Erythrocyte $^{22}$Na efflux and urinary sodium excretion in essential hypertension

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Summary

1. The rate constant for total $^{22}$Na efflux from erythrocytes was examined in patients with mild to moderate hypertension and in normotensive controls. No difference in $^{22}$Na efflux rate constant was found when the cells from both groups were incubated in artificial medium. When the cells from both groups were incubated in their own plasma, the rate constant for Na efflux was significantly elevated for hypertensive patients compared with controls ($0.40 \pm 0.02$, $0.36 \pm 0.01$ respectively; $P < 0.05$).

2. In hypertensive patients sodium efflux rate constant varied inversely with 24 h urinary sodium excretion when erythrocytes were incubated in artificial medium ($r = -0.34$, $P < 0.05$) or in plasma ($r = -0.42$, $P < 0.05$). No association between sodium efflux rate constant and urinary sodium excretion occurred in normotensive subjects.

3. These findings provide further evidence that sodium is an important aetiological factor in hypertension. In ‘salt-sensitive’ individuals dietary sodium may interact with the regulation of cellular sodium transport via both humoral and cellular mechanisms to elevate blood pressure.

Key words: erythrocytes, hypertension, sodium diet, sodium efflux.

Introduction

Intracellular sodium content is increased in leucocytes obtained from patients with essential hypertension (Edmondson, Thomas, Hilton, Patrick & Jones, 1975; Araoye, Khatri, Yao & Freis, 1978), which is associated with a depression of the rate constant for active sodium efflux (Edmondson et al., 1975). It is possible that a high salt intake could cause intracellular sodium to rise through an alteration in the rate constant for sodium efflux and lead to the development of hypertension in susceptible individuals. The aim of this study was to determine whether the rate constant of sodium efflux from erythrocytes differed between hypertensive patients and a control group of normotensive subjects matched for age and sex, and to determine whether there is a relationship between sodium intake and the rate constant for erythrocyte sodium efflux in hypertensive patients.

Methods

Blood pressure was measured using a mercury sphygmomanometer with the patients or subject supine for 10 min. Blood (35 ml) was then collected from an antecubital vein for the determination of erythrocyte $^{22}$Na efflux rate constant and for the measurements of plasma levels of sodium, potassium and creatinine.

Measurement of $^{22}$Na efflux rate constant

The blood was centrifuged and the plasma was removed and stored at 4°C until required. Theuffy coat was removed by aspiration. The erythrocytes were washed three times by centrifugation at 1600 g for 10 min at 4°C with a balanced salt solution (BSS) NaCl 110 mmol/l; sodium acetate 30 mmol/l; CaCl$_2$ 1.2 mmol/l; MgCl$_2$ 1.2 mmol/l; K$_2$HPO$_4$ 2.5 mmol/l; glucose 20 mmol/l (pH 7.4).

After the third wash, the erythrocytes were resuspended to a packed cell volume of $40.0 \pm 0.5\%$ with cold BSS and 2.0 μCi of $^{22}$NaCl was added to 10 ml of the resuspended cells. The cells were then incubated with radio-
active buffer for 18 h at 4°C. After this pre-incubation period, the samples were centrifuged. The supernatant was removed, the cells were washed three times with cold BSS and the volume was restored to 10 ml. The suspension was vortexed and 2 ml aliquots were added to either 5 ml of BSS or 5 ml of plasma (from the original blood sample) in duplicate. The suspensions were vortexed and incubated at 37°C in a shaking water bath was commenced. Immediately after the start of the incubation, and at 120 min, 500 µl of suspension was removed from each incubating sample and transferred to a separate counting tube. Also immediately after the start of the incubation and at 30 min intervals for 120 min, 1 ml aliquots were withdrawn from each of the incubations and centrifuged at 1600 g for 10 min at 4°C, after which 500 µl of the supernatant was transferred to a separate counting tube.

The 22Na content of each counting tube was measured in a Packard gamma-counter.

The efflux rate constants (k,.) can be determined under steady-state conditions by using equation ln(1 – Nt/No) = –k,t, where Nt = 22Na present per unit volume of suspension of incubating cells (mean of the counts/unit volume of suspension taken at time 0 and 120 min), No = 22Na present per unit volume of supernatant at time (h).

The best estimate of k, was taken as the slope of the linear relation between –ln(1 – Nt/No) and t, calculated by the least-squares method.

Reproducibility of measurement of k, and k,t

To assess the reproducibility of the technique a sample of blood was taken from three healthy volunteer subjects over a 2 week period on 3 days of each week and k, was measured in duplicate for the erythrocytes incubated in their own plasma and in BSS.

The within-person and between-person coefficients of variation were for BSS 11.2 ± 2.1 and 13.5 ± 2.4% respectively; for plasma they were 10.0 ± 2.5 and 9.0 ± 1.3% respectively.

Patients and subjects

Patients were diagnosed as having hypertension if the supine resting diastolic pressure of duplicate measurements was greater than 90 mmHg (Korotkoff phase IV) on two consecutive measurements at least 24 h apart. Dietary sodium intake was estimated by measuring the 24 h urinary sodium excretion, in urine collected on the day of the test. Renal function was determined by plasma creatinine estimation (Technicon autoanalyser) and/or by creatinine clearance.

Statistical analysis was performed by unpaired t test; probability values greater than 0.05 were considered non-significant.

Results

All results are expressed as mean ± SE.

The 22Na efflux rate constant of 21 untreated hypertensive patients (mean blood pressure 154 ± 4/101 ± 2 mmHg) was measured and compared with that of 21 age- and sex-matched normotensive controls (mean blood pressure 115 ± 3/77 ± 2 mmHg). Renal function as determined by the plasma creatinine level was similar in both groups.

No difference was found between the k, for hypertensive patients compared with normotensive subjects when the erythrocytes were incubated in BSS (0.34 ± 0.02, 0.31 ± 0.02 respectively). There was significant increase in k, when the cells from the hypertensive subjects were incubated in their own plasma compared with results from controls (0.40 ± 0.02, 0.36 ± 0.01 respectively; P < 0.05).

The 22Na efflux rate constant was also measured on 32 occasions in 20 untreated hypertensive patients on a measured dietary sodium intake. Dietary sodium was varied twice in eight of these patients and three times in two of the hypertensive patients and the sodium efflux was measured on each occasion. The 22Na efflux rate constant was measured on 34 occasions in 22 normotensive subjects on a measured dietary sodium intake. Twelve of these normotensive subjects were studied twice on different dietary sodium intakes.

Renal function (creatinine clearance 95 ± 6 ml/min for the hypertensive patients, 104 ± 5 ml/min for the normotensive subjects) and the mean and range of urinary sodium excretion per 24 h was similar between the groups (144 ± 11, range 42–307: 123 ± 11. range 21–271 mmol/24 h respectively).

There was a significant inverse correlation between urinary sodium excretion per 24 h and k, for hypertensive patients when the 22Na-loaded erythrocytes were incubated in BSS (r = -0.34, P < 0.05) and in plasma (Fig. 1: r = -0.42, P < 0.05).

However, there was no correlation between k, and urinary sodium excretion per 24 h for the normotensive controls when erythrocytes were incubated in BSS (r = +0.06, N.S.) or plasma (Fig. 1: r = +0.08, N.S.).
**Discussion**

Total rate constant for $^{22}\text{Na}$ efflux from erythrocytes incubated in artificial medium did not differ between patients with mild to moderate hypertension and controls. This finding contrasts with a previous report for sodium transport across the leucocyte cell membrane (Edmondson et al., 1975) but supports the observation reported by Postnov, Orlov, Shevchenko & Adler (1977).

The degree of hypertension may reflect the extent that sodium transport across cell membranes is altered compared with that in normotensive subjects. Garay & Meyer (1979) found that net $\text{Na}^+$ efflux from erythrocytes was significantly reduced in cases of severe hypertension compared with controls whereas erythrocyte net $\text{Na}^+$ efflux of moderate hypertensive patients was not different from control subjects.

In the present study, only mild to moderate hypertensive patients were examined.

When cells were incubated in plasma, $k_e$ was significantly elevated for the hypertensive patients compared with their normotensive controls.

This finding suggests either that plasma from hypertensive patients contains a factor which increases the efflux rate constant of sodium from erythrocytes or that a factor responsible for regulating the sodium efflux rate constant in the plasma of normotensive subjects is absent from plasma of hypertensive patients.

A significant inverse association was observed between erythrocyte $k_e$ and urinary sodium excretion per 24 h for hypertensive patients when the cells were incubated in both BSS and plasma.

No such association was observed for the normotensive subjects.

This finding suggests a role for increased dietary sodium intake in the aetiology of essential hypertension through an increase in the intracellular sodium as a consequence of a decrease in $k_e$ for sodium efflux. Hypertension could be initiated by the effect of an increased intracellular sodium content or by the rise in intracellular calcium which accompanies the increased intracellular sodium (Blaustein, 1977).

The range over which dietary sodium intake varied was partly accounted for in the age- and sex-matched hypertensive patients and control subjects by obtaining the mean of $k_e$ on different sodium diets when this was measured more than once in any individual.

It is proposed that dietary sodium intake may interact with the regulation of cellular sodium transport via both humoral and cellular mechanisms to precipitate hypertension in 'salt-sensitive' individuals.

**References**


