Hyperinsulinemia Is a Determinant of Membrane Fluidity of Erythrocytes in Essential Hypertension

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In the present study, to determine a possible role of insulin in the regulation of membrane functions, we have examined the relationship between plasma insulin level and membrane fluidity of erythrocytes in patients with essential hypertension and normotensive subjects. Membrane fluidity of erythrocytes obtained from hypertensive and normotensive subjects were evaluated by means of an electron paramagnetic resonance and spin-labeling method. The order parameter (S for 5-nitroxide stearate) and the peak height ratio (h₀/h⁻¹ for 16-nitroxide stearate) obtained from electron paramagnetic resonance spectra of erythrocyte membranes were significantly higher in patients with essential hypertension than in normotensive subjects. The finding indicated that the erythrocyte membrane fluidity was lower in essential hypertension than in normotensive controls. The plasma concentration of insulin while fasting was also significantly greater in hypertensive patients than in normotensive subjects. In addition, the plasma insulin level was significantly correlated with the values of the order parameter (S) and the peak height ratio (h₀/h⁻¹), which showed that the higher plasma insulin was associated with the lower membrane fluidity of erythrocytes. These results support the hypothesis that insulin may actively participate in the regulation of membrane fluidity of erythrocytes in essential hypertension. Am J Hypertens 2001;14:419–423 © 2001 American Journal of Hypertension, Ltd.

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Recent studies have shown that hyperinsulinemia may be associated with hypertension. It was demonstrated that fasting and postprandial insulin levels were significantly higher in patients with essential hypertension than in normotensive subjects. However, the role of insulin in the pathogenesis of hypertension is not fully understood. With regard to the relationship between insulin and membrane function, it was shown that insulin influenced several transmembrane ionic transport systems, including the Ca²⁺-ATPase, the Na⁺-Ca²⁺ exchange systems, the Na⁺,K⁺-ATPase, and the Na⁺,H⁺ transport systems.

In addition, insulin itself has primary direct cellular ionic actions to increase intracellular Ca²⁺ and Mg²⁺, not only in vascular smooth muscle cells, but also in platelets and erythrocytes.

It has been proposed that cell membrane abnormalities are an etiologic factor in hypertension. Recently, an electron paramagnetic resonance (EPR) and spin-labeling method have been developed to elucidate the membrane fluidity and perturbations of the membrane function by external agents. The membrane fluidity is a physicochemical feature of biomembranes, and has an important role in modulating cell functions such as rheologic behavior and membrane microviscosity. Using the EPR method our previous studies showed that in an in vitro study insulin decreased the membrane fluidity of erythrocyte, which indicated that insulin may increase the membrane microviscosity. In the present study, we have examined the relationship between plasma insulin level and membrane fluidity of erythrocytes and further determined the possible role of insulin in the regulation of membrane functions in patients with essential hypertension by means of the EPR method.
Methods

Subjects

Sixteen patients with untreated essential hypertension were studied and compared with 17 age-matched normotensive control subjects (Table 1). Consent was obtained from all participants after the nature and objective of the study was explained. The hypertensive group consisted of borderline and mild hypertensive patients and they had taken no medication for at least 2 weeks before the study. They had no other diseases such as hematologic or hepatic disorders. Blood sampling was performed by venipuncture after a minimum of 30 min of bed rest while fasting. After plasma and buffy coat were carefully removed by centrifugation at 155 g for 10 min at 4°C, 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%. One hundred microliters of the solution containing fatty acid spin-label agents (5-nitroxide stearate [5-NS] and 16-nitroxide stearate [16-NS], $5 \times 10^{-5}$ mol/L) was added to 200 μL of erythrocyte suspension, and the mixed solution was then incubated for 2 hours at 37°C with gentle shaking.

The plasma insulin was measured by radioimmunoassay, and other routine laboratory findings were obtained at the same time as the erythrocyte sample collection. In our assay system of insulin, the cross-reactivity with proinsulin was approximately 15%.

EPR Measurements

The EPR measurements were performed using an EPR spectrometer (model Jeol JES-FE2XG, Nihon Denshi, Tokyo, Japan) with a microwave unit (model Jeol ES-SCXA, Nihon Denshi) as described previously. The microwave power was 5 mW, and the modulation frequency was 100 KHz with a modulation amplitude of 2.0 gauss. The temperature of the measurement was controlled at 30°C. The receiver scan width was 3280 ± 50 gauss with a sweep time of 8 min, and receiver gain was 4.0 to 7.9 ± 10^3 with a response time of 1.0 sec. The fatty acid spin-label agents are believed to be anchored at the lipid–aqueous interface of the cell membranes by its carboxyl end, whereas the nitroxide group moves rapidly through a restricted angle around the point of attachment. Therefore, the EPR spectra of the fatty acid spin-label agents are used to detect an alteration in the freedom of motion in biological membranes and to provide an indication of membrane fluidity.

Drugs

The spin-label agents 5-NS and 16-NS were purchased from Aldrich Co., Ltd. (Milwaukee, WI). All other drugs used were standard laboratory reagents of analytical grade.

Statistics

Values are expressed as mean ± SEM. The differences between hypertensive and normotensive subjects were an-
alyzed by the Mann-Whitney U test. Linear regression analysis was used to identify the correlation between plasma insulin level and membrane fluidity indexes of erythrocytes. A $P$ value less than .05 was accepted as the level of significance.

**Results**

**Membrane Fluidity of Erythrocytes and Plasma Insulin Level in Patients With Essential Hypertension and Normotensive Subjects**

The values of the order parameter (S) and the peak height ratio ($ho/h-1$) of the EPR spectra were significantly greater in patients with essential hypertension (HT) than in normotensive subjects (NT) (S value, HT $0.716 \pm 0.005$, $n = 16$, NT $0.698 \pm 0.004$, $n = 17$, $P < .05$; $ho/h-1$ value, HT $5.30 \pm 0.09$, $n = 16$, NT $5.02 \pm 0.08$, $n = 17$, $P < .05$).

The findings indicated that the erythrocyte membrane fluidity was decreased in patients with essential hypertension when compared with normotensive subjects. The fasting plasma insulin level was significantly higher in patients with essential hypertension than in normotensive subjects (HT $9.1 \pm 1.0 \mu U/mL$, $n = 16$, NT $5.6 \pm 0.5 \mu U/mL$, $n = 17$, $P < .05$), although other routine laboratory findings were not different between hypertensive and normotensive subjects (Table 1). Fig. 1 demonstrates the relationship between the basal plasma insulin level while fasting and membrane fluidity of erythrocytes in patients with essential hypertension and normotensive subjects. The plasma insulin concentration was significantly correlated with the values of the order parameter (S) and the peak height ratio ($ho/h-1$) of the EPR spectra. The finding might indicate that the higher plasma insulin was associated with lower membrane fluidity of erythrocytes.

**Discussion**

There has been much evidence showing that hyperinsulinemia may actively participate in the pathophysiology of hypertension. In the present study we determined a role of insulin in the regulation of membrane fluidity of erythrocytes in patients with essential hypertension and normotensive subjects by means of the EPR and spin-labeling method. The values of the order parameter (S) and the peak height ratio ($ho/h-1$) obtained from the erythrocyte EPR spectra were significantly greater in patients with essential hypertension than in the normotensive subjects. The results are consistent with the idea that the membrane fluidity of erythrocytes was lower in essential hypertension than in normotensive subjects, and confirm our previous reports showing that the cell membranes were stiffer and less fluid in primary hypertension. This study showed that the plasma basal insulin level while fasting was significantly higher in patients with essential hypertension than in normotensive subjects, and confirm our previous reports showing that the cell membranes were stiffer and less fluid in primary hypertension. This study showed that the plasma basal insulin level while fasting was significantly higher in patients with essential hypertension than in normotensive subjects.

The precise mechanisms underlying the insulin effects on the membrane fluidity are still uncertain. There has been much evidence showing the importance of Ca$^{2+}$ in
the mechanisms of insulin actions in several tissues. By using the nuclear magnetic resonance method, Barbagallo et al. demonstrated that insulin significantly elevated the cytosolic-free Ca\(^{2+}\) level of human erythrocytes in a dose- and time-dependent manner. With regard to the interactions between Ca\(^{2+}\) and the membrane fluidity, it was observed that Ca\(^{2+}\) strongly decreased the membrane fluidity of erythrocytes and other cells. We also reported that the treatment of erythrocytes with Ca\(^{2+}\) and Ca\(^{2+}\)-ionophore A23187 reduced the erythrocyte membrane fluidity. In an in vitro study, we demonstrated that insulin in combination with Ca\(^{2+}\) increased the values of the order parameter (S) and the peak height ratio (ho/h-1) of the EPR spectra. It is likely that the insulin-evoked decrease in the membrane fluidity may be partially mediated by the increased intracellular Ca\(^{2+}\) concentration. Furthermore, the result indicated that insulin lowered the membrane fluidity of erythrocytes, and is consistent with the above-mentioned finding that hyperinsulinemia was associated with the lower membrane fluidity of erythrocytes. Because membrane fluidity is a reciprocal value of membrane microviscosity, the decreased membrane fluidity of erythrocytes might cause a disturbance in blood rheologic behavior and microcirculation, which could, at least in part, contribute to the pathophysiology of hypertension.

In summary, the results of the present study showed that hyperinsulinemia was associated with the lower membrane fluidity of erythrocytes. Although further studies should be conducted to assess the precise mechanisms underlying the insulin action on the membrane fluidity and their participation in blood pressure control, the results support the hypothesis that hyperinsulinemia may constitute a crucial effect on the physicochemical properties of the cell membranes in hypertension.

References