Abnormal Erythrocyte Cation Transport in Primary Hypertension
Clinical and Experimental Studies
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SUMMARY Several abnormalities concerning sodium (Na+) transport in erythrocytes of essential hypertensive patients have been recently observed. An abnormal extrusion of an erythrocyte Na+ load was described in our laboratory. This defect appeared to be specific for essential hypertension since it was absent in the secondary forms of the disease. The present investigation was performed on 194 Caucasian subjects with essential hypertension or born of hypertensive parents, 86 normotensive controls, and 14 families (78 subjects) studied over two to three generations. The distribution pattern of the erythrocyte defect is compatible with the expression of a single gene transmitted according to an autosomal and dominant mode. To confirm the genetic association between the red blood cell abnormality and primary hypertension, genetically hypertensive rats were investigated in parallel to our clinical studies. A reduction in the net Na+ efflux from red blood cells was found in two varieties of genetic hypertension (SHR and H-prone-Na+-sensitive Sabra rats). The abnormality could be detected before the development of a significant hypertension. When these various rat substrains were acutely or chronically loaded with Na+ (either intraperitoneally or orally), a significant increase in erythrocyte Na+ content was observed only in those substrains having a genetic propensity to develop hypertension.

This finding, which appears to be a consequence of the reduction in net Na+ efflux, is of interest for several reasons. It confirms the existence of a close association between a genetic predisposition to develop high blood pressure and cell Na+ retention in the presence of an excess Na+ intake. It draws attention to the possible role of intracellular Na+ in the pathogenesis of primary hypertension. Of more practical importance, the abnormal Na+ handling in erythrocytes may be a genetic marker of primary hypertension.

KEY WORDS • heredity • erythrocytes • genetics • essential hypertension • sodium transport

In previous publications, we reported a defective extrusion of a Na+ load in erythrocytes from essential hypertensive patients and in some of their young normotensive offspring. This abnormality seems to be a consequence of an inherited defect in a Na+, K+ cotransport system. We also reported a diminished erythrocyte net Na+ extrusion in two strains of hypertensive rats.

In this paper, we present further clinical and experimental evidence indicating that the red blood cell defect is likely to be inheritable and transmitted in close association with primary (or essential) hypertension. In particular, we observed that erythrocytes from rats having a genetic propensity to develop hypertension are poorly tolerant to a Na+ load, which results in an increase in the intracellular Na+ concentration. Thus, an abnormal Na+ handling in erythrocytes further appears as a promising genetic marker of hypertension.

Methods and Materials
Patients
We studied 194 Caucasian subjects with essential hypertension or born of hypertensive parents, 86 normotensive controls, and 14 families (78 subjects) followed over two to three generations, characterized by a high frequency of essential hypertension. Blood pressure (BP) value was the mean of two to three measurements performed in the supine position using a mercury manometer or a Doppler device; in children, cuff bladders of adequate size were used.
Subjects over the age of 18 years were considered hypertensives when systolic BP was over 160 mm Hg and/or diastolic BP over 95 mm Hg. For those under 18 years, hypertension was recognized when the BP was over the 95th percentile of age-BP distribution of French children. The diagnosis of essential hypertension was made after routine clinical and biological investigation. All subjects were on a free diet, and none of the hypertensives had been given any antihypertensive drug before blood sampling.

Rat Hypertension

This study was performed on three groups of male rats: 1) unselected Wistar rats; 2) Okamoto/Kyoto spontaneously hypertensive rats (SHR) and male Wistar/Kyoto normotensive controls (WKY) derived from the National Institutes of Health (NIH) stock; and 3) rats supplied by the Hadassah Medical School (Jerusalem, Israel) that were divided into three subgroups: a) Sabra rats belonging to the Hebrew University Sabra strain; b) hypertension-prone rats (H); and c) hypertension-resistant rats (N). The two substrains were studied at 4 and 8-10 weeks of age. Sabra rats were associated with a 2% Na+ diet for the last 3 days. Some rats were subjected to a high Na+ diet (Na+ rats) consisting of 1% NaCl to drink for 10 days in association with a 2% Na+ diet for the last 3 days. Other rats, previously uninephrectomized, were subjected to deoxycorticosterone mepivaleate (DOC) in-jections (25 mg/kg i.m.) for 8 weeks, and received dur-

Acute Na+ Load

Rats were anesthetized with ether, and a catheter was inserted in the carotid artery. Three hours later a blood sample was taken to measure basal intraerythrocyte Na+ content. Then NaCl 2 mmoles/l 00 g body weight in a 15 ml volume was injected intra-

Human Erythrocytes

Net sodium (Na+) and potassium (K+) fluxes were measured in Na+ loaded/K+ depleted erythrocytes according to a procedure described in a previous publication. Freshly drawn venous blood was collected in heparinized tubes and centrifuged; plasma and Buffy coat were removed by aspiration. The washed erythrocytes were then suspended in the sodium-loading medium containing 0.02 mM 2,5-p-

Rat Erythrocytes

Net Na+ and K+ Fluxes

Some modifications were found to be particularly important for rat red blood cells. The composition of the loading solution was as follows (mM): NaCl 150, MgCl2 1, Na+ phosphate 2.5 (pH 7.2), PCMBS 0.02. Carefully washed (with NaCl 155 mM) erythrocytes were incubated in the loading solution during 21 hours (at 4°C) under constant agitation. The solution was changed after the first 3 hours of incubation. Erythrocytes were subsequently incubated for 1 hour at 37°C in the "recovering" solution containing (mM) NaCl 150, KCl 5, MgCl2 1, glucose 10, Na+ phosphate 3, cysteine 4, HCl adenine 2, inosine 5, Tris 3. The Na+ and K+ fluxes were measured in erythrocytes subsequently immersed in a physiological solution containing (mM) NaCl 150, KCl 5, MgCl2 1, glucose 10, Na+ phosphate 3. The times of incubation were 0, 1, 3, and 5 hours.

Erythrocyte Na+ Concentration

A method derived from that proposed in man by Smith, which ensures no loss of intracellular Na+...
ABNORMAL ERYTHROCYTE CATION TRANSPORT/De Mendonca et al.

FIGURE 1. Representation of a three-generation hypertensive family (essential hypertension). The HLA haplotypes of some members are indicated to show that there is no close relationship between the major complex of histocompatibility and hypertension. Black symbols = hypertension patients; open symbols = normotensive subjects; © = positive erythrocyte test; © = normal erythrocyte test; © = subjects not investigated.

During erythrocyte washing, was used in the present investigation. Heparinized arterial blood was centrifuged at 4°C and plasma and buffy coat subsequently discarded. Erythrocytes were then washed three times at 4°C with a solution containing 112 mM MgCl₂ and 10⁻⁴ M ouabain. Hemolysis was performed with distilled water (v/v), and erythrocyte membranes discarded after centrifugation of the sample.

The concentration of Na⁺ was determined by flame photometry on the supernatant. The number of erythrocyte per volume unit was calculated with the use of hematocrit, hemoglobin determination and also with a Coultercounter. Systolic arterial BP was recorded by tail plethysmography.

Results

Human Hypertension

The results of the population sample study are given in table 1. The frequency of positive erythrocyte subjects was influenced by the presence of hypertension in parents and reached 73% when both were hypertensives. The investigation of the 14 families indicated that hypertension was frequent (25%) in the youngest generation comprising 39 subjects with a mean age of 19 ± 2 years. Occurrence of hypertension was, as expected, more prevalent in the two older generations (mean age 51 ± 2.2 years; 39 subjects; 53% hypertensives). In the families where three consecutive generations were available for study, at least one member with a positive erythrocyte test was observed in each generation (fig. 1).

All subjects with clinical hypertension had a positive erythrocyte test, and one of their parents also had a positive test. This positive parent was usually hypertensive. However, in some rare cases (3 of 39), the positive parent had normal BP at the time of the examination but had experienced transitory episodes of hypertension in the past (fig. 2). A positive erythrocyte test was seen to occur with approximately the same frequency in males and females.

Rat Hypertension

Blood pressure values are indicated in table 2. Several observations are of interest: 1) the BP of young 4-week-old SHR was not significantly augmented as compared to WKY controls; 2) a 10-day exposure to a high Na⁺ diet did not increase BP in the adult SHR; and 3) the BP of (N) rats remained strictly normal despite considerable Na⁺ loading.

Net Na⁺ efflux was significantly reduced in SHR erythrocytes as compared to controls, both at 4 and 8–10 weeks. Net erythrocyte Na⁺ efflux was also reduced in (H) as compared to (N), WKY, and Sabra rats. After DOC administration, Na⁺ content of red blood cells increased in (Sa) and (H) rats, but remained unchanged in (N) rats (fig. 3).

After an acute Na⁺ load, a 20 to 25% increase in erythrocyte content was observed in SHR as compared to 0 to 5% in WK. The time-course variations are shown in figure 4.

<table>
<thead>
<tr>
<th>TABLE 1. Influence of Parents' Blood Pressure on the Frequency of a Positive Erythrocyte Test in Children</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>Both normotensive</td>
</tr>
<tr>
<td>No.</td>
<td>86</td>
</tr>
<tr>
<td>No. with © test</td>
<td>3</td>
</tr>
<tr>
<td>% with © test</td>
<td>3.5</td>
</tr>
</tbody>
</table>

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TABLE 2. Systolic Blood Pressure (mm Hg) in Rats Fed a High Na+ Diet Without [Na+] or in Association With DOC Administration [DOC-Na+]

<table>
<thead>
<tr>
<th>Rats</th>
<th>Control</th>
<th>Na+</th>
<th>DOC-Na+</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY-4W</td>
<td>105 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY-8W</td>
<td>125 ± 2</td>
<td>125 ± 5</td>
<td></td>
</tr>
<tr>
<td>(n = 15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR-4W</td>
<td>115 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR-8W</td>
<td>178 ± 3</td>
<td>179 ± 3</td>
<td></td>
</tr>
<tr>
<td>(n = 24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabra N</td>
<td>126 ± 2</td>
<td>123 ± 2</td>
<td>137 ± 4</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(n = 8)</td>
<td></td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Sabra H</td>
<td>120 ± 2</td>
<td>126 ± 3</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>(n = 7)</td>
<td></td>
<td>(n = 8)</td>
</tr>
<tr>
<td></td>
<td>146 ± 5</td>
<td>146 ± 5</td>
<td>171 ± 7</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(n = 8)</td>
<td></td>
<td>(n = 7)</td>
</tr>
</tbody>
</table>

WKY-4W = Wistar-Kyoto rats studied at 4 weeks; SHR-8W = spontaneously hypertensive rats studied at 8 weeks. Each value represents mean ± SEM. A similar number of 4 week (4W) and 8-10 week (8-10W) animals was used in the WKY and SHR subgroups.

TABLE 3. Absolute Values of Na+ Extrusion from Na+ Loaded - K+ Depleted Rat Erythrocytes

<table>
<thead>
<tr>
<th>Time of study</th>
<th>Japanese rats</th>
<th>Sabra rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>4 weeks</td>
<td>73 ± 7</td>
<td>39 ± 3*</td>
</tr>
<tr>
<td>8-10 weeks</td>
<td>17 ± 0.9</td>
<td>11.5 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
</tbody>
</table>

Na+ efflux in mmole 1⁻¹.h⁻¹. *p < 0.001.

Discussion

The present data show that net Na+ extrusion from Na+ loaded/K+ depleted erythrocytes is reduced both in human essential hypertension and in several varieties of genetically transmitted hypertension in the rat. It seems, therefore, that primary hypertension and the erythrocyte abnormality are closely linked. The influence of the parents' BP on the frequency of the erythrocyte abnormality, and the finding that the abnormality is observed in one or several members of each consecutive generation of hypertensive families, suggest a monogenic and dominant transmission. However, it is clear that such a conclusion can be reached only after the investigation of a larger number of subjects. The defective extrusion of an erythrocyte Na+ load shown here is the likely explanation for the slight elevation of erythrocyte Na+ concentration previously reported in some essential hypertensive patients.11,12

To avoid in vitro and possibly artificial conditions, the present investigation was extended to genetically hypertensive rats subjected in vivo to acute and chronic Na+ loading. It appears difficult to draw any conclusion on the basal erythrocyte Na+ contents, given the high interstrain or substrain differences possibly reflecting genetic variations. On the other hand, the changes in the internal Na+ content of erythrocytes induced by a chronic Na+ load only occurred in hypertensive rats or in animals presenting a genetic propensity to develop hypertension. The most striking result was the constancy of intracellular Na+ in the (N) rats in parallel with the absence of BP elevation.

Acute Na+ loading can be considered as a dynamic test associating in vivo the two phenomena of Na+ entry and Na+ extrusion which are studied separately in vitro conditions. The present study indicates that erythrocytes of genetically hypertensive rats are selectively enriched in their Na+ content after an acute Na+ load.

Primary hypertension is thus characterized by the presence of erythrocyte membrane abnormalities which lead to a poor tolerance of excess Na+ intake.
Membrane alteration appears to be genetically transmitted in close association with hypertension, drawing attention to the importance of intracellular Na\(^+\) excess. Second, these alterations allow the consideration that the erythrocyte defect ensuring a poor tolerance to a Na\(^+\) load could be a genetic marker of hypertension.

Primary hypertension of the rat results from the action of a limited number of genes, and possibly from a single one, the Ht gene.\(^1\) One may suggest, given the functional consequences of intracellular Na\(^+\) enrichment,\(^11\) that the erythrocyte membrane defect expresses the abnormal gene.

The genetic transmission of human primary (or essential) hypertension is more debatable since it has been attributed either to a monogenic\(^1\) or to a polygenic mechanism.\(^1\) This uncertainty is related to the complexity of the disease, which proceeds from interactions between genetic and multiple environmental factors. Among the latter, excess of alimentary Na\(^+\) is the best identified.\(^1\)\(^\text{ and 18}\) Our clinical investigation suggests that in human beings, as in rats, erythrocyte abnormality may indicate a genetic propensity to develop hypertension since it expresses a genetically determined tendency of intracellular Na\(^+\) enrichment with excess Na\(^+\) intake.

The results of the present investigation therefore imply that primary hypertension could be related to an inborn error of transmembrane Na\(^+\) fluxes, resulting in a poor cellular tolerance to chronic and acute Na\(^+\) load.

Acknowledgments

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References

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