CONCISE REPORT

Assessment of Red Cell Sodium Transport in Essential Hypertension

By John R. Mahoney, Nina L. Etkin, John D. McSwigan, and John W. Eaton

Abnormal erythrocyte Na⁺ transport has been reported in patients with essential hypertension and some first-degree relatives. The two major techniques now employed for estimating Na⁺ transport—Na⁺/Li⁺ countertransport and Na⁺/K⁺ cotransport—are rather intricate and time consuming. Furthermore, the precise nature of the transport processes being measured is not clear. We have developed a simpler, more direct technique based on measurement of 22Na⁺ accumulation by erythrocytes. 22Na⁺ uptake by red cells from patients with essential hypertension averages twice normal. Indeed, of 21 patients with essential hypertension, only 2 patients had values within the upper end of the normal range. In 12 patients with secondary hypertension and no family history of essential hypertension, erythrocyte 22Na⁺ accumulation was within normal limits. Control experiments indicate that our technique for estimating red cell 22Na⁺ uptake is highly reproducible and shows little day-to-day variation. This procedure for the assessment of erythrocyte Na⁺ transport should be useful in differential diagnosis and the presymptomatic identification of individuals genetically prone to essential hypertension.

An inherited predisposition to essential hypertension and elevated dietary Na⁺ intake are thought to be predominant factors in the genesis of this prevalent disease. Recently, Na⁺ transport has been shown to be altered in red cells from patients with essential hypertension.1 The abnormality in Na⁺ transport appears to have a genetic basis; it is present in many first-degree relatives of patients with essential hypertension and may follow an autosomal dominant mode of inheritance.3-6 In addition, the transport defect is largely absent in patients suffering from secondary hypertension,1-3 indicating that it is not acquired simply as a result of elevated blood pressure. Although the molecular basis of this alteration is as yet unclear, it may ultimately provide important diagnostic and prognostic information.

The techniques available for determining hypertension-related abnormalities in Na⁺ transport are complex, lengthy, and not well suited for clinical use.1-4 One, developed by Canessa et al.,1 relies on the measurement of Na⁺-driven efflux of Li⁺ from red cells loaded with Li⁺ during several hours' preincubation. This test, most patients with essential hypertension have Na⁺-driven Li⁺ efflux which is about twice as rapid as normal. A second test, developed by Garay and coworkers,4,7 measures the rate of Na⁺ efflux from erythrocytes preloaded with Na⁺ during incubation with the organic mercurial, p-chloromercuriphenylsulfonic acid. Subsequently, the rate of ouabain-insensitive Na⁺/K⁺ cotransport is determined. The results indicate a decreased rate of cotransport in erythrocytes from patients with essential hypertension.7

In an effort to develop a test more amenable to clinical use, we have devised a rapid, simple, and reproducible procedure for the estimation of red cell Na⁺ transport. This test successfully discriminates most patients with essential hypertension from normotensive and secondarily hypertensive individuals. In addition, it may allow the identification of individuals with a congenital predisposition to the development of hypertension.

MATERIALS AND METHODS

Patients

In all patients studied, the diagnosis of essential hypertension was based on a supine diastolic pressure >95 mm Hg (×2) and a variety of exclusionary criteria including normal renal function and normal intravenous pyelogram. The 12 patients with secondary hypertension had non-neoplastic renal diseases. Controls were defined by normal blood pressure (supine diastolic pressure >85 mm Hg) and no family history of hypertension. All subjects were white, the majority being of northern European descent. In all cases, informed consent was obtained prior to venipuncture.

Methods

Venous blood (10 ml) was collected in heparinized vacutainers. The blood was washed 3 times in a buffer comprised of 145 mM KCl, 5 mM NaCl, 10 mM Tris(hydroxymethyl)aminomethane (Tris), 10 mM morpholinopropanesulfonic acid (MOPS), 10 mM d-glucose, and 0.1 mM ouabain, pH 7.40, at 37°C. The buffy coat was removed during the washing procedure. Of washed, packed erythrocytes of known hematocrit (75 vol %), 0.5 ml was admixed with 0.5 ml of the same buffer containing 0.1 μCi of 22Na⁺ (New England Nuclear, Boston, Mass.) (final specific activity approxi-
Incubation of the cell suspension was carried out in a shaking water bath at 37°C for 30 min, at which time the cells were rapidly washed 3 times (total time ~ 9 min) in 5 volumes of ice-cold wash buffer containing 75 mM MgCl₂, 85 mM sucrose, 10 mM D-glucose, 10 mM Tris, and 10 mM MOPS, pH 7.4, at 37°C. Of washed packed cells, 0.3 ml was combined with 0.6 ml of 10% trichloroacetic acid, vortexed vigorously, and centrifuged for 5 min at 1000 g. Of the clear supernatant, 0.3 ml was removed for liquid scintillation counting, and the hematocrit of the remaining packed cells was determined. From the known specific activity of \(^{22}\)Na \(^+\) in the 1-ml incubation volume, the unidirectional \(\text{Na}^+\) influx was calculated as follows:

1. \(1000 \mu\text{l (total incubation volume)} - \mu\text{l RBC in incubation} = \text{extracellular volume (usually 600-650 } \mu\text{l)}\)
2. extracellular volume \(\times 0.005 \text{ (mmole Na}^+/\text{ml)} = \text{extracellular Na}^+ \text{ (}{\mu\text{mole)}\)
3. \(\text{total cpm }^{22}\text{Na}^+ \text{ in incubation} \div \text{extracellular Na}^+ \text{ (}{\mu\text{mole)} = \text{cpm}/\text{mole Na}^+\)
4. \(\frac{\text{cpm}/300 \mu\text{l TCA extract}}{\text{hematocrit of washed RBC/100}} \times 20 \text{ (volume and time)} = \text{cpm/ml RBC/hr}\)
5. \(\frac{\text{cpm/ml RBC/hr}}{\text{mmole Na}^+ \text{/liter RBC/hr}} = \text{mmole Na}^+ \text{/liter RBC/hr (apparent Na}^+ \text{ influx)}\)

**RESULTS**

The accumulation of \(^{22}\text{Na}^+\) by red cells is linear over a period of at least 4 hr (Fig. 1). This linearity is consistent with the fact that the internal \(^{22}\text{Na}^+\) concentration is only 1%-3% of the external \(^{22}\text{Na}^+\) concentration after a 30-min period. Therefore, the flux of \(^{22}\text{Na}^+\) is largely unidirectional and unopposed by backflux of intracellular isotope.

The results of studies of patients with essential and secondary hypertension and normal controls are shown in Fig. 2. The average difference in the rate of \(^{22}\text{Na}^+\) influx between patients with essential hypertension and normal controls is approximately twofold (\(t = 8.87; p < 0.001\)). In fact, of the 21 patients with essential hypertension, only two have values that overlap the upper range for normal controls. In contrast, patients with secondary hypertension have normal values for \(^{22}\text{Na}^+\) influx. Sixteen of the patients with essential hypertension were receiving antihypertensive medications, and 5 were not. Comparison of the mean \(^{22}\text{Na}^+\) influx values for these two groups (0.537 versus 0.507 mmole \(\text{Na}^+\)/liter RBC/hr; \(t = 0.402, p > 0.30\)) indicates that such therapy has no consistent effect on the \(^{22}\text{Na}^+\) permeability of washed erythrocytes.

The reproducibility of the \(^{22}\text{Na}^+\) influx technique was assessed through repeated determinations on labo-

![Fig. 1. Na⁺ flux in erythrocytes from a patient with essential hypertension (— — —) and a normotensive control (— — —) during 6-hr incubation. The measurement and calculation of Na⁺ accumulation were performed as described in Materials and Methods.

![Fig. 2. Comparison of erythrocyte \(^{22}\text{Na}^+\) flux values for 21 patients with essential hypertension, 12 with secondary hypertension, and 21 normal controls. The average rate of red cell Na⁺ influx for patients with essential hypertension is twice that for normal controls (0.527 ± 0.128 versus 0.286 ± 0.044 mmole \(\text{Na}^+\)/liter RBC/hr; \(t = 8.87; p < 0.001\)). The average value for patients with secondary hypertension (0.233 ± 0.060 mmole \(\text{Na}^+\)/liter RBC/hr) is close to that for normal controls.](http://www.bloodjournal.org)
ratory personnel over several months. As shown in Table 1, little temporal fluctuation occurs in either the assay itself or the values obtained for each individual.

Finally, to determine whether interindividual differences in rates of $^{22}\text{Na}^+$ influx might be related to variations in Na$^+$/Li$^+$ countertransport, we carefully measured these two parameters in a series of 5 normal controls and 2 patients with essential hypertension. These limited investigations suggest an excellent correlation between the two procedures (Fig. 3).

**DISCUSSION**

The results indicate that measurement of $^{22}\text{Na}^+$ influx is reproducible and readily identifies most patients with essential hypertension. In addition, it appears that our procedure may also detect individuals with a genetic predisposition to essential hypertension. Investigations of 34 members of three kindreds, each founded by a progenitor with essential hypertension, indicate that approximately 50% of the offspring (F$_1$ and F$_2$) have increased red cell Na$^+$ permeability. Many of these latter are not yet hypertensive (results not shown).

Our procedure for determining red cell Na$^+$ transport abnormalities that may be linked with essential hypertension compares favorably with the other published techniques.$^{3,7}$ Furthermore, the alternative approaches involve prolonged loading of erythrocytes with Li$^+$ or Na$^+$ and, in the latter procedure, altering membrane permeability with an organic mercurial. In both cases, the red cells are incubated from 3 to 20 hr before any determinations of cation transport are made. Thus, neither of the previously published techniques is easily adapted to clinical use.

It should be pointed out that the accumulation of $^{22}\text{Na}^+$ by red cells under our conditions may reflect a combination of several (ouabain-insensitive) transport processes. Nonetheless, when our procedure and that of Canessa et al.$^3$ are applied to individual blood samples, the results correlate very well. It should be noted that we have employed a K$^+$-rich buffer system in order to minimize competition between $^{22}\text{Na}^+$ and Na$^+$. This permits the use of smaller amounts of isotope, but does not alter the relative values obtained. Thus, the performance of this procedure in a Na$^+$-rich buffer or plasma also yields a twofold difference in apparent Na$^+$ influx between patients with essential hypertension and those with secondary hypertension or normotensive controls. Therefore, the differences that we report are not due to K$^+$-induced changes in membrane Na$^+$ permeability.

In sum, our assay for determining red cell Na$^+$ influx is simple, reproducible, and rapid. It requires minimal expertise, can be adapted readily by clinical laboratories, and discriminates most patients with essential hypertension from those with secondary hypertension. This technique should be of utility in the diagnosis of essential hypertension and the identification of genetically predisposed individuals.

**ACKNOWLEDGMENT**

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**REFERENCES**


Table 1. Results of Repeated Determinations of $^{22}\text{Na}^+$ Influx in Erythrocytes From Normotensive (1-5) and Hypertensive (6) Individuals

<table>
<thead>
<tr>
<th>Subject</th>
<th>$n$</th>
<th>Mean (mmole Na$^+$/liter RBC/hr)</th>
<th>Range (mmole Na$^+$/liter RBC/hr)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.214</td>
<td>0.200–0.247</td>
<td>0.013</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.235</td>
<td>0.205–0.299</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.255</td>
<td>0.214–0.280</td>
<td>0.030</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>0.318</td>
<td>0.259–0.372</td>
<td>0.041</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.265</td>
<td>0.231–0.287</td>
<td>0.022</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>0.494</td>
<td>0.466–0.539</td>
<td>0.023</td>
</tr>
</tbody>
</table>

The assay was performed the number of times indicated ($n$) over a period of 60 days.


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