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MEMBRANE FLUIDITY OF ERYTHROCYTES AND ITS MODULATION BY OUABAIN IN ESSENTIAL HYPERTENSION –AN ELECTRON PARAMAGNETIC RESONANCE STUDY–

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The purpose of the present study was to examine alterations in membrane fluidity of erythrocytes in essential hypertension by means of an electron paramagnetic resonance (EPR) and a spin labeling method. In addition, we investigated the effects of ouabain on the fluidity of erythrocytes, and elucidated a possible role of Na^+, K^+-ATPase in the regulation of membrane fluidity in hypertension. Erythrocytes obtained from patients with essential hypertension were examined compared with those from age-matched normotensive subjects. The EPR spectra for 5-nitroxide stearate incorporated into erythrocyte membranes were studied.

The values of the outer hyperfine splitting and order parameter (S) of the EPR spectra were significantly higher in patients with essential hypertension than in normotensive subjects. This finding shows that the membrane fluidity of erythrocytes might be lower in essential hypertension. Ouabain-loading of erythrocytes decreased the membrane fluidity (S value was increased). The ouabain-induced changes were significantly greater in essential hypertension than in normotensive subjects.

These results demonstrate that the membrane fluidity of erythrocytes might be lower in essential hypertension than in normotensive subjects. Furthermore, the membrane fluidity might be highly dependent on the Na^+, K^+-ATPase activity in essential hypertension, which would suggest an abnormality in Na^+-related cellular functions in hypertension.

KEY WORDS: Membrane fluidity, erythrocytes, essential hypertension, electron spin resonance, spin labeling, ouabain.

INTRODUCTION

Much evidence has indicated that biochemical and biophysical abnormalities of cell membranes are important factors in the pathogenesis of hypertension[1,2]. The abnormalities appear to be involved not only in vascular smooth muscle cells but also in circulating blood cells such as erythrocytes, lymphocytes and platelets. As well as functional abnormalities of electrolyte-handling, structural and physical properties of the cell membranes have been studied in hypertension. It has been shown that the viscosity and rigidity of erythrocyte membranes were increased in spontaneously hypertensive rats (SHR) and in patients with essential hypertension[3,4]. Recently, electron paramagnetic resonance (EPR) spectrometry and spin-labeling techniques have been used to assess the physicochemical properties of

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the cell membranes and perturbations of the membranes by external agents[5-9]. There are suggestions that the membrane fluidity can be modified by various factors, eg, calcium, sodium and their pump activity on the membranes[10]. To gain further information concerning the membrane abnormalities in hypertension, we examined alterations in the membrane fluidity of erythrocytes in patients with essential hypertension. Furthermore, we studied the effects of ouabain on erythrocyte membrane fluidity, and elucidated the possible role of Na^+, K^+-ATPase activity in the regulation of membrane fluidity in hypertension.

MATERIALS AND METHODS

Eighty-six patients with untreated essential hypertension (male 43, female 43, aged 52.5 ± 1.4 years old, blood pressure after 30 min bed-rest 162.8 ± 2.2/97.0 ± 1.2 mmHg, mean ± SEM) were studied in comparison with age-matched normotensive subjects (male 22, female 15, aged 52.8 ± 2.7 years old, $n = 37$, blood pressure 120.7 ± 2.7/72.7 ± 2.2 mmHg).

All hypertensive patients had stage I or II hypertension (World Health Organization classification) and they had no medication at least 2 weeks before the study. The level of serum protein, cholesterol, triglyceride, sodium, and potassium and other routine laboratory findings were similar in the hypertensive subjects and the normotensive subjects.

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 modified Ringer-Locke solution (mmol/L: NaCl 120.7, KCl 5.9, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 15.5, NaH_2PO_4 1.2 and glucose 11.5, pH 7.4, 37°C) at a hematocrit of 50%. The buffer (50 μl) containing ouabain (2×10^{-5} mol/L) or vehicle was added to the 200 μl of erythrocyte suspension, which was incubated for 5 hours at 37°C. Then, a solution containing a fatty acid spin label agent (5-nitroxide stearate: 5×10^{-5} mol/L; 100 μl) was added to the erythrocyte suspension, and the mixed solution was incubated for 2 hours at 37°C with gentle shaking.

The EPR measurements were performed using an EPR spectrometer (Nihon Kohden, 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 (G). The temperature of the measurement was controlled at 30°C or 37°C. The receiver scan width was 3280 ± 50 G, and the sweep time was 8 min. The receiver gain was 4×10^3 to 7.9×10^3 with a response time of 1.0 sec.

For indicators of the membrane fluidity, we evaluated the values of outer and inner hyperfine splitting ($2T'_{\parallel}$ and $2T'_{\perp}$ in G) from each EPR spectrum, and calculated the order parameter (S) from $2T'_{\parallel}$ and $2T'_{\perp}$[5,6]. The greater values of $2T'_{\parallel}$ and S mean the lesser membrane fluidity[5,6].

Drugs

The spin label agent, 5-nitroxide stearate, was purchased from Syva Associates (Palo Alto, CA, USA) and kept as a stock solution of 0.01 mol/L in 99.5% ethanol. The label agent was diluted in NaCl-Tris buffer just before the experiment

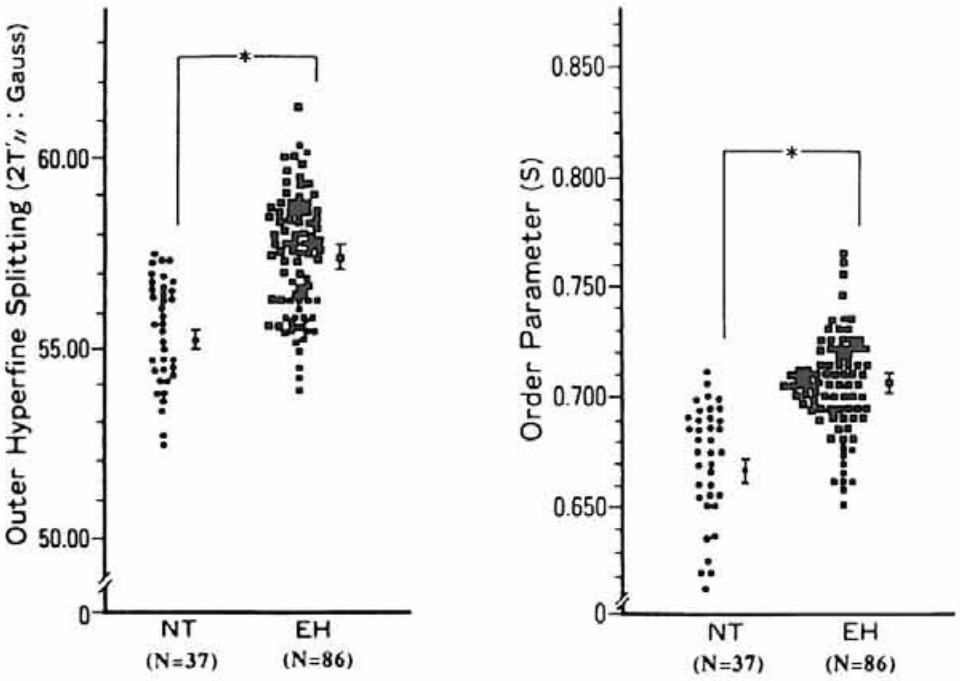

FIGURE 1 Electron paramagnetic resonance spectrum parameters for 5-nitroxide stearate (at 37°C) in hypertensive patients and normotensive subjects (EH: patients with essential hypertension, NT: normotensive subjects, mean ± SEM, * $P < 0.05$).

(5×10^{-5} mol/L). All other drugs used were standard laboratory reagents of analytical grade.

Statistics

All values were expressed as mean ± SEM. Statistical significances were determined by Student's *t*-test. A *p* value less than 0.05 was considered significant.

RESULTS

Membrane Fluidity of Erythrocytes in Essential Hypertension

As shown in Figure 1, the values of $2T'_{\parallel}$ and of S obtained from the EPR spectra were significantly higher in erythrocytes of essential hypertension than in erythrocytes of normotensive subjects ($2T'_{\parallel}$ at 37°C, EH 57.50 ± 0.16 G, $n = 86$, NT 55.47 ± 0.22 G, $n = 37$, $P < 0.05$, $2T'_{\parallel}$ at 30°C, EH 58.95 ± 0.43 G, $n = 16$, NT 57.22 ± 0.37 G, $n = 15$, $P < 0.05$; S value at 37°C, EH 0.707 ± 0.002, $n = 86$, NT 0.668 ± 0.005, $n = 37$, $P < 0.05$, S value at 30°C, EH 0.735 ± 0.009, $n = 16$, NT 0.708 ± 0.005, $n = 15$, $P < 0.05$). The results show that the erythrocyte membrane fluidity might be lower in patients with essential hypertension than in

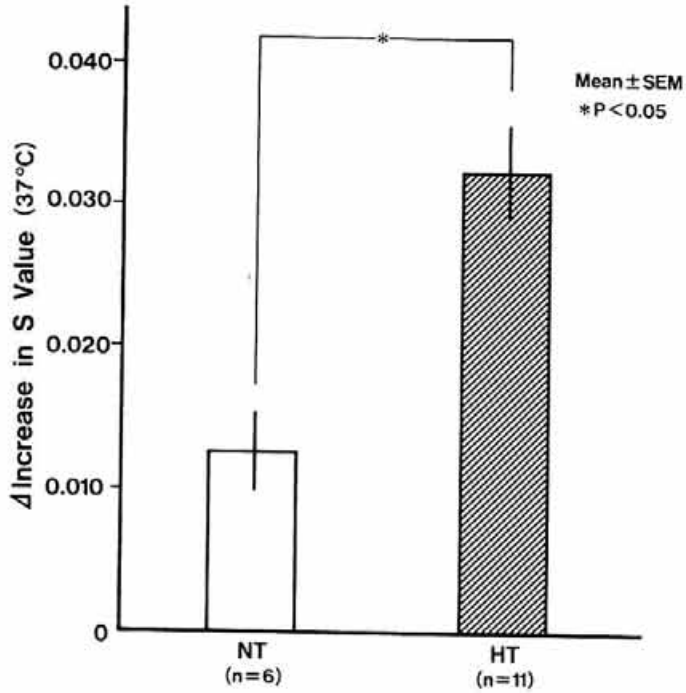

FIGURE 2 Effects of ouabain on membrane fluidity of erythrocytes (S value in EPR spectra at 37°C) in patients with hypertension (HT) and normotensive subjects (NT).

normotensive subjects. Additionally, there are no significant sex-differences in the fluidity both in the hypertensive patients and normotensive controls.

Effects of Ouabain on Membrane Fluidity of Erythrocytes in Essential Hypertension

When ouabain was loaded into erythrocytes, the order parameter (S) of the EPR spectra was increased both in patients with essential hypertension and normotensive subjects (Figure 2). The finding indicates that the membrane fluidity of erythrocytes was decreased by ouabain application. The ouabain-induced change was significantly greater in essential hypertension than in normotensive subjects (changes in S value: 37°C, EH 0.032 ± 0.004, $n = 11$, NT 0.012 ± 0.003, $n = 6$, $P < 0.05$, 30°C, EH 0.033 ± 0.004, $n = 16$, NT 0.006 ± 0.003, $n = 12$, $P < 0.05$). Furthermore, the change was pronounced in older patients (over 60 years old) with essential hypertension (Figure 3).

DISCUSSION

The present results demonstrate that the erythrocyte membrane fluidity was decreased in patients with essential hypertension compared with normotensive

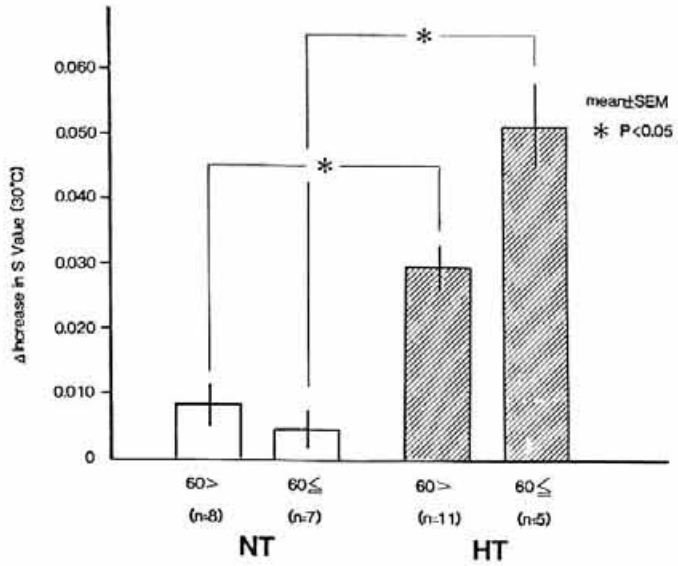

FIGURE 3 Effects of aging on ouabain-induced changes in membrane fluidity of erythrocytes in patients with essential hypertension (Data were obtained at 30°C).

subjects. The finding suggests that the cell membrane was stiffer and less fluid in hypertension.

It has been proposed that the fluidity could be, at least in part, affected by the Na^+, K^+-ATPase activity on the membranes[10]. Our study showed that ouabain, a potent Na^+, K^+-ATPase inhibitor, decreased the membrane fluidity of erythrocytes. Furthermore, the change was more pronounced in patients with essential hypertension, particularly in aged patients than in normotensive control subjects. Although the precise mechanisms responsible for the fluidity changes by ouabain remain unclear, the results suggest that the membrane fluidity might be highly dependent on Na^+, K^+-ATPase activity in essential hypertension. Previous studies reported that the Na^+, K^+-ATPase activity was increased in erythrocyte ghost of essential hypertensive patients[11]. We also demonstrated that the sensitivity to ouabain was increased in the mesenteric vasculatures of SHR[12]. These observations would be consistent with the present finding that the Na^+, K^+-ATPase activity might be enhanced in hypertension, which might suggest that alterations in sodium-metabolism at cellular levels have a possible role in the regulation of membrane fluidity in hypertension.

It has been reported that the changes in cholesterol-phospholipid ratio of erythrocyte membranes could affect membrane fluidity as well as contransport of Na^+ and K^+ membrane permeability to several substances, or the diffusion of substances across the membranes[13]. It was also shown that cholesterol content of erythrocyte membranes was less in SHR[14]. Naftilan *et al.* studied the lipid component of platelet membranes in patients with essential hypertension, and reported that the membranes in hypertension contained less linoleic acid than in normotensive subjects[15]. Thus, it is possible that abnormalities in lipid composition might cause the changes in the Na^+, K^+-ATPase activity in hypertension.

Recently, much evidence has been accumulated that endogenous digitalis-like factor (DLF) might contribute, at least partially, to elevating blood pressure by inhibiting Na^+, K^+-ATPase in some hypertensive patients[16]. Such an endogenous compound may serve as a specific modulator of the sodium-pump activity of the membranes, and may be implicated in the regulation of sodium-balance in the cells. Hamlyn *et al.* have reported that the circulating DLF level was significantly greater in essential hypertension than in normotensive control subjects[16]. The enhanced sensitivity to ouabain shown in this study might represent that Na^+, K^+-ATPase activity could be largely affected by DLF in essential hypertension. Further studies should be required to determine more precise role of Na^+, K^+-ATPase in the regulation of membrane fluidity in hypertension.

In summary, the results of the present study show that the erythrocyte membrane fluidity was lower in patients with essential hypertension. Ouabain decreased the membrane fluidity of erythrocytes more markedly in patients with essential hypertension than in normotensive subjects, which might suggest that Na^+, K^+-ATPase activity has a crucial role in the regulation of physicochemical properties of the cell membranes in hypertension.

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