocytes and plasma before and after saline infusion (Figs. 1, 2).

DISCUSSION

In spite of the unknown aetiology of essential hypertension, much evidence suggests that an abnormal sodium metabolism in connection with excessive salt intake[1-4] and abnormal cell transport[5-8] may have some significant role at the onset. A close connection between the erythrocyte total ^{22}Na efflux rate constant and sodium ingestion has been found in hypertensive patients[9]. Recently, excessive salt intake has been reported to produce a circulting sodium transport inhibitor, thus influencing cell membrane sodium transport in hypertensive adults[13].

The authors have previously found abnormal erythrocyte sodium transport in normotensive children with a family history of essential hypertension[12], the same findings as previously reported in hypertensive adults[10]. Recently, the circulating sodium transport inhibitor was demonstrated to be increased by acute salt load in plasma of hypertensive adults[13,14]. As yet, it is not known in children, while abnormal erythrocyte sodium transport observed in childhood did not result from high sodium intake[17].

In the present study, no remarkable changes in Bmax and Kd were observed after the acute saline infusion. Children's plasma after the infusion did not affect the Bmax and Kd. These findings suggest that a circulating sodium inhibitor may not appear in the plasma of children unlike adults when salt is loaded acutely.

There may be a cntroversy about the method of salt load adopted in the present study. There in children is no fixed idea on how much saline should be infused to achieve our desired end. We infused 15 ml of physiological saline per kg body weight for 60 min, which is almost equivalent to the volume adopted in adults[14].

The authors have previously reported that the number of sodium pump sites in erythrocytes decreases in normotensive children with a family history of essential hypertension[12]. The same abnormality has been reported in hypertensive adults[10,11], a condition which may be partially caused by a circulatiog sodium transport inhibitor in connection with much salt intake[13,14]. However, an abnormal erythrocyte sodium trans-

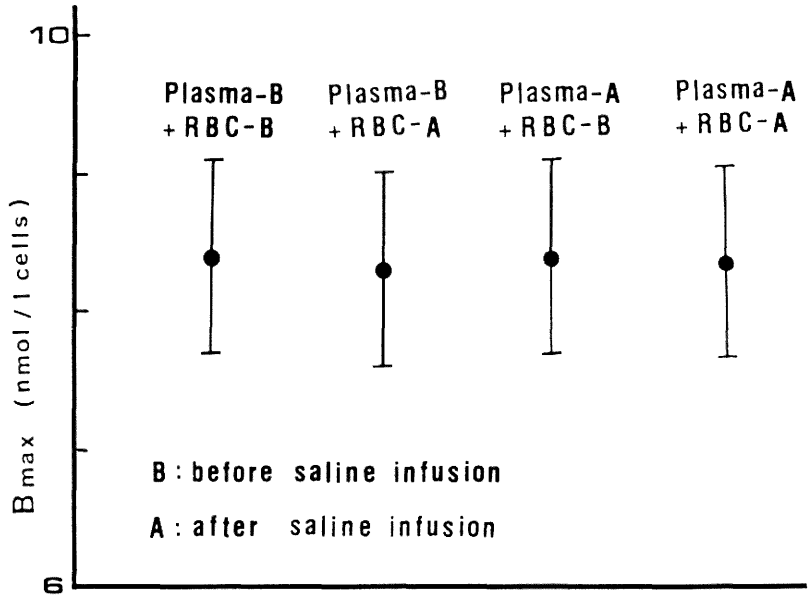
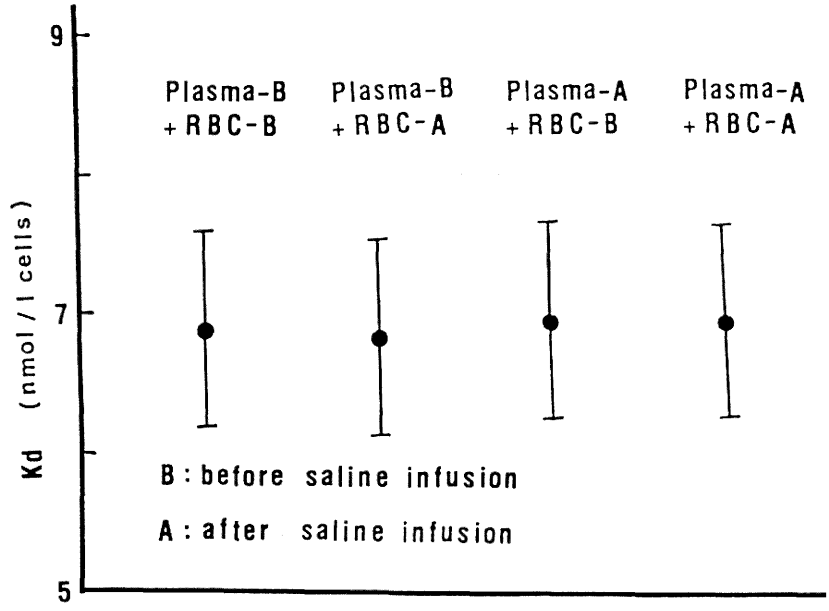


Fig. 1 Bmax in varioun combinations of erythrocytes and plasma before and after saline infusion



Figz 2 Kd in various combinations of erythrocytes and plasma before after saline infusion

port found in children may not be acquired the way it is in adults since there was no evidence of the appearance of a circulating sodium transport inhibitor in the present study.

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