Preliminary Observations on Abnormalities of Membrane Structure and Function in Essential Hypertension

ALLEN J. NAFTILAN, VICTOR J. DZAU, AND JOSEPH LOSCALZO

SUMMARY To test the hypothesis that structural abnormalities exist in the cell membrane in persons with essential hypertension and that these abnormalities affect membrane-related cellular functions, we examined several membrane-dependent phenomena and membrane lipid composition in the blood cells of subjects with essential hypertension. We analyzed platelet aggregability, membrane fluidity, membrane fatty acid composition, and erythrocyte deformability in four normolipidemic subjects with untreated essential hypertension and in five age-matched normotensive controls. As compared with the controls, the subjects with essential hypertension had platelets that aggregated at lower concentrations of adenosine 5'-diphosphate, platelet membranes that were less fluid, and erythrocytes that were more deformable. Lipid analysis of the membranes of platelets from the two study groups showed that although the cholesterol content was identical, the membranes from the essential hypertension group contained significantly less linoleic acid (18:2) than did those from the normotensive controls. Given the known effects of cis-unsaturated fatty acyl composition on membrane fluidity and membrane-related cellular functions, these data suggest that one factor contributing to essential hypertension is an inherent structural membrane abnormality that alters the physical and functional properties of the cell membrane. (Hypertension 8 [Suppl II]: II-174-II-179, 1986)

KEY WORDS platelets • membrane fluidity • fatty acids • atherosclerosis • essential hypertension

ESSENTIAL hypertension has been associated with various functional membrane abnormalities in humans and animals. This diversity of membrane-associated disturbances suggests that an intrinsic structural abnormality of the cell membrane that is important for a variety of disparate membrane protein activities may be a key etiological factor. One such global membrane property is microenvironmental fluidity, variations in which have been shown to affect a number of different membrane functions, including lymphocyte capping, platelet aggregation, macrophage phagocytic activity, and sodium-potassium adenosine triphosphatase (ATPase) activity. Factors that have been shown to influence membrane fluidity include the cholesterol content and fatty acid composition of the membrane bilayer; the fluidity increases with decreasing cholesterol content or increasing cis-unsaturated fatty acyl composition.

To test the hypothesis that a structural membrane abnormality exists in the cells of persons with essential hypertension and that this abnormality affects the physical state and functional properties of the membrane, we examined two membrane-dependent cell functions, platelet aggregability and erythrocyte deformability, in normolipidemic subjects with untreated hypertension and in age-matched normotensive controls. We also measured membrane fluidity in the platelets of these individuals, as well as the cholesterol content and fatty acyl composition of the platelet membranes, in an attempt to correlate functional abnormalities with structural alterations. The preliminary results from a small group of individuals demonstrate that inherent abnormalities of membrane composition and function are present in the blood cells of persons with essential hypertension. Structural membrane abnormalities that affect the physical state and function of cell membranes may be a generalized phenomenon in essential hypertension, and these abnormalities may be either primary or secondary to the hypertensive process.
Materials and Methods

Materials

1,6-Diphenyl-1,3,5-hexatriene (DPH) and adenosine 5'-diphosphate (ADP) were obtained from Sigma Chemical (St. Louis, MO, USA). High pressure liquid chromatography-grade hexane, 2-propanol, chloroform, methanol, petrol ether, diethyl ether, and acetyl chloride were purchased from Aldrich Chemical (Milwaukee, WI, USA). Fatty acid methyl esters were obtained from Nu Chek Prep (Elysian, MN, USA). All other chemicals used were reagent grade or better. Deionized water was used throughout.

Experimental Groups

The populations studied included four normolipidemic subjects with newly diagnosed essential hypertension (two white men and two white women, three of whom had family histories of hypertension) who had received no prior medical therapy, and five agematched normotensive controls (three white men and two white women). Various characteristics of the two groups are listed in Table 1. Individuals were defined as hypertensive if on three consecutive occasions the systolic blood pressure was higher than 140 mm Hg or if the diastolic blood pressure was higher than 90 mm Hg. The subjects were on their usual diets, which appeared to be comparable, on the basis of informal dietary histories.

Platelet and Erythrocyte Preparation

Fifty milliliters of venous blood was obtained from each subject and anticoagulated with 13 mM sodium citrate. Platelet-rich plasma was prepared from whole blood by centrifuging the anticoagulated blood at 160 g for 10 minutes. Washed platelets were prepared by centrifuging the platelet-rich plasma at 800 g for 20 minutes, resuspending the platelet pellet in platelet washing buffer (10 mM sodium citrate, 0.15 M NaCl, 1 mM ethylenediaminetetraacetic acid, pH 7.0), centrifuging the platelets at 800 g for 20 minutes, repeating the suspension in washing buffer (0.13 M NaCl, 2.7 mM KCl, 12 mM NaHCO₃, 0.42 mM NaH₂PO₄, pH 7.4). Platelet counts were determined with a Coulter counter (Model F; Coulter Electronics, Hialeah, FL, USA).

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Erythrocytes were centrifuged at 800 g for 20 minutes, resuspended in modified Tyrode’s solution, centrifuged, and resuspended twice more. The packed erythrocytes were then diluted with modified Tyrode’s solution for the filtration experiments described below.

Platelet Aggregation

Platelet aggregation was monitored by a standard nephelometric technique in which 0.4-ml aliquots of platelet-rich plasma were incubated at 37°C and stirred at 900 rpm in a Payton dual-channel aggregometer (Payton Associates, Buffalo, NY, USA). Aggregation was induced by the addition of ADP (over a range of concentrations), and changes in light transmittance were recorded with an Omniscribe recorder (Houston Instruments, Austin, TX, USA). Aggregation was quantitated by measuring the maximal rate of change in light transmittance.

Fluorescence Measurements

DPH₁₃⁻₁₅ at a final concentration of 2 µM was incubated at room temperature with a suspension of washed platelets in modified Tyrode’s solution at 1 x 10¹⁰/µl for 20 minutes, after which the platelets were centrifuged and resuspended in modified Tyrode’s solution twice.

Fluorescence measurements were performed with a Perkin-Elmer Model 44B spectrofluorometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a thermostat-controlled cell holder and Hitachi polarizers. The steady-state fluorescence polarization was measured by exciting the cell suspension at 360 nm and recording the emission at 430 nm. The polarization of fluorescence emission was calculated from the equation P = (Iᵥ - Gᵥ)/[Iᵥ + Gᵥ], where P is polarization, I is the fluorescence intensity, the first and second subscripts refer to the plane of polarization of the excitation and emission beams, respectively (V = vertical, H = horizontal), and G = Gᵥ/G₇. For each measurement of P, the emission intensities were corrected for contributions due to scattering by measuring the intensity of these intensities in a blank containing all the solution constituents except the fluorophore.

Erythrocyte Deformability Measurements

Erythrocyte deformability was assessed by measuring the flow time of a suspension of erythrocytes through 4.7-micron Nucleopore filters (Hemafil polycarbonate membranes; Nucleopore, Pleasanton, CA, USA) under a constant negative pressure of 20 cm H₂O, according to the method of Reid and colleagues.

Lipid Extraction and Analysis

Platelet membrane lipids were extracted from washed platelets with hexane-2-propanol, as described by Hara and Radin. Cholesterol was determined with o-phthalaldehyde, by the method of Rudel and Morris.

Table 1. Characteristics of the Normotensive and Hypertensive Study Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive (n = 5)</th>
<th>Hypertensive (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>170 ± 8</td>
<td>180 ± 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67 ± 9</td>
<td>85 ± 15</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.78 ± 0.08</td>
<td>2.05 ± 0.08</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29 ± 6</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Total plasma cholesterol (mg/dl)</td>
<td>182 ± 17</td>
<td>184 ± 28</td>
</tr>
<tr>
<td>Platelet count (x 10¹³/µl)</td>
<td>278 ± 31</td>
<td>272 ± 20</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>118 ± 8/74 ± 6</td>
<td>145 ± 5/99 ± 4</td>
</tr>
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</table>

Values are means ± SD.
Platelet membrane phospholipids were transesterified with methanolic-HCl. The fatty acid methyl esters were extracted into petroleum ether and separated and identified by gas chromatography with a Chrompack CP Sil 88 column (Chrompack, Bridgewater, NJ, USA) in a Varian chromatograph (Varian Associates, Palo Alto, CA, USA) equipped with a solid injector and a flame ionization detector connected to a computer integrator. Fatty acid methyl esters were identified by comparison with known standards and were quantitated by integration of peak areas.

**Statistical Analysis**

The data are expressed as means ± SD. Statistical comparisons were performed with the nonpaired Student's t test, and the null hypothesis was rejected for p values less than 0.05.

**Results**

**Platelet Aggregation**

The platelets from subjects with essential hypertension aggregated at lower concentrations of ADP than did those from the normotensive controls. The dose-response data presented in Figure 1 indicate that the 50% effective concentration (EC50) for ADP-induced aggregation was reduced approximately 2.5-fold, from 5.0 μM to 2.0 μM in the hypertensive group (p < 0.01). The difference between the two groups was not uniform throughout the range of concentrations of ADP tested but appeared to be more marked at higher concentrations of the agonist.

**Platelet Membrane Fluidity**

The fluorescence polarization of DPH incorporated in the platelet membrane bilayer was measured as a function of temperature in suspensions of platelets prepared from the hypertensive and normotensive subjects. DPH fluorescence polarization decreases with increasing microenvironmental fluidity. The data in Figure 2 demonstrate that at temperatures higher than 20°C, the fluorescence polarization of DPH was significantly greater in platelets from the hypertensive subjects than in those from the normotensive controls (p<0.001 at temperatures higher than 35°C). This difference in polarization became more pronounced as the temperature was increased, suggesting that the microenvironment of DPH in platelet membranes from hypertensive persons is considerably less fluid than that in platelets from normotensive persons.

**Erythrocyte Deformability**

The deformability of erythrocytes from the two groups was compared by measuring the flow rate of a range of concentrations of cells through polycarbonate membranes of 4.7-micron pore size (Figure 3). The more deformable the erythrocyte, the faster it will flow through the membrane. The data demonstrate that erythrocytes from the hypertensive subjects were less deformable than those from the normotensive controls (p<0.01 at hematocrits ≥0.1). Over a range of erythrocyte concentrations, the flow rate of the cell suspension was slower for cells from hypertensive subjects than for those from controls, with differences between the two groups being most marked at hematocrits above 0.1 (i.e., 10%).

**Platelet Lipid Analysis**

Since the structural elements of bilayer membranes that influence fluidity most dramatically are cholesterol and phospholipid fatty acyl chain saturation, we analyzed the cholesterol content and fatty acid composition of platelet membranes in the two experimental groups. The cholesterol content of platelets did not differ significantly between the two groups (12 ±2 μg/10⁸ platelets in the hypertensive subjects and 13 ± 2 μg/10⁸ platelets in the normotensive controls). In contrast, the data listed in Table 2 indicate that there was significantly less linoleic acid (18:2) in the platelet membranes from the hypertensive subjects than in those from the normotensive controls, with 44% less cis-unsaturated fatty acid in the former group (p<0.01). In addition, less oleic acid (18:1) was de-
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Figure 2. 1,6-Diphenyl-1,3,5-hexatriene fluorescence polarization as a function of temperature (T) for fluorophore incorporated in intact platelets from hypertensive (O) and normotensive (●) subjects. Each point represents the mean value (± SD) for steady-state fluorescence polarization, derived as described in Materials and Methods.

Figure 3. Erythrocyte deformability assessed by measuring the flow rate of erythrocyte suspensions through 4.7-micron-pore polycarbonate filters as a function of the erythrocyte concentration in hypertensive (O) and normotensive (●) subjects. The flow rate is normalized to that for buffer (modified Tyrode's solution). Each point represents the mean value (± SD) for the entire group of hypertensive or normotensive measurements.

Table 2. Platelet Membrane Fatty Acid Composition in Normotensive and Hypertensive Subjects

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<td>14.3 ± 2.3</td>
<td>18.8 ± 10.9</td>
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<tr>
<td>18:0</td>
<td>19.8 ± 4.1</td>
<td>18.5 ± 1.3</td>
</tr>
<tr>
<td>18:1</td>
<td>17.5 ± 9.5</td>
<td>11.9 ± 2.7</td>
</tr>
<tr>
<td>18:2</td>
<td>10.5 ± 5.8</td>
<td>5.9 ± 1.4*</td>
</tr>
<tr>
<td>18:3</td>
<td>1.01 ± 0.55</td>
<td>0.62 ± 0.66</td>
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<tr>
<td>20:4</td>
<td>26.9 ± 0.5</td>
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*p < 0.01.

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Discussion

Few data exist on cellular membrane structure and function in essential hypertension. Two groups have demonstrated increased microviscosity in platelet and erythrocyte ghosts in the spontaneously hypertensive...
rat model of essential hypertension. In addition, McGregor and colleagues showed that platelets from spontaneously hypertensive rats aggregated in response to lower concentrations of thrombin or ADP than did platelets from Wistar-Kyoto controls and that this difference was potentiated by feeding the animals diets rich in saturated fats. A decrease in erythrocyte deformability and an increase in erythrocyte membrane microviscosity have been reported in patients with essential hypertension. Nara and colleagues have also recently demonstrated that platelets aggregated in response to lower concentrations of ADP in men with a family history of essential hypertension than in those with no such history.

Our data support the concept that the cell membranes of persons with essential hypertension are structurally abnormal. This alteration in structure produces a stiffer, less fluid membrane that modulates surface-dependent cellular physical properties and functions. Platelets with surface membranes made less fluid by exogenous increases in the cholesterol content or the saturated fatty acid composition become hyperaggregable. Similarly, less fluid erythrocyte membranes produce less deformable erythrocytes, as we have noted in this study. An alternative explanation for the decreased deformability of erythrocytes in essential hypertension is, of course, that they have lower Na\(^+\)-K\(^+\)-dependent ATPase activity, thereby increasing cell volume. One study failed to demonstrate any correlation between Na\(^+\)-K\(^+\)-dependent ATPase activity and erythrocyte filterability in hypertensive persons. Conversely, Yeagle has shown that erythrocyte membrane lipid composition markedly affects Na\(^+\)-K\(^+\)-dependent ATPase activity, with increased cholesterol resulting in reduced ATPase activity and diminished membrane fluidity.

From the data presented here, we cannot determine whether the observed changes in membrane composition are causative or a consequence of the shear stress engendered by elevated blood pressure in hypertensive individuals. Two recent studies, however, support the former hypothesis. Bianchi and colleagues have shown that an alteration in erythrocyte Na\(^+\)-K\(^+\) co-transport observed in the Milan hypertensive rat strain could be transferred to normotensive controls by bone marrow transplantation, and Nara and co-workers have reported increased platelet aggregability in response to ADP in healthy normotensive men with a family history of hypertension.

Interpreted in the context of these studies, our data support the hypothesis that one factor contributing to or potentially causing essential hypertension is an inherent membrane structural abnormality that alters the physical state of the membrane and thereby modifies membrane-related cellular functions. Although we have studied only platelets and erythrocytes, this abnormality may be global, affecting the membranes of all cells, including endothelial and smooth muscle cells and the surface effectors of their functions that are important for blood pressure regulation. If a single genetic defect is responsible for this abnormality, one might speculate that a defective fatty acid desaturase or elongase may be a critical determinant of the hypertensive state. Failure to incorporate fatty acyl chains of the appropriate length and degree of unsaturation would lead to changes in membrane fluidity and consequent changes in membrane protein conformation and function.

Two critical lipid determinants of fluidity in cell membranes have been defined, cholesterol composition (and the cholesterol/phospholipid ratio) and cis-unsaturated fatty acyl group content (and the saturated/cis-unsaturated fatty acyl group ratio). Exogenous modification of cholesterol content has been shown to alter the membrane bilayer fluidity and membrane protein function in several systems. A 41% decrease in the cellular cholesterol/phospholipid ratio produces a 57% reduction in lymphocyte capping. A 54% increase in the platelet cholesterol/phospholipid ratio leads to a 47% reduction in the EC\(_50\) of thrombin-induced platelet aggregation, with a concomitant 73% increase in high-affinity thrombin receptors. In contrast, a 30% depletion of the erythrocyte cholesterol content induces a 40% increase in the apparent maximal rate of sodium efflux, a 45% decrease in the apparent dissociation constant of K\(^+\) from the pump, and an 88% increase in the apparent dissociation constant of Na\(^+\) from the pump. Similar kinds of studies using cell membrane fatty acyl group modifications have been performed less frequently. A 12 to 15% increase in macrophage stearate content reduces erythropagocytic activity by 76%, as compared with control cells. Similarly, an approximate 5% increase in platelet membrane linoleate content is associated with a 10% decrease in DPH fluorescence polarization and an 80% inhibition of platelet aggregation. These data indicate that although changes in cholesterol content must be rather extensive to produce marked changes in membrane protein function, similar effects may be produced by much less dramatic alterations in fatty acyl group composition. The biophysical reasons for this differential sensitivity are not known but may relate to the notion that specific annular or boundary lipids relatively inexchangeably bound to membrane proteins form local microdomains that can dramatically alter local protein conformation or function without markedly changing global membrane properties. This principle was espoused as early as 1963, when Jurshuk et al. showed that optimal activation of mitochondrial d(-)-\(\beta\)-hydroxybutyric acid dehydrogenase requires an unsaturated acyl lecithin in its microenvironment.

It is this kind of mechanism that may be invoked to explain the myriad of transport abnormalities and other surface membrane changes that have been described in hypertension. Some of the transport abnormalities themselves could also lead to changes in the amount and type of fatty acyl chains incorporated in the membrane phospholipids, thereby further altering microdomain composition, fluidity, and membrane protein function. Further studies are under way to address these complex issues concerning cellular structure-function relationships in hypertension.
Acknowledgments

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References

11. Lokesh BR, Wrann M. Incorporation of palmitic and oleic acid into macrophage lipid exerts differential effects on the function of normal mouse peritoneal macrophages. Biochim Biophys Acta 1983;792:141–148
30. Kuyper IA, Foelefson B, van Kamp JAF, Van Deenen LLM. The membrane of intact erythrocytes tolerates only limited changes in the fatty acid composition of its phosphatidylcholine. Biochim Biophys Acta 1984;769:337–347

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