Effect of Protein Kinase C Activation on Cytoskeleton and Cation Transport in Human Erythrocytes

Reproduction of Some Membrane Abnormalities Revealed in Essential Hypertension

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SUMMARY Certain manifestations of alterations of membrane cytoskeleton, protein kinase C activity, and ion transport were revealed in erythrocytes of patients with essential hypertension: 1) the average volume of erythrocytes is reduced by 4%; 2) about 7% of the total number of erythrocytes is represented by cup-shaped forms compared with 1.5 to 3.0% in the control group; 3) basal phosphorylation of Band 4.9 protein is increased 1.6-fold to 1.8-fold; 4) activity of protein kinase C is increased by 60 to 70%; 5) the rate of proton electrochemical gradient (ΔμH+)—induced Na+-H+ exchange is increased twofold. Treatment of erythrocytes of healthy donors with protein kinase C activator (12-O-tetradecanoylphorbol-13-acetate) leads to similar but more marked changes in cell shape (17% of cup-shaped forms), volume reduction (by 7%), an increase of Band 4.9 protein phosphorylation (threefold), and an increase in the rate of Na+-H+ exchange (fourfold). Protein kinase activation does not modify Na+-Li+ exchange and slightly increases (by 20–50%) Na+-K+ pump activity, Na+-K+ cotransport, and the rate of 45Ca influx. It may be assumed that the increase of protein kinase C activity is one of the most probable molecular mechanisms conditioning abnormalities of the membrane skeleton and Na+-H+ exchange in primary hypertension.

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KEY WORDS • essential hypertension • erythrocytes • cytoskeleton • protein kinase C • phosphorylation • Na+-H+ exchange

ERYTHROCYTES are the first cells (other than contractile cells of the cardiovascular system) in which membrane cation transport abnormalities were observed in primary hypertension. Further research on erythrocytes characterized the principal manifestations of this defect, which appeared to be common to a whole series of cells.1 Enhanced phosphoinositide metabolism in erythrocytes of spontaneously hypertensive rats (SHR)2 and patients with essential hypertension3 described earlier, indicated possible changes in both protein kinase C activity and membrane skeleton proteins, which are substrates phosphorylated by this protein kinase. The existence of abnormalities in the erythrocyte membrane skeleton in primary hypertension is borne out by the decreased deformability of these cells in essential hypertension4 and the reduction of erythrocyte volume described earlier in rats with spontaneous hypertension, the SHR5 and Milan hypertensive6 strains. Taking these data into account, we undertook the study of membrane skeleton protein phosphorylation, as well as cell shape and volume (as cytoskeleton-dependent parameters) in erythrocytes of patients with essential hypertension. We also attempted to characterize the state of the Na+-K+ pump, the system of facilitated diffusion of univalent cations (Na+-K+ cotransport, Na+-Li+ and Na+-H+ countertransport), and the 45Ca influx rate. All the data obtained were compared with similar data obtained under protein kinase C stimulation by 12-O-tetradecanoylphorbol-13-acetate (TPA) in the erythrocytes of healthy subjects. We also performed a direct estimation of protein kinase C activities in...
membrane-free lysate of erythrocytes of hypertensive and normotensive subjects.

**Subjects and Methods**

**Donors**

Blood samples were taken from the following groups 1) 49 patients with clinically established hypertension, World Health Organization Stages II and III (40 men and 9 women; mean age, 49.0 ± 2.4 years, blood pressure range, 170–215/105–115 mm Hg); 2) 18 patients with hypertension of renal origin (12 men and 6 women; mean age, 55.2 ± 4.7 years; blood pressure range, 190–220/100–125 mm Hg); and 3) 43 healthy normotensive subjects (36 men and 7 women; mean age, 47.2 ± 4.6 years; blood pressure range, 110–120/70–85 mm Hg), used as controls. In all patients in the second group, hypertension was caused by glomerulonephritis without renal insufficiency.

Blood samples were obtained (after subjects had fasted 10–12 hours) and stored with heparin (30–40 IU/ml) on ice no more than 3 hours before experiments were begun.

**Erythrocytes**

After sedimentation of blood samples (2000 g for 10 minutes at 0–2 °C), plasma and white blood cells were removed and erythrocytes were washed twice with 5 ml of a medium containing 150 mM of NaCl and 5 mM of sodium phosphate buffer (pH 7.4). To determine the rate of Na⁺-Li⁺ countertransport, erythrocytes were washed with a medium containing 75 mM MgCl₂, 85 mM sucrose, 10 mM glucose, and 10 mM HEPES-Tris (pH 7.4).

**Phosphorylation of Erythrocyte Membrane Proteins**

The aliquots of erythrocytes washed with Medium A were preincubated for 1.5 hours at 38 °C in the same medium containing 0.7 to 0.9 mCi/ml of [³²P]orthophosphate (hematocrit, 30%). Medium A consisted of 130 mM NaCl, 0.01 mM CaCl₂, 0.01 mM MgCl₂, 20 mM glucose, and 35 mM Tris HC1 (pH 7.4). Protein kinase A and C were activated during a 10-minute incubation with 1 mM of dibutyryl-cyclicadenosine 3',5'-monophosphate (cAMP) and 1 μM β-phorbol ester (TPA), respectively. After incubation, the samples were diluted with 40 volumes of 5 mM sodium phosphate buffer (pH 8.0, 2–4 °C) and centrifuged at 25,000 rpm for 25 minutes. Erythrocyte ghosts were washed under the same conditions, transferred in 2.5% sodium dodecyl sulfate containing 1% 2-mercaptoethanol, 20% glycerin, 1 mM EDTA, 10 mM Tris HCl (pH 7.4; Medium B) and kept in boiling water for 5 minutes. Proteins were resolved in 5 to 13% gradient polyacrylamide gel in the presence of sodium dodecyl sulfate, transferred to nitrocellulose sheets, and autoradiographed on x-ray film.

As can be seen from Figure 1, the addition of dibutyryl-cAMP resulted in a drastic increase of phosphorylation of Band 4.9. In contrast, with the addition of TPA the incorporation of ³²P was more pronounced in Band 4.1 than in Band 4.9. Inactive α-phorbol has no effect on membrane protein phosphorylation (data not presented). These results are in accordance with data reported by Horne et al.⁷ The synergistic effect of dibutyryl-cAMP and TPA (see Figure 1, Lane IV) can be explained by the data on the difference in tryptic fragments phosphorylated in these proteins by kinase A and C.⁷

We found the quantitative comparison of basal phosphorylation of Band 4.1 between essential hypertensive and normotensive subjects to be difficult because of the more extensive ³²P incorporation in Band 3 protein (see Figure 1, Lane I). To investigate Band 4.9 phosphorylation in erythrocytes of patients with essential hypertension, strips of nitrocellulose membrane corresponding to this protein stained with Amidoschwarz 10B were cut and placed in Bray’s solution. To minimize the possible differences between the activity of endogenous proteases, the stage of erythrocyte ghosts preparation was omitted and the reaction of ³²P incorporation was terminated by washing the erythrocytes in cold Medium A and subsequently transferring them to Medium B.

**Protein Kinase C Activity**

Packed erythrocytes were transferred in one volume of medium containing 10 mM sodium phosphate (pH 7.4), 100 mM 2-mercaptoethanol, 4 mM EGTA, 0.2% Triton X-100, 2 mM phenantholine, 20 μM pepstatin, 20 μM leupeptin, 20 μM antipain, 200 μM...
zbestatin, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged at 120,000 g for 45 minutes. Protein kinase C of the supernatant was partially purified by CM-Sephadex and diethylaminoethylcellulose chromatography.

Protein kinase activity was determined by measuring the incorporation of $^{32}$P from labeled adenosine 5'-triphosphate (ATP) into calf thymus core histones. The samples (150 µl) containing 10 to 15 µg of protein were mixed with 50 µl of the assay medium (0–2 °C) containing 200 mM Tris HCl (pH 6.0), 40 mM magnesium acetate, 8 mM CaCl$_2$, 50 µM ATP and [γ-$^{32}$P]ATP (0.5–1 × 10$^6$ cpm), and 400 µg of core histones. The reaction was terminated by transferring 200 µl of the sample on Whatman GF/F filters (Clifton, NJ, USA) with 1 ml of 25% trichloracetic acid and placed into vials filled with Bray’s solution. The kinetics of $^{32}$P incorporation in histones was linear up to 30 minutes. In experiments the incubation time was limited by 15 minutes. Protein kinase C activity was determined as an increase of histone $^{32}$P-labeling in the presence of phosphatidylserine (100 µg/ml) and TPA (2.5 × 10$^{-7}$ M).

**Erythrocyte Volume and Shape**

The erythrocyte volume was measured with the Coulter counter (Model ZBIC-1000, Coultronics, Margency, France) at 20 °C. Influence of protein kinase activators on cell volume was determined after incubation of the erythrocytes at 37 °C in Medium C, consisting of 120 mM NaCl, 1.2 mM MgCl$_2$, 1 mM CaCl$_2$, 5 mM glucose, 20 mM HEPES NaOH (pH 7.4; $K_0$ = 0.5). The incubation was terminated by transfer of 100 µl of cell suspension into 900 µl of cold Medium C; 10 µl of this suspension was placed into 10 ml of isotonic solution (Isoton II, Coultronics).

Microscopic observation of erythrocyte shape was done with phase contrast optics. After incubation in Medium C with or without protein kinase activators, cells were fixed with 2.5% glutaraldehyde dissolved in the incubation medium. To calculate the number of cup-shaped cells, the samples were placed in the counting chamber.

$^8$Rb Influx

To measure $^8$Rb influx, 200 µl of packed erythrocytes was transferred into 1 ml of Medium D, containing 130 mM NaCl, 3 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 1 mM K$_2$HPO$_4$, 10 mM glucose, and 30 mM HEPES-Tris (pH 7.4; 37 °C), and preincubated for 30 minutes at 37 °C. The erythrocyte suspension was centrifuged (5000 rpm for 1–2 minutes; FP-9 centrifuge, Helsinki, Finland), and 0.8 ml of Medium D containing 4 µCi/ml $^8$Rb, used as a radioactive analogue of potassium, was added. In some cases, 0.2 mM ouabain and 0.25 mM furosemide were added. To determine the $^8$Rb content in erythrocytes, 200 µl of cell suspension was placed into 1 ml of washing solution containing 150 mM choline chloride and 10 mM Tris HCl (pH 7.4; 2–4 °C). The erythrocytes were sedimented, washed twice under the same conditions, and treated with 0.5 ml of 0.5% Triton X-100 and 0.5 ml of 10% trichloroacetic acid. After protein sedimentation, 0.8 ml of supernatant was transferred into Bray’s solution. The kinetics of $^8$Rb influx was linear up to 75 minutes of incubation. In accordance with these results the rate of the unidirectional ion flux was calculated as $V = (A_2 - A_1)/a.m.t$, where $A_2$ and $A_1$ are the radioactivity of $m$ liters of erythrocytes at 45 and 15 minutes of incubation (cpm), $a$ is the specific radioactivity of the incubation medium (cpm/µmol), and $t$ is the incubation time (30 minutes).

The activity of the Na$^+$-K$^+$ pump and Na$^+$-K$^+$ cotransport were determined as the rates of ouabain-inhibited and ouabain-sensitive furosemide-inhibited components of $^8$Rb influx, respectively.

Na$^+$-H$^+$ Countertransport and Na$^+$-Li$^+$ Countertransport

In erythrocytes, $^9$, $^{10}$ as well as in other resting cells, $^{11}$ the Na$^+$-H$^+$ exchanger is quenched. To activate this ion transport system, we undertook the acidification of cytoplasm according to methods described by Escobales and Canessa. $^{12}$ First, 100 µl of packed erythrocytes was placed into 1.9 ml of medium consisting of 130 mM NaCl, 1 mM KCl, 1 mM MgCl$_2$, and 10 mM glucose. In some cases this medium contained 1 µM of TPA. After 5 minutes of incubation (37 °C) the pH value was adjusted to 6.35–6.45 by a 0.2 N solution of HC1 in 150 mM NaOH. After addition of anion transport inhibitor (200 µM 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid; DIDS), the pH of incubation medium was increased to 7.95–8.05 by a 0.05 N solution of NaOH in 150 mM NaCl. In some experiments 0.5 mM amiloride was added before medium alkalization. The kinetics of proton efflux was indicated by a 91-15 electrode (Orion, Cambridge, MA, USA) connected to a PHM-64 (Radiometer, Copenhagen, Denmark). The rate of Na$^+$-H$^+$ exchange was determined as $(\Delta p_{H_1} - \Delta p_{H_2})$ • $b$ • $m$ • $t$. Here, $\Delta p_{H_1}$ and $\Delta p_{H_2}$ are the initial rates of medium acidification without and with amiloride, respectively; $b$ is the buffer capacity of the incubation medium (µmol of H$^+$ per pH unit); $m$ is the erythrocyte content in suspension (0.0001 L); $t$ is the incubation time (1 minute). Na$^+$-Li$^+$ countertransport was determined as described by Canessa et al. $^{13}$ TPA (1 µM) was added both in Na$^+$-containing and in Mg$^{2+}$-containing mediums.

$^{4}$Ca Influx

The rate of $^{4}$Ca uptake by fresh erythrocytes is extremely low due to high Ca$^{2+}$ pump activity and insignificant intracellular Ca$^{2+}$ buffer capacity. $^{14}$ To enhance the last parameter, erythrocytes were loaded with quin 2 by methods described previously, $^{15}$ with slight modifications. Next, 200 µl of packed erythrocytes was preincubated for 90 minutes at 37 °C in 800 µl of Medium D containing 1%
bovine serum albumin and 100 μM quin 2AM, washed with Medium D, and incubated for 30 minutes at 37 °C in the same medium. After sedimentation, erythrocytes were resuspended in four volumes of Medium D containing 4CaCl2, 4 μCi/ml, and washed with Medium D, and incubated for 30 minutes at 37 °C in the same medium. After sedimentation, erythrocytes were resuspended in four volumes of Medium D containing 4CaCl2, 4 μCi/ml, and incubated at 37 °C. At the fixed incubation times, the aliquots of suspension were transferred into 1 ml of the cold medium containing 4CaCl2, 4 μCi/ml, and incubated at 37 °C. At the fixed incubation times, the aliquots of suspension were transferred into 1 ml of the cold medium containing 4CaCl2, 4μCi/ml, and incubated at 37 °C.

Reagents

NaCl, KCl, MgCl2, CaCl2, Na2HPO4, NaH2PO4, K2HPO4, Tris, HCl, NaOH, and ATP Na2 were obtained from BDH (London, UK). Glycerin and glucose were obtained from Merck (Darmstadt, FRG). EDTA, HEPES, and PMSF were obtained from Serva (Heidelberg, FRG). Dibutyryl-cAMP was obtained from Boehringer (Mannheim, FRG). Amiloride, furosemide, ouabain, phenanthroline, pepstatin, leupeptin, antipain, bestatin, phosphatidyl-L-serine, TPA, phosphatidyl-L-serine, TPA, a-phorbol ester (4α-phorbol, 12,13-didecanoate), and quin 2AM were obtained from Sigma Chemical (St. Louis, MO, USA). DIDS was obtained from Calbiochem, San Diego, CA, USA. [γ-32P]ATP, 86RbCl, and 43CaCl2 were obtained from Amersham (Buckinghamshire, UK). Calf thymus core histones were obtained from the Department of Molecular Biology, Moscow State University (Moscow, USSR).

Results

Phosphorylation of Band 4.9 Protein

As can be seen from Table 1, the basal 32P incorporation into Band 4.9 protein of erythrocytes of patients with essential hypertension was increased 1.6-fold to 1.8-fold as compared with that in normotensive subjects. TPA treatment increased this protein phosphorylation by 300 to 400%. Under these conditions we failed to see any differences between normotensive and hypertensive groups.

Table 1. Basal and TPA-Stimulated 32P Incorporation in Band 4.9 Protein of Erythrocytes of Patients with Essential Hypertension and of Normotensive Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>32P (cpm)</th>
<th>Control</th>
<th>+TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>132 ± 23</td>
<td>485 ± 31</td>
<td></td>
</tr>
<tr>
<td>EH (n = 12)</td>
<td>210 ± 25*</td>
<td>469 ± 32</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± SE are given. TPA = 12-O-tetradecanoylphorbol-13-acetate; EH = essential hypertensive.

Erythrocyte Volume and Shape

We found a 4% decrease in the average volume of erythrocytes in essential hypertension (Table 2). While our manuscript was in preparation, we learned that similar results had already been obtained by Bruschi et al.,16 who also reported a decrease in erythrocyte volume in essential hypertension. As can be seen from Table 2, the average volume of the erythrocytes of patients with renal hypertension did not differ from that of normotensive subjects. As can be seen in Table 2 and Figure 2, a similar but more marked decrease of cell volume may be caused by the treatment of erythrocytes of healthy donors with TPA, but not with dibutyril-cAMP.

As can be seen in Figure 3, the addition of TPA induced monoconcave cup-shaped cell formation and, during a 10-minute incubation, the quota of...
these cells increased sevenfold to ninefold (see Table 2). Unexpectedly, an approximately threefold increase in the number of monoconcave cup-shaped erythrocytes was observed in patients with essential hypertension (see Table 2).

**Protein Kinase C Activity**

As can be seen in Table 3, basal protein kinase activity in erythrocytes did not significantly differ between normotensive and essential hypertensive groups. The addition of phosphatidylserine and TPA resulted in an increase in protein kinase activity of 50 and 80%, respectively, in these groups. Protein kinase C activity in erythrocytes of patients with essential hypertension was increased by 60 to 70% as compared with that in the control group (see Table 3).

**Ion Fluxes**

The rate of Na\(^+\)-Li\(^+\) countertransport in erythrocytes of patients with essential hypertension was increased by 70 to 80% (Table 4). This observation is in accordance with data reported by Canessa et al.\(^{13}\) and later confirmed in many laboratories (see reviews in References 17 and 18). We also found that the rate of Na\(^+\)-K\(^+\) cotransport (ouabain-insensitive furosemide-inhibited component of \(^{86}\)Rb influx) in erythrocytes of patients with essential hypertension was increased by 80%. Data on the increase of this component of \(^{86}\)Rb influx in erythrocytes of patients with essential hypertension have been reported by Bin Talib et al.\(^{19}\) and Wambach et al.\(^{20}\) To our knowledge, this is the first report of the twofold increase in the rate of proton electrochemical gradient (\(\Delta\mu_H^+\))-induced Na\(^+\)-H\(^+\) countertransport in erythrocytes of patients with essential hypertension (see Table 4). The rates of Na\(^+\)-K\(^+\) pump activity and \(^{45}\)Ca influx in erythrocytes of normotensive and hypertensive patients did not differ.

As can be seen from Table 5, the addition of TPA resulted in a fourfold increase in the rate of Na\(^+\)-H\(^+\) exchange (\(p < 0.0001\)). The same results have been obtained for Na\(^+\)-H\(^+\) exchange of Swiss 3T3 fibroblasts, sea urchin eggs, human epidermal carcinoma, human leukemic cells, human fibroblasts, HeLa cells, neuroblastoma, porcine neutrophils, and rat thymocytes and myoblasts (see review in Reference 11). With the addition of TPA, the rates of Na\(^+\)-K\(^+\) pump activity, Na\(^+\)-K\(^+\) cotransport, and \(^{45}\)Ca influx were increased by 20 to 50% (\(p = 0.12, p < 0.05,\) and \(p < 0.05,\) respectively). We failed to observe any effects of this protein kinase C activator on the rate of Na\(^+\)-Li\(^+\) countertransport. It should be stressed, however, that under lithium loading both the basal and TPA-induced levels of \(^{32}\)P incorporation in erythrocyte membrane proteins are decreased twofold to fourfold (data not presented).

**Discussion**

The data obtained in this study show that the addition of protein kinase C activator (TPA) results in an increase of \(^{32}\)P incorporation in Band 4.1 and 4.9 proteins as well as in an erythrocyte shape change and in a decrease of average cell volume. Similar but less marked alterations of these parameters were observed in erythrocytes of patients with essential hypertension (see Tables 1 and 2), in which activity of this enzyme was found to be increased (see Table 3). Does the protein kinase C act on erythrocyte shape and volume through the phosphorylation of Band 4.9, Band 4.1, or some minor membrane proteins (or all three)? To resolve this problem, additional experiments should be carried out.

In the mid-1970s, we showed that the passive (ouabain-insensitive) permeability of erythrocyte membrane for univalent cations is increased both in SHR\(^{21}\) and in patients with essential hypertension.\(^{22}\) Subsequent studies revealed that this abnormality is probably due to an increase of the rate of Na\(^+\)-Na\(^+\) exchange.

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**Table 3.** Protein Kinase C Activity in Erythrocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>−PS</th>
<th>+PS</th>
<th>ΔPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>20.7 ± 1.4</td>
<td>32.1 ± 2.8</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>EH (n = 11)</td>
<td>22.9 ± 1.6</td>
<td>41.8 ± 3.3*</td>
<td>18.9 ± 1.3†</td>
</tr>
</tbody>
</table>

Means ± SE are given (in pmol \(^{32}\)P/min/mg protein). Protein kinase was measured in the presence (+ PS) or absence (− PS) of phosphatidylserine and TPA as described under Subjects and Methods. ΔPS refers to the phosphatidylserine and TPA-induced kinase activity (i.e., protein kinase C). See Table 2 for key to other abbreviations.

\( p < 0.05, \) \( p < 0.001,\) compared with respective value in normotensive subjects.

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**Figure 3.** Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the shape of erythrocytes of normotensive patients during the control period (A) and 10 minutes after the addition of TPA (B). (Final magnification \( \times 1300.\) )
The data displayed in Table 4 are in agreement with this assumption. The rates of both Na\(^+\)-Li\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport were increased about twofold in patients with essential hypertension. However, the maximal activation of protein kinase C by TPA did not modify Na\(^+\)-Li\(^+\) countertransport and enhanced the rate of Na\(^+\)-K\(^+\) cotransport by only 30% (see Table 5). Thus, the alteration of these ion transport systems found in hypertensive patients with acidified cytoplasm; i.e., \(\Delta\mu_{\text{H}^+}\)--induced Na\(^+\)-H\(^+\) exchange, being enhanced twofold in hypertension, showed a clearer connection to protein kinase C activation. The influence of TPA on protein kinase C resulted in enhanced activity (fourfold) of this carrier.

Activation of the Na\(^+\)-H\(^+\) exchanger due to the influence of protein kinase C in cells other than erythrocytes has also been described (the data are summarized in Reference 11). An increased rate of \(\Delta\mu_{\text{H}^+}\)--induced Na\(^+\)-H\(^+\) exchange similar to the one described in this report has been shown for platelets of essential hypertensive patients\(^{23}\) and thymocytes of SHR.\(^{24}\) Together with our data, this shows the presence of widespread abnormalities in the Na\(^+\)-H\(^+\) exchanger, which can also be explained by increased protein kinase C activity.

The addition of TPA resulted in a 50% increase of the rate of \(^{43}\)Ca influx in quin 2--loaded human erythrocytes (see Table 5). The rate of calcium influx in ATP-depleted erythrocytes of SHR also was found to be slightly increased.\(^{25}\) However, we failed to observe any significant alteration of this parameter in quin 2--loaded erythrocytes of patients with essential hypertension (see Table 4).

The activity of protein kinase C in cells is controlled by the level of diacylglycerol formed as a product of phosphoinositide breakdown in a reaction catalyzed by phospholipase C.\(^{26}\) Recently, it was shown that the increase of free intraerythrocyte calcium concentration is accompanied by an increase of membrane-bound protein kinase C.\(^{27}\) Numerous data on alterations both in phosphoinositide metabolism and in intracellular calcium distribution in primary hypertension were recently summarized.\(^{1-17, 18, 28}\) Further, the activity of protein kinase C may be enhanced, because of its limited proteolysis, by the calcium-dependent proteinase calpain.\(^{29}\) Evidence for enhanced activity of this enzyme in rat erythrocytes and the kidney cortex of the Milan hypertensive strain,\(^{30, 31}\) as well as in erythrocytes of patients with essential hypertension,\(^{32, 33}\) was recently demonstrated.

In accordance with these data, several explanations of the enhanced protein kinase C activity in primary hypertension are offered:

1. Alteration of enzyme activities controlling the phosphoinositide metabolism in cell membranes (for example, phosphoinositide kinases);
2. Permanent activation of protein kinase C by the natural agonists, including compounds similar to growth factors, or by Ca\(^{2+}\)--dependent proteinase;
3. Increase of the content of membrane-bound protein kinase C as a result of permanent or excitation--induced increase of intracellular Ca\(^{2+}\) concentration;
4. Genetically determined alteration of protein kinase C gene expression or alteration of cell oncogenes coding the other protein kinases;
5. Primary alterations of cytoskeleton proteins and their interaction with protein kinase C.

Further research is needed to determine which of these alternative explanations gives the most correct picture of the protein kinase C activation mechanism.

Thus, activation of protein kinase C is able to bring about changes in the erythrocyte membrane skeleton, as well as in the function of some ion carriers, and these changes are, in principle, similar to those observed in primary hypertension. This

### Table 5. Effect of TPA (1 \(\mu M\)) on the Rate of Na\(^+\)-K\(^+\) Pump Activity, Na\(^+\)-K\(^+\) Cotransport, Na\(^+\)-H\(^+\) Countertransport, and \(^{43}\)Ca Influx in Erythrocytes of Normotensive Subjects

<table>
<thead>
<tr>
<th>Normotensive group</th>
<th>Na(^+)-K(^+) pump</th>
<th>Na(^+)-K(^+) cotransport</th>
<th>Na(^+)-H(^+) countertransport</th>
<th>Na(^+)-Li(^+) countertransport</th>
<th>(^{43})Ca influx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>18.0 ± 1.5</td>
<td>2.70 ± 0.25</td>
<td>95.2 ± 16.9</td>
<td>4.15 ± 0.54</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>TPA (n = 6)</td>
<td>23.6 ± 3.2</td>
<td>3.51 ± 0.20*</td>
<td>399.8 ± 12.51</td>
<td>3.97 ± 0.61</td>
<td>0.54 ± 0.06*</td>
</tr>
</tbody>
</table>

Mean values ± SE are given (in \(\mu\)mol/L of cells/min). TPA = 12-O-tetradecanoylphorbol-13-acetate.

\(*p < 0.05, \dagger p < 0.0001, \ddagger p < 0.05\), compared with respective control value.
finding provides evidence that the same mechanism is involved both in the case of essential hypertension and in vitro TPA action. Apparently, this mechanism in primary hypertension is also present in other types of cells in which, as mentioned, membrane alterations have been observed, and the presence of increased protein kinase C activity in SHR platelets confirms this assumption.34

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