ABNORMALITIES of erythrocyte membrane sodium transport in patients with essential hypertension have now been reported by several investigators. Similar abnormalities have been observed in the normotensive relatives of such patients. The exact nature of the transport defect remains unclear, with conflicting results obtained from different laboratories. There are now several techniques available for assessing erythrocyte membrane sodium transport, including "cold" flux studies after preincubation of the cells with a sulphydryl inhibitor to load them with high concentrations of sodium, radioactive $^{22}$Na flux studies at more physiologic concentrations of cell sodium, and measurement of Na,K-ATPase activity in the cell membrane. We have used these three techniques to assess membrane sodium transport in four groups of subjects. Group A consisted of patients with untreated essential hypertension. Group B consisted of patients with essential hypertension whose blood pressure had been controlled by medication. Group C consisted of normotensive subjects with a family history of hypertension. Group D consisted of control subjects with no family history of hypertension.

Methods

Selection of study subjects. Nine patients with newly diagnosed untreated essential hypertension were selected for detailed studies of erythrocyte sodium transport. They ranged in age from 20 to 54 years. Diastolic blood pressure varied from 95 to 115 mm Hg (mean 106) and systolic blood pressure from 140 to 190 mm Hg (mean 156). To determine the cause of the hypertension, the following studies were performed in all patients: serum electrolytes, hemogram, urinalysis, urine culture and sensitivity, serum thyroxine, 24 hr creatinine clearance, peripheral vein renin, and hypertensive intravenous pyelogram. On the basis of these studies all patients were judged to have essential hypertension. One patient had polycystic kidney dis-
ease and one patient had a small stone in the left renal pelvis; both these patients had normal renal function. Peripheral vein renin related to the 24 hr urinary sodium excretion was not elevated in any of the patients in the study. After informed consent was obtained, the patients were asked to come to the laboratory, where blood was drawn for "cold" flux studies and erythrocytes membranes were prepared for ATPase assay. The patients returned 1 week later and had a second blood sample drawn that was used for radioactive flux studies and determination of intracellular sodium concentration. The untreated newly diagnosed hypertensive patients are referred to subsequently as group A. Group B consisted of eight patients with essential hypertension whose blood pressure was controlled with medication. All had had hypertension for more than 5 years and were being treated with a thiazide diuretic. Most were also receiving a vasodilator and/or a sympathetic blocking agent. The mean blood pressure of this group of patients at the time of the study was 134/91 mm Hg.

Group C consisted of nine subjects whose parents had a history of essential hypertension. Group D subjects were normal controls whose first-degree relatives were, as far as the subjects were aware, normotensive. Group C and D subjects were all normotensive at the time of the study.

**Radioactive flux studies.** Washed erythrocytes were loaded with $^{22}$Na by incubating them for 3 hr at 37°C in a flask containing 50 $\mu$L of 120 mM phosphate buffer (pH 7.4), 0.45 ml of 0.16 M NaCl, 10 $\mu$L of 50% glucose and 10 $\mu$L of $^{22}$Na. After the loading period the sodium content of the erythrocytes was determined on a flame photometer by lysing the cells with 50 volumes of 15 mM lithium chloride and relating the sodium content of the hemolysate to the hematocrit of the original suspension. Then, 200 $\mu$L of the erythrocytes was transferred to three pairs of flasks containing basic flux medium, basic flux medium plus 10 $^{-4}$M ouabain, or basic flux medium plus 10 $^{-4}$M ouabain and 10 $^{-3}$M ethacrynic acid. The basic flux medium consisted of 5 mM KCl, 160 mM NaCl, 1.2 mM phosphate buffer (pH 7.4), 8 mM glucose and 10% isotonic glycylglycine-MgCO$_3$ buffer (pH 7.4). A 5 ml aliquot of the erythrocyte suspension was removed at 20 min intervals between 0 and 60 min and the $^{22}$Na in the supernate was determined with a Packard auto gamma spectrometer. The total amount of sodium pumped out of the erythrocytes each hour was then determined from the formula:

$$\text{Na}^{+}_{e} - \text{Na}^{+}_{b} = \ln \left( \frac{1 - \text{Na}_{a}/\text{Na}_{b}}{1 - \text{Fractional hemolysis}} \right)$$

where $\text{Na}_{a}$ is the activity of the supernate (cpm/ml) and $\text{Na}_{b}$ is the activity of the hemolyzed sample (cpm/ml). Additional details of this technique can be found in previous publications.

**ATPase assay.** Erythrocyte membranes were prepared by an osmotic hemolysis technique. Maximal ATPase activity was determined by the release of Pi from ATP in the presence of 75 mM NaCl, 25 mM KCl, 1 mM MgCl$_2$, 0.1 mM EGTA, and 25 mM Tris (pH 7.45).

Ouabain-sensitive Na,K-activated ATPase activity was determined by adding 1 mM ouabain to the assay. The ATPase activity was related to dry weight of membrane suspension, determined on a Cahn electrobalance after 24 hr drying in a vacuum oven. Details of this technique have been published previously.

**Cold flux studies.** Net sodium and potassium fluxes after preincubation of the cells with parachloromercuriphenyl-sulfonic acid (PCMBS) were determined with a Garay technique. Twenty milliliters of fresh heparinized blood was centrifuged at 1750 g for 10 min and the plasma anduffy coat were discarded. The remaining erythrocytes were washed twice with 10 volumes of cold 150 mM NaCl; 7.5 ml of erythrocytes was incubated for 24 hr at 4°C in a loading medium that consisted of 150 mM NaCl, 1 mM MgCl$_2$, 2.5 mM NaPO$_4$ buffer (pH 7.4) and 0.02 mM PCMBS. The loading medium was replaced once during the incubation. After the loading period the erythrocytes were incubated for 1 hr at 37°C in a solution containing 2 mM adenosine, 3 mM inosine, 5.4 mM NaPO$_4$ buffer (pH 7.4), 145 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 10 mM glucose, and 4 mM neutralized cytoine to reverse the action of the PCMBS. The erythrocytes, which now contained an average of 46 mM of sodium, were centrifuged and resuspended in a flux medium consisting of 145 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 5 mM NaPO$_4$ (pH 7.4), and 10 mM glucose. Red cell sodium and potassium was measured, as outlined above, at 0, 1, 2, 3, 4, and 5 hr. The rate constant for sodium efflux and potassium influx was calculated as the slope of the line relating time to intracellular electrolyte concentration. All determinations were done in duplicate.

Studies of radioactive sodium flux, red cell sodium determination, and ATPase assay were all started within 10 min of the time the blood was drawn. Initially each patient was paired with a control, blood was drawn at the same time, and all steps in the assays were carried out in a tightly paired fashion. Subsequent analysis of the data revealed that this pairing was unnecessary and the data were unpaired with patient and control values being expressed separately. Statistical analysis of all data was performed with an unpaired Student’s t test. Results are expressed as mean ± SEM.

**Results**

The result of measuring red cell sodium in the four groups of patients is shown in figure 1. The mean cell sodium in the group A untreated hypertensive patients was 8.3 ± 1.0 mM Na/l RBCs, compared with 6.2 ± 0.4 mM Na/l RBCs in the control group. The difference was statistically significant at p < .05. There was no significant difference in cell sodium between groups B, C, or D.

The results of the $^{22}$Na efflux studies are summarized in table 1. The total net sodium efflux was 2.12 ± 0.17 mM Na/l RBCs/hr in the group A patients compared with 1.55 ± 0.12 mM Na/l RBCs/hr in group D. Total sodium efflux was partitioned into an ouabain-sensitive and ouabain-insensitive pump. Both components of sodium efflux were significantly increased in the group A patient compared with group D controls. Unlike the group A patients, the group B patients did not had a statistically significant increase in either component of sodium efflux. Adding both ethacrynic acid and ouabain to the flux medium inhibits an additional component of active sodium efflux. This component is designated ethacrynic acid–sensitive sodium efflux in table 1 and is measured by subtracting the sodium efflux in the presence of maximal inhibitory concentrations of ethacrynic acid and ouabain from the sodium efflux in the presence of ouabain alone. Ethacrynic acid–sensitive sodium efflux was the same in all four groups. Sodium efflux that persists in the presence of ethacrynic acid and ouabain (labeled residual in table 1)
ATPase activity was measured in fragmented erythrocyte membranes from the four groups of study subjects. The results are shown in table 2. The total ATPase activity was identical in group A and group D subjects. Partitioning the enzyme activity into an ouabain-sensitive and ouabain-insensitive component revealed that in the group A subjects, the Na,K-activated ouabain-sensitive ATPase activity was $7.5 \pm 1.5 \times 10^{-8}$ M Pi/mg dry wt. of membrane suspension/hr compared with $8.4 \pm 0.9 \times 10^{-8}$ M Pi/mg dry wt. of membrane suspension/hr in the group D controls. The Na,K-insensitive, ouabain-insensitive ATPase activity was $5.2 \pm 1.4 \times 10^{-8}$ M Pi/mg dry wt of membrane suspension/hr in group A subjects, and $4.4 \pm 0.6 \times 10^{-8}$ M Pi/mg dry wt. of membrane suspension/hr in group D. None of these differences was statistically significant.

Net sodium and potassium fluxes were measured in the four groups of study subjects, after preloading of the erythrocytes with high concentrations of sodium. Despite uniform loading conditions, the red cell sodium concentration varied widely at the end of the loading period from 20.4 mM/l RBCs (in a group D subject) to 76.0 mM/l RBCs (in a group A subject).

As shown in figure 2, there was a significant correlation between postload cell sodium and the rate of sodium efflux ($r = .53$, $p < .001$). The mean postload cell sodium was not significantly different in the four groups. Net sodium efflux at these high concentrations of intracellular sodium was not statistically different in any of the four groups (table 3). Calculations of net potassium influx also failed to reveal any differences between the four groups of study subjects. The net Na/K flux ratio, which Garay et al.\textsuperscript{12} reported to be reduced in patients with essential hypertension, is shown in the last column of table 3. This ratio calculated separately for each experiment averaged 1.37 $\pm$ 0.17 in the group D controls, and 1.13 $\pm$ 0.26 in the group

**TABLE 1**

<table>
<thead>
<tr>
<th>Isotopic flux studies of erythrocyte sodium transport</th>
<th>Net sodium efflux (mM Na/l RBCs/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n Total Ouabain-insensitive Ouabain-insensitive Ethacrynic acid-sensitive Residual</td>
<td></td>
</tr>
<tr>
<td>Group A 9 2.12 $\pm$ 0.17 1.38 $\pm$ 0.09 0.74 $\pm$ 0.09 0.22 $\pm$ 0.05 0.52 $\pm$ 0.06</td>
<td></td>
</tr>
<tr>
<td>Group B 8 1.68 $\pm$ 0.18 1.04 $\pm$ 0.11 0.64 $\pm$ 0.08 0.23 $\pm$ 0.04 0.41 $\pm$ 0.05</td>
<td></td>
</tr>
<tr>
<td>Group C 9 1.65 $\pm$ 0.18 1.10 $\pm$ 0.11 0.55 $\pm$ 0.03 0.23 $\pm$ 0.02 0.32 $\pm$ 0.02</td>
<td></td>
</tr>
<tr>
<td>Group D 22 1.55 $\pm$ 0.12 1.04 $\pm$ 0.07 0.51 $\pm$ 0.05 0.21 $\pm$ 0.03 0.31 $\pm$ 0.03</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistical significance compared with group D.
A patients. The difference was not statistically significant.

Discussion

Abnormalities in erythrocyte membrane sodium transport in patients with hypertension have been recognized for many years. Present interest in this topic was sparked by a series of papers by Garay et al. in which he reported a decreased net Na/K flux ratio in patients with essential hypertension but not in patients with renovascular hypertension. Similar abnormalities were observed in some of the normotensive offspring of hypertensive patients. Subsequently, decreased Na K cotransport out of the erythrocyte in patients and some of the relatives of patients with essential hypertension but an accompanying increase in net sodium extrusion was reported. It was suggested that such studies could be used to differentiate between essential and secondary hypertension and could be used to detect normotensive offspring of hypertensive parents who had a potential risk of developing hypertension. These findings have not been confirmed by all investigators, with some groups reporting an increase in Na K cotransport in patients with essential hypertension and others reporting no change.

For studies such as these to be technically feasible, it is necessary to load the erythrocytes with high concentrations of sodium by preincubation with PCMBS, a powerful sulfhydryl inhibitor that poisons the normal transport pathways of the cell. It can be argued that the observed changes in membrane transport in the erythrocytes of patients with hypertension may partly result from a differing susceptibility to sulfhydryl inhibition.

A more physiologic method of studying erythrocyte sodium transport is to use radioactive tracers, which allow sodium flux studies to be performed at approximately the same concentration of intracellular sodium as exists in vivo. With isotopic flux techniques sodium transport can be compartmentalized into ouabain-sensitive and ouabain plus ethacrynic acid–sensitive components. Hoffman has labeled the former component of sodium transport pump I, and the latter pump II. The efflux of sodium that remains in the presence of maximal inhibitory concentrations of ouabain and ethacrynic acid is thought to represent passive outward leakage. With these techniques we have shown an increase in total sodium efflux in patients with essential hypertension when compared with normal controls. The addition of inhibitors of active transport to our flux medium revealed a significant increase in both ouabain-sensitive and ouabain-insensitive components of sodium efflux. The most marked increase in sodium transport was in the efflux that remained after the addition of both ouabain and ethacrynic acid to the flux medium. This latter observation would tend to confirm the work of Mahoney et al., who found a significant increase in erythrocyte Na22 uptake in patients with essential hypertension. Fitzgibbon et al., with a slightly different technique, showed increased total sodium efflux in patients with essential hypertension.

Other investigators have measured a furosemide-sensitive ouabain-insensitive sodium-lithium countertransport system in sodium loaded erythrocytes and found this to be increased in patients with hypertension. This finding may be related to our observation of increased ouabain-insensitive Na22 efflux, although it is difficult to draw any firm conclusion on this point, since we did not measure sodium-lithium countertransport per se.

The enzyme system responsible for ouabain-sensitive sodium efflux from the erythrocyte is a ouabain-sensitive Na,K-activated ATPase. We measured the maximal activity of this enzyme system in fragmented erythrocyte membranes from patients with essential hypertension and found, in agreement with the studies of Swarts et al., that ATPase activity was normal.

![Figure 2](http://circ.ahajournals.org/)

**FIGURE 2.** Relationship between net sodium efflux, measured over a 5 hr period and intracellular sodium concentration (Nac) after 48 hr of loading in a high sodium medium (150 mM Na) containing 0.02 mM PCMBS. Groups as defined in figure 1. Regression line calculated by method of least squares.
This suggests that the increase in ouabain-sensitive sodium efflux in essential hypertension involves a change in the control mechanism for this component of the sodium pump rather than a change in the underlying enzyme activity.

A third component of our study involved measuring net sodium and potassium fluxes after sodium loading of the erythrocytes in the presence of a sulfhydryl inhibitor. Despite efforts to keep the loading conditions as uniform as possible, a wide variation in cell sodium at the end of the loading period was noted (figure 2 and table 3). Because of the strong correlation between the rate of sodium efflux and the intracellular sodium, this variation may have obscured differences in net sodium efflux between the four groups of study subjects. However, we were unable to confirm the findings of Garay et al.12

In addition to studying membrane sodium transport in patients with untreated essential hypertension, we performed similar studies in treated patients and in normal individuals with a family history of hypertension. In the latter group of individuals we were unable to demonstrate any significant changes in erythrocyte membrane transport. In most of the studies that have been done on this group, the observed changes in membrane transport were less pronounced than in patients with essential hypertension. It may have been that the techniques that we were using were not sufficiently specific to detect an abnormality in this group.

Very few studies of erythrocyte sodium transport have been done in patients with treated essential hypertension, despite the obvious clinical interest in studying this group of patients. In our study of eight hypertensive patients whose blood pressure had been controlled with medication for at least 5 years, we found that the abnormalities in membrane sodium transport, which were noted in patients with untreated essential hypertension, were not present. This suggests that treatment of hypertension reverses the underlying changes in membrane transport. All our patients were receiving a thiazide diuretic and most were receiving vasodilators and/or sympathetic blocking agents. Additional studies will be necessary to determine how quickly normalization of erythrocyte membrane sodium transport occurs and whether all antihypertensive agents have the same effect.

The interactions of the various components of sodium efflux in the cell membrane are complex and as yet poorly understood. In this study we made no effort to directly examine Na K cotransport or sodium lithium counter transport. The effect of treatment of hypertension on these two components of sodium efflux remains to be studied, as does the interaction between these components of sodium efflux and ouabain-sensitive Na-K exchange.

We thank Mrs. Florence McGrail for her expert technical assistance.

References


Table 3

Sodium and potassium fluxes after preloading with high concentration of sodium

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Postload Na⁺</th>
<th>Postload K⁺</th>
<th>Net Na⁺ efflux*</th>
<th>Net K⁺ influx*</th>
<th>Na/K flux ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>46.3 ± 4.7</td>
<td>31.7 ± 2.7</td>
<td>2.20 ± 0.37</td>
<td>2.50 ± 0.38</td>
<td>1.13 ± 0.26</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>51.1 ± 2.6</td>
<td>26.6 ± 3.3</td>
<td>2.74 ± 0.37</td>
<td>2.69 ± 0.57</td>
<td>1.25 ± 0.23</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>46.4 ± 3.4</td>
<td>33.0 ± 2.3</td>
<td>2.59 ± 0.41</td>
<td>2.39 ± 0.43</td>
<td>1.58 ± 0.39</td>
</tr>
<tr>
<td>D</td>
<td>22</td>
<td>46.1 ± 2.2</td>
<td>31.0 ± 1.6</td>
<td>2.67 ± 0.20</td>
<td>2.62 ± 0.30</td>
<td>1.37 ± 0.17</td>
</tr>
</tbody>
</table>

*Expressed as mM electrolyte/1 RBCs/hr.
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