Ca\textsuperscript{2+} binding and membrane fluidity in essential and renal hypertension

SERGEI N. ORLOV AND YUVENALI V. POSTNOV

Central Research Laboratory of the Ministry of Public Health of the U.S.S.R., Moscow, U.S.S.R.

(Received 21 January 1982; accepted 14 April 1982)

Summary

1. Ca\textsuperscript{2+}-binding ability and membrane structure of red blood cells of patients with essential and renal hypertension were studied.

2. Ca\textsuperscript{2+}-binding ability of the erythrocyte membrane of patients with essential hypertension was found to be reduced by 30% compared with that of normotensive controls.

3. The rate of lateral diffusion of pyrene in the erythrocyte membrane of patients with essential hypertension was reduced both in the lipid bilayer and in the region of annular lipid compared with that of normotensive patients.

4. There are no differences either in Ca\textsuperscript{2+}-binding ability or in fluidity of the erythrocyte membrane of patients with chronic renal hypertension compared with that of normotensive individuals.

Key words: hypertension, erythrocytes, membrane structure, calcium.

Introduction

Recent studies have revealed a number of functional alterations of the erythrocyte membrane in essential hypertension and its experimental analogue, spontaneous hypertension of rats (SHR, Kyoto–Wistar). In particular, it was demonstrated in both forms of primary hypertension that permeability of the erythrocyte membrane for univalent cations is increased [1–3], and Ca\textsuperscript{2+}-binding ability is decreased [4, 5]. It was found also that erythrocytes of spontaneously hypertensive rats show certain alterations in their membrane structure [6–8] and have an increased intracellular concentration of Ca\textsuperscript{2+} [9].

The present investigation deals with the Ca\textsuperscript{2+}-binding ability and certain structural characteristics of the erythrocyte membrane, both in essential and in chronic renal hypertension.

Material and methods

Venous blood samples were taken from the following groups.

1. Nine patients with clinically established essential hypertension

This group consisted of four males and five females (mean age 51; range 38–68 years), hospitalized for chronic vascular disease (without acute myocardial infarction), frequent crises or for investigation of high blood pressure. The duration of known hypertension was 4–12 years: blood pressure on hospitalization was 145–190/90–105 mm Hg. Renal or endocrine pathology, which might have been a cause of secondary hypertension, was not detected on clinical examination.

2. Eleven patients with chronic hypertension of renal origin (chronic glomerulonephritis or pyelonephritis)

This group consisted of three males and eight females (mean age 50; range 35–61 years). The duration of hypertension was 6–15 years, blood pressure was 170–220/110–100 mm Hg. We
express our gratitude for the clinical examination of this group to Professor I. E. Tareeva and Dr P. V. Klepikov (Problem Laboratory of Nephrology, Setchenov First Medical Institute, Moscow).

3. Thirteen normotensive individuals

This group consisted of six males and seven females (mean age 47; range 32–61 years) without any history of hypertension; some of them were hospitalized for chronic vascular disease (without myocardial infarction), the others being healthy volunteers.

Erythrocyte ghosts

The erythrocytes were sedimented by 1000 g centrifugation for 10 min and washed three times with a solution containing 150 mmol of NaCl/l and 5 mmol of sodium phosphate/l (pH 8.0; 0–2°C). The washed erythrocytes were haemolysed in 20 volumes of sodium phosphate (5 mmol/l) buffer (pH 8.0; 0–2°C) and sedimented at 25000 g for 30 min. The sediment was washed three times under the same conditions. Lowry’s method was used to determine the protein concentration [10].

Ca\(^{2+}\) binding

The method used in this study has been described previously [4]. The incubation medium contained (mmol/l): NaCl 140; EGTA, 0.2; CaCl\(_2\), 0.18; imidazole HCl 20 (pH 7.4); \(^{42}\)CaCl\(_2\), \(\mu\)Ci/ml; protein concentration 200 \(\mu\)g/ml. The free calcium concentration in the incubation medium, calculated for \(pK\) of Ca...EGTA = 6.3 was 4.5 \(\mu\)mol/l. After 30 min of incubation the samples were transferred on filters (SM, Millipore) and washed three times with a solution containing NaCl (140 mmol/l) and imidazole/HCl (20 mmol/l) (pH 7.4; 0–2°C).

It is known that erythrocyte ghosts contain both right-side-out and inside-out vesicles and unresealed membrane fragments [11]. To make both sides of the membrane accessible to Ca\(^{2+}\), Ca\(^{2+}\) ionophore A23187 (2 \(\mu\)mol/l) was added to the incubation medium.

Fluorescent probe

Structural properties of the erythrocyte membrane were studied, using the fluorescent probe pyrene, which was embedded in the membrane. The intensities of monomer (I\(^m\); emission wavelength, 373 nm) and eximer (I\(^e\); emission wavelength, 450 nm) fluorescence were measured and the ratio I\(^m\)/I\(^e\) was calculated. This ratio is in inverse proportion to the rate of lateral diffusion of pyrene molecules in the membrane [12]. The fluorescence of pyrene was excited both directly (excitation wavelength, 325 nm) and by excitation of tryptophan residues in membrane proteins (excitation wavelength, 285 nm). In the first case it is possible to estimate the integral characteristics of the pyrene absorption site, whereas in the second case one can characterize the rate of lateral diffusion near the protein molecules only (in the region of annular lipids). The methodical details were described previously [7]. The incubation medium content (mmol/l) was: imidazole/HCl, 40 (pH 7.4; 37°C); NaCl, 140; MgCl\(_2\), 2; EGTA, 1; CaCl\(_2\), up to 1.2; pyrene, 0.01; protein concentration, 100 \(\mu\)g/ml.

Results

As can be seen from Table 1, Ca\(^{2+}\)-binding ability of the erythrocyte membrane of patients with essential hypertension is reduced by 30% compared with that of normotensive controls. The rate of lateral diffusion in the erythrocyte membrane of essential hypertensive patients is reduced compared with normotensive patients (an increase in the I\(^m\)/I\(^e\) ratio) (Fig. 1).

There are no differences either in the Ca\(^{2+}\)-binding ability of the erythrocyte membrane or in its structure between patients with chronic renal hypertension and the normotensive group.

As can be seen from Fig. 1, an increase in Ca\(^{2+}\) concentration in the incubation medium results in a change in the lateral diffusion rate of pyrene in the erythrocyte membrane of normotensive individuals and patients with renal hypertension. No influence of Ca\(^{2+}\) on the erythrocyte structure of patients with essential hypertension was observed.

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Ca(^{2+}) (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normotensive patients</td>
<td>13</td>
<td>1.22 \pm 0.07</td>
</tr>
<tr>
<td>2. Patients with essential hypertension</td>
<td>9</td>
<td>0.87 \pm 0.05</td>
</tr>
<tr>
<td>3. Patients with chronic renal hypertension</td>
<td>11</td>
<td>1.23 \pm 0.10</td>
</tr>
</tbody>
</table>

\(P_{0.05} < 0.005\)  
\(P_{0.01} \) N.S.
Discussion

The results of the present study confirm the decrease in Ca^{2+}-binding ability of the erythrocyte membrane previously established in patients with essential hypertension [4] and demonstrate the absence of this alteration in hypertension of renal origin.

The study of fluorescence spectra of pyrene in membrane preparations also demonstrates the presence of structural alterations in the erythrocyte membrane obtained from essential hypertensive patients, but not from those with renal hypertension.

It has become evident that the membrane defect in erythrocytes, which we regarded previously as part of widespread membrane alterations [13, 14], is specific to primary hypertension. This assumption is supported by experimental studies in which decreases in Ca^{2+} binding [4], alterations in univalent-cation permeability [15] and changes in the erythrocyte membrane structure [7] were observed in spontaneously hypertensive rats, but were not present in rats with renal and deoxycorticosterone acetate/salt hypertension. This suggests a profound pathogenetic similarity between human essential hypertension and spontaneous hypertension in rats (Kyoto–Wistar hypertensive rats).

As we showed previously [14] for spontaneously hypertensive rats, the alteration in the Ca^{2+}-binding ability of the erythrocyte membrane is located at the inner surface, i.e. to the part of membrane-bound Ca^{2+} that determines membrane permeability to univalent cations [2]. The same alteration is likely to take place in essential hypertension, and this may explain enhanced erythrocyte membrane permeability to Na^+, K^+ and Li^+ found in essential hypertensive humans, as well as the absence of this membrane functional abnormality in renal hypertensive patients [3]. As can be seen from the data on pyrene fluorescence in the erythrocyte membrane (Fig. 1), both direct and indirect excitation produces an increase in the \( \frac{I^m/I^e}{Ca^{2+} } \) ratio for essential hypertensive patients compared with the normotensive group as well as the renal hypertensive group. These data indicate a decreased rate of lateral diffusion both in the membrane lipid bilayer and in the region of annular lipids, i.e. an indication of increased microviscosity (rigidity) in the erythrocyte membrane in essential hypertension.

A comparison of the data on direct and indirect excitation of pyrene fluorescence (Fig. 1) reveals a certain irregularity in the structural alteration of the membrane. It is known that in the case of the sarcoplasmic reticulum of skeletal muscles the rates of pyrene lateral diffusion at physiological temperatures (37°C) in the lipid bilayer and in the region of annular lipids, i.e. an indication of increased microviscosity (rigidity) in the erythrocyte membrane in essential hypertension.
of pyrene in the annular lipid regions was decreased considerably compared with the lipid bilayer. This suggests that the protein molecules in the erythrocyte membranes of these patients are functioning in structurally different lipid environments.

Fig. 1 shows that the increase in Ca\(^{2+}\) concentration in the incubation medium (accompanied by increasing membrane-bound Ca\(^{2+}\)) results in a decrease in the rate of pyrene lateral diffusion in the erythrocyte membrane of both normotensive and renal hypertensive patients. This phenomenon might be caused by the clostretation of acid lipids induced by calcium [17].

In the case of essential hypertension, where lateral diffusion in the membrane is initially decreased, this phenomenon cannot be clearly seen. This may demonstrate the existence of altered interaction of membrane lipids with Ca\(^{2+}\). Further studies should reveal the specific mechanism responsible for the formation of the structural defect in the erythrocyte membrane and, probably, in the plasma membrane of other types of cells and prove its connection with membrane function alterations in essential hypertension.

Acknowledgments

We thank Dr A. M. Adler and Dr Ilya Y. Postnov for the clinical examination of the patients, and Mr V. M. Boitsov and Ms Z. V. Karagodina for computer calculation.

References


