Age-related Changes in Membrane Fluidity of Erythrocytes in Essential Hypertension
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In the present study, age- and calcium-related changes in the membrane fluidity of erythrocytes were examined in patients with essential hypertension by use of electron spin resonance method (ESR).

The erythrocytes were obtained from patients with essential hypertension. We examined the ESR spectra for a fatty acid spin label agent (5-nitroxy stearate) incorporated into the erythrocyte membranes. The values of outer hyperfine splitting and order parameter (S) were significantly higher in subjects with essential hypertension than in the normotensive subjects. This finding indicates that the membrane fluidity of erythrocytes was lower in essential hypertension. Calcium-loading of erythrocytes with the Ca-ionophore A23187 decreased the membrane fluidity (S value was increased) more strongly in essential hypertension than in the normotensive subjects. Furthermore, this Ca-induced change in membrane fluidity was significantly correlated with age in essential hypertension. These results demonstrate that the membrane fluidity of erythrocytes is markedly decreased by calcium, especially in essential hypertension in older patients. This suggests an increased calcium-sensitivity of cell membranes in the aged hypertensive patient. Am J Hypertens 1990;3:714-716

KEY WORDS: membrane fluidity, electron spin resonance, erythrocytes, essential hypertension, age, calcium.

It has been proposed that abnormalities of cell membranes are an etiological factor in hypertension. Such changes are not only functional abnormalities, such as transmembrane ionic transport, but also structural abnormalities. Montenay-Garestier et al. reported that the viscosity of erythrocyte membranes was increased in spontaneously hypertensive rats (SHR). In a clinical study, it was observed that the rigidity of erythrocyte membranes was increased in patients with essential hypertension, but not in patients with renal hypertension.

Electron spin resonance (ESR) spectroscopy and spin-labelling techniques have been used recently to probe the structures of cell membranes. Gulak et al. have observed the ESR spectra of erythrocyte membranes for a maleimide spin-label agent, and reported that the protein structure of erythrocytes might be changed in SHR. We have also found that the membrane fluidity of erythrocytes was lower in SHR and in patients with essential hypertension by using a fatty acid spin-label agent (5-nitroxy stearate).

In the present study, in order to obtain further insights into membrane abnormalities in hypertension we have examined age- and calcium-related changes in the membrane fluidity of erythrocytes in patients with essential hypertension by means of the ESR method.

MATERIALS AND METHODS
Thirty-five patients with untreated essential hypertension (mild to moderate, aged 48.1 ± 1.7 years old, blood pressure 160.0 ± 3.5/99 ± 3.2 mm Hg, mean
subjects (aged 44.0 ± 1.8 years old, blood pressure 126.3 ± 3.4/77.9 ± 2.3 mm Hg, n = 9).

Blood sampling was performed by venipuncture after at least 30 min of bed rest. After plasma and buffy coat were carefully removed by centrifugation, washed erythrocytes were resuspended in the isotonic buffer (140 mmol/L NaCl, 20 mmol/L Tris-HCl, pH 7.4) at a hematocrit of 50%. A solution containing a fatty acid spin label agent (5-nitroxy stearate: 5 × 10⁻⁵ mmol/L) (200 μL) was added to 400 μL of erythrocytes, and the mixed solution was then incubated for 2 h at 37°C with gentle shaking.

The ESR measurements were performed using an ESR spectrometer (Nihon Denshi, Model JEOL JES-FE2XG, Tokyo, Japan) with a microwave control unit (Model JEOL ES-SCXA). The microwave power was 5 mW, and the modulation frequency was 100 kHz with an amplitude of 2.0 gauss. The temperature of the measurement was controlled at 30°C. The receiver scan width was 3280 ± 50 gauss, and the sweep time was 8 min. The receiver gain was 4 × 10³ to 7.9 × 10³ with a response time of 1.0 sec.

To examine the calcium-related changes in membrane fluidity, erythrocytes were preincubated with Ca-ionophore A 23187 (0.9 μmol/L) and CaCl₂ (1.0 mmol/L) for 30 min at 37°C. Then, 200 μL of spin-label–containing solution were added. The ESR spectra were obtained by the same procedures as described above.

All values were expressed as mean ± SEM. Statistical significances were determined by paired or unpaired Student’s t test. A value of P < .05 was considered significant.

RESULTS

For the indicators of the membrane fluidity, we evaluated the values of outer and inner hyperfine splitting (2T'∥, 2T'⊥ in gauss) in each ESR spectrum, and calculated the order parameter (S) from 2T'∥∥ and 2T'∥⊥. The greater values of 2T'∥ and S mean the lesser membrane fluidity.

The values of 2T'∥ and S of the ESR spectra were significantly higher in patients with essential hypertension than in the normotensive subjects (2T'∥: EH 58.20 ± 0.27 gauss, n = 35; NT 56.41 ± 0.25 gauss, n = 9, P < .05; S: EH 0.737 ± 0.003, n = 35; NT 0.695 ± 0.016, n = 9, P < .01). This finding shows that the membrane fluidity of erythrocytes was decreased in essential hypertension. Furthermore, this calcium-induced change was significantly correlated with age in essential hypertension (r = 0.44, n = 35, P < .05) (Figure 1). In normotensive subjects, there was no correlation between age and the Ca-induced change in membrane fluidity of erythrocytes.

DISCUSSION

Our previous reports showed that membrane fluidity of erythrocytes might be decreased in SHR and in patients with essential hypertension. The present study confirms the hypothesis and suggests that cell membranes might be stiffer and less fluid in essential hypertension. In addition, we observed that the fluidity in patients with secondary hypertension was not different from that in normotensive subjects. Thus, it seems likely that the lower membrane fluidity might be a genetically determined abnormality of essential hypertension.

It is well known that membrane fluidity is influenced by the nature of lipid composition, water content or divalent cations. Especially, it has been recognized that calcium strongly decreases the membrane fluidity. We have already reported that treatment of erythrocytes with Ca-ionophore (A 23187) reduced the erythrocyte membrane fluidity, and that the change was more pronounced in essential hypertension than in normotensive subjects. Furthermore, the fluidity response to Ca was blocked by Ca-antagonists, such as verapamil and diltiazem. In the present study, the Ca-induced decrease in membrane fluidity of erythrocytes (Δ increase in S value in ESR spectra) was significantly correlated with age in essential hypertension. This result may indicate that the Ca-sensitivity of the cell membranes was enhanced in elderly patients with essential hypertension. Previously, it was shown that the decrease in membrane fluidity by Ca-loading was prominent in patients with the low renin type of essential hypertension. Elderly patients with essential hypertension, in general, tend to have lower plasma renin activity, which may partially explain the finding that the fluidity response by Ca-loading was pronounced in elderly and/or low renin patients. Müller et al have reported that Ca-antagonists are more effective in lowering blood pressure in aged and low renin essential hypertensive patients, and proposed the Ca-abnormalities in these patients. Thus, it is highly likely that abnormalities in the Ca-handling of the cell membranes have a crucial role in the pathogenesis of essential hypertension in the elderly and patients with low renin.

The lower membrane fluidity of erythrocytes may be a genetically determined change in essential hypertension, although the fluidity is also influenced by the salt intake of the patients. Further studies are required to...
determine the factors modulating the membrane fluidity in hypertension.

In summary, the present results demonstrate that the decrease in membrane fluidity of erythrocytes by Ca-loading was pronounced in elderly patients with essential hypertension, suggesting the increased Ca-sensitivity of the cell membranes in senile hypertension.

REFERENCES


