Increased Rigidity of Red Blood Cell Membrane in Young Spontaneously Hypertensive Rats

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SUMMARY The micropipette test was used to study the effects of age on the elasticity of red blood cell (RBC) membrane in spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY), ranging from 3 to 23 weeks of age. The development of hypertension in the SHR started at 3 weeks and was fully established at 7 to 8 weeks. In the developmental phase of hypertension (3–5 weeks), the SHR showed a significant increase in RBC membrane elastic modulus (i.e., a decrease in RBC membrane deformability) when compared with the age-matched normotensive control rats (WKY). After the establishment of hypertension (7–8 weeks), however, the deformability of the RBC membrane of SHR improved and became comparable to that of the WKY. These results indicate that abnormal erythrocyte membrane elasticity is an early event in SHR and that adaptive recovery occurs when hypertension is fully developed. (Hypertension 10: 603–607, 1987)

Key Words • erythrocyte deformability • erythrocyte membrane elasticity • hypertension • spontaneously hypertensive rats

SPONTANEOUSLY hypertensive rats (SHR) are regarded as the best animal model of human essential hypertension. In patients with essential hypertension, blood viscosity can be correlated with both systolic and diastolic pressure. Moreover, in essential hypertensive patients with high plasma renin activity, blood viscosity is significantly elevated, and the increase has been attributed in part to reduced red blood cell (RBC) deformability. Erythrocyte filterability is reduced in subjects with untreated essential hypertension. The diameter of RBCs is generally larger than that of precapillaries or capillaries. Under hypertensive conditions with an increase in vessel tone, RBC deformability could be a limiting factor in RBC transit through the microcirculation. Therefore, the present experiments were undertaken to investigate if RBC deformability is altered in the animal model of hypertension (SHR), as determined by measuring the RBC membrane elasticity with the micropipette technique.

Materials and Methods

Preparation of Red Blood Cell Suspensions

Male SHR (Tac:N[SHR]fBR) and Wistar-Kyoto control rats (Tac:N[WKY]fBR) were obtained from Taconic Farms (Germantown, NY, USA). Three series of experiments were performed. In the first series, two age groups were studied: 4 and 8 weeks old. Ten animals of each group were killed, and the exsanguinated blood was heparinized; the organs were used for studies on calcium-binding protein. In the second series of experiments, to further document the age-related variation of RBC mechanical properties, blood was drawn through venous puncture from 10 awake SHR and 10 awake WKY and pooled into heparinized tubes. The animals were kept alive and fed a nutritionally complete pellet diet (Camm Maintenance Rodent Diet, Camm Research Institute, Wayne, NJ, USA; 0.9% Ca, 0.8% P), with water ad libitum. Blood was drawn from these animals at intervals beginning at the age of 3 weeks and continuing to 23 weeks of age. In the third series of experiments, blood was taken from anesthetized animals by heart puncture from 3 SHR and 3 WKY at 3, 5, and 7 weeks of age and organs were isolated for studies on calcium-binding protein. Systolic blood pressure (SBP) was measured by tail sphygmomanometry, after the awake animals had been warmed for 15 minutes (29°C). On each occasion, two determinations of SBP were performed and...
the values were averaged. For the micropipette test, the pooled blood was centrifuged at 1500 g for 5 minutes and the plasma and buffy coat were removed. The erythrocytes were washed three times in a standard wash buffer composed of 8 mM sodium phosphate, 145 mM NaCl, and 5 mM KCl (pH 7.4). The washed erythrocytes were resuspended in the wash buffer containing 0.0025% bovine serum albumin to yield a hematocrit of 0.01%.

The mean corpuscular volume (MCV) of RBCs was calculated from the hematocrit value determined in a microcentrifuge (15,000 g for 5 minutes) divided by the RBC count measured in an electronic counter (Model ZB, Coulter Electronics, Hialeah, FL, USA).

**Micropipette Technique**

The micropipette technique has been described elsewhere. Micropipettes with an internal radius of 0.45 to 0.70 μm were prepared with a micropipette puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan). The micropipette was filled with the buffer solution and mounted on a micromanipulator (Narishige). The wide end of the micropipette was connected to a pressure-regulating system, which consisted of two reservoir bottles and a damping chamber. By adjusting the relative heights of the reservoir bottles with a micrometer device, desired pressure levels were preset and then imposed on the micropipette by turning a stopcock. The applied pressure was measured with a transducer (Model 23 BC, Statham Instruments, Oxnard, CA, USA) and recorded with an amplifier-recorder system (Gould, Cleveland, OH, USA). A suspension of erythrocytes at a hematocrit level of approximately 0.01% was placed in a small round chamber located on the stage of a Nikon inverted microscope (Ehrenreich Photo-Optical, Garden City, NY, USA). The erythrocytes were viewed with the use of a 100 x objective and a 20 x eyepiece. The image was recorded with a video camera and a tape recorder system (Panasonic, division of Matsuchita Electric Corp. of America, Franklin Park, IL, USA). The micropipette tip was manipulated for positioning at the surface of the erythrocyte membrane. A small portion of the erythrocyte was aspirated by a preset negative pressure for 20 seconds. The length of the aspirated tongue and the radius of the pipette were measured on a television screen. The membrane elastic modulus, which reflects the steady state resistance to deformation, was calculated from the relationship between the stress applied, \((\Delta P)R_p\), and the strain induced, \(D_m/R_p\), where \(\Delta P\) is the applied negative pressure, \(R_p\) is the internal radius of the micropipette, and \(D_m\) is the maximum length of the aspirated portion within the micropipette. When the aspiration pressure was removed, the deformed erythrocyte segment in the micropipette decreased in length with time and the cell recovered its original shape. All measurements were made at room temperature (21-24°C). For each experiment the same micropipette was used to test RBCs from SHR and WKY. Statistical analysis of the data was performed using a Wilcoxon rank sum test and a paired Student's t test.

**Results**

The increase in SBP with age is shown in Figure 1. These data were obtained from the same sets of animals studied over a 20-week period starting at the age of 3 weeks. The only test performed on the rats was drawing 0.2 to 0.8 ml of blood from tail vein puncture at intervals. The SBP was always higher in SHR than in WKY, and the difference was already significant at 5 weeks of age \((p<0.005)\). After the sharp initial rise in pressure over the first 10 weeks, the blood pressure increased more slowly thereafter.

The variation in MCV is shown in Figure 2. MCV declined with age in both WKY and SHR. Both sets of animals exhibited a sharp initial decrease in MCV until the age of 9 weeks, followed by a plateau. The MCV of the erythrocytes from SHR was always smaller than that from the WKY (from 3 to 10 weeks of age; \(p<0.05\)).
are the means of two or three experiments performed with different sets of rats on different dates. For each of these experiments the results exhibited the same trend as that of the means (Figure 3). Comparisons of the values for the elastic modulus between SHR and WKY were made at each age group. In Figure 4 the ratio of the elastic modulus for the erythrocyte membrane of SHR to the value of the corresponding WKY control is plotted against the age of the rats. Comparison of these ratios not only facilitates the assessment of the difference between SHR and WKY at different ages but also minimizes any possible experimental errors (e.g., those due to variations in the radius of the pipette used in the different experiments), since the same pipette was used for individual experiments on both groups. The results of Figure 4 show that the membranes of erythrocytes from the young SHR were more rigid than those of the age-matched WKY and that this difference disappeared at about 7 weeks of age.

**Discussion**

No consensus exists as to the cause and effect relationship between the elevation in blood pressure and the associated rheological and microcirculatory disturbances during the evolution of the hypertensive syndrome. Blood and plasma viscosities are increased in patients with essential hypertension, even in the early phase of borderline elevation of arterial pressure. In the animal model of hypertension, the SHR, marked cardiovascular alterations occur in the neonatal stage. These findings suggest that changes begin in the prehypertensive stage and that some genetic factor may be involved. Our finding of a significant increase of RBC membrane rigidity in the SHR before the definitive establishment of the hypertension indicates that a change in the membrane of the erythrocyte presages the established hypertension. The viscoelasticity of the RBC membrane is determined mainly by the protein skeleton lining the cytoplasmic side of the membrane. We did not find any significant difference in the RBC membrane protein profile between SHR and WKY, as determined by sodium dodecylsulfate gel electrophoresis of the RBC membrane (data not shown). Thus, proteins of the membrane skeleton are present in equal amounts in SHR and WKY. The major protein of the RBC membrane skeleton is spectrin, and the state of spectrin self-association appears to be of importance for the viscoelasticity of the membrane. We therefore used nondenaturing gel electrophoresis to determine the state of protein oligomerization in the membrane. We did not find any difference between SHR and WKY at ages 3, 5, 7, and 23 weeks (data not shown). Abnormalities of calcium binding to the RBC membrane have been detected in the SHR. A number of calcium abnormalities have been observed in the plasma membrane of various cell types, even in young animals before the rise in blood pressure. These changes may increase the amount of ionized calcium available for cross-linking of membrane proteins and consequently influence the rigidity of the RBC membrane skeleton.

In our study the SHR, in comparison with WKY, had a smaller MCV and a greater RBC membrane rigidity prior to 7 to 9 weeks of age, the developmental stage of arterial hypertension (see Figures 1–4), sug-

### Table 1. Elastic Modulus of RBC Membrane

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Elastic modulus (10^-3 dyn/cm)</th>
<th>No. of cells</th>
<th>SHR/WKY ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.5±0.8</td>
<td>20</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>4</td>
<td>5.0±0.3</td>
<td>28</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>5</td>
<td>4.8±0.3</td>
<td>26</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>7</td>
<td>7.3±0.6</td>
<td>22</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>8</td>
<td>5.1±0.3</td>
<td>20</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>10</td>
<td>3.6±0.2</td>
<td>15</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>14</td>
<td>4.3±0.5</td>
<td>12</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>19</td>
<td>5.2±0.5</td>
<td>10</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>23</td>
<td>2.8±0.3</td>
<td>12</td>
<td>0.9±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.05, compared with values for WKY (by Wilcoxon rank sum test).
FIGURE 4. Membrane elastic modulus of erythrocytes from SHR versus age. The elastic modulus is expressed as the ratio of the value for SHR to that for the normotensive WKY.

suggesting an association between these parameters. Because of their greater rigidity, the RBCs from the SHR may have to exit from the bone marrow at a smaller MCV. This early abnormal membrane rigidity may also result in an abnormal blood rheology and may account for the ventricular hypertrophy present in SHR at a very early age. In human essential hypertension, the left ventricular mass has been found to be significantly correlated with blood viscosity. The increased rigidity of the RBC membrane of SHR does not seem to affect the survival of the cells, since the RBC half-life survival time is identical for SHR and WKY.

Our finding provides new evidence in support of a genetic predisposition in the SHR. The later recovery of RBC membrane elasticity may be an adaptive process similar to that found for arteriolar distensibility. Karr-Dullien et al. found an enhanced distensibility of the arterioles of newborn SHR, whereas others have observed a decreased distensibility in adult SHR. Karr-Dullien et al. also proposed that the early increase in vessel wall distensibility may lead to arteriolar wall thickening (adaptive response), which later results in decreased distensibility. An increased activity of the sympathetic nervous system has been observed in young SHR, as shown in particular by the elevation of the plasma level of dopamine-β-hydroxylase (DβH). In contrast, the plasma level of DβH is identical in 14-week-old SHR and WKY. Although the age-dependent release of DβH in the plasma parallels the change in RBC membrane rigidity, the effect of DβH, or other factors, on RBC rigidity remains to be investigated.

Our results show the importance of studying the RBC membrane in the early stage of hypertension. By contributing to the increase in vascular resistance, an abnormal RBC membrane deformability may be a cause of the initial increase in blood pressure. At a later stage, however, the RBC membrane deformability is normalized and does not continue to contribute to the sustained elevation of arterial pressure.

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References

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