The goal of this brief review is to show the possibility of a new approach to the understanding of what we believe is the major pathogenetic basis behind the primary forms of hypertension* (i.e., a certain type of membranopathy that is characteristic of this cardiovascular pathology). This approach is based on our 15-year experience in the study of cell membrane properties in hypertension, as well as on the data of other groups working in this field. By "membranopathy," we mean the presence of widespread abnormalities in the ion transport function of the cell plasma membrane and changes in its ultrastructure as well as in certain physico-chemical properties, resulting in insufficient membrane control over intracellular calcium (the "membrane defect").

The major pathogenetically significant consequence of these alterations is the appearance of increased concentrations of cytoplasmic free calcium under physiological action on the mechanisms of cell calcium homeostasis. At least three known membrane abnormalities should be considered as a source of this free calcium excess: increased calcium influx rate in various types of cells (which occurs in hypertension despite the difference in transport systems maintaining the calcium influx in different cells), the decrease of calcium binding by the inner surface of the plasma membrane, and the alteration of adenosine triphosphate (ATP)-dependent, calmodulin-stimulated outward calcium transport (for review, see Reference 2).

The discovery of widespread membrane alterations seems to clarify the key pathogenetic mechanisms in primary hypertension: changes in the sensitivity to various agonists and excessive contractility of vascular smooth muscles, increased neuromediator charge in sympathetic terminals, and changed sensitivity and reactivity of certain other tissues (for reviews, see References 3–5).

From the outset, our own approach to the problem considered membrane alterations of cardiovascular contractile cells as only a fragment of a more generalized alteration of the cation transport in plasma membranes that would be present in several different types of cells. This point of view was first stated in my paper "Essential hypertension as membrane pathology" published in Russian in 1975.10 Then, as data (obtained in our laboratory and in others) accumulated, the idea was developed in joint papers and reivews with S. Orlov.11–13

We started in 1974 and were soon able to show (both in erythrocytes of SHR and essential hypertensive [EH] patients) the presence of a nearly twofold increase in the ouabain-independent component of Na+–Na+ exchange, which characterizes the "passive" permeability of the plasma membrane to Na+.14–16

In later studies, these data were supplemented by characteristics of other transport systems, namely sodium-potassium cotransport17 and lithium-sodium18 and Na+–H+19 countertransport. This confirmed the initial conclusion about the increased permeability to univalent cations of the erythrocyte membrane in primary hypertension. Already in the first stages of our

*Here we use the term primary hypertension both for essential hypertension in humans and its experimental model, spontaneous hypertension of rats of the Okamoto-Aoki strain (SHR).
research, the decreased calcium-binding ability of the inner part of the membrane was shown in erythrocytes of both SHR and EH patients.\(^{20}\) The latter phenomenon was also observed in plasma membranes of cardiomyocytes and adipocytes of SHR,\(^{13}\) and an increase of intracellular exchangeable calcium was revealed in adipose tissue in both types of primary hypertension.\(^{21,22}\)

Thus, by 1978–1980 certain alterations of cation transporting functions in plasma membranes had been shown in various cells (other than the cardiovascular contractile elements) in both forms of primary hypertension.\(^{11–13}\) This showed the widespread character of membrane alteration. This important trait of primary hypertension was also pointed out by Meyer’s group in 1982,\(^{23}\) with respect to SHR. Subsequently, increased voltage-dependent Ca\(^{2+}\) influx (measured by \(^{45}\)Ca) in platelets\(^{24}\) and in brain synaptosomes,\(^{25}\) increased rate of Ca\(^{2+}\) influx in quin-2–loaded erythrocytes\(^{26}\) of SHR as well as increased Ca\(^{2+}\) currents through voltage-sensitive Ca\(^{2+}\) channels in isolated vascular smooth muscle cells of SHR (patch clamp technique) were demonstrated.\(^{27}\)

At the same time, decreased activity of the calmodulin-dependent component of calcium pump was revealed in erythrocytes\(^{28}\) and platelets\(^{29}\) of EH patients and in erythrocytes,\(^{30}\) synaptosomes,\(^{25}\) and cardiomyocytes\(^{31}\) of SHR. These studies were followed up by the finding of increased cytoplasmic free calcium concentration in platelets of both EH patients\(^{32}\) and SHR,\(^{33,34}\) in erythrocytes,\(^{35}\) in brain synaptosomes\(^{26}\) of SHR, in lymphocytes\(^{37}\) and in vascular smooth muscle cells\(^{38,39}\) of SHR.

The relation between alterations of cell calcium homeostasis and abnormalities in the transport systems of univalent cations in the plasma membrane is not always sufficiently clear. The possibility of reproducing, in erythrocytes, a significant part of the membrane phenomenology characteristic of primary hypertension by means of protein kinase C (PKC) activation (see below) seems to indicate a parallel and independent (at least partly) development of changes in the univalent cation transport systems and alterations in membrane calcium handling, that are due to a common cause. On the other hand, the partial depolarization of the membrane observed in both forms of primary hypertension in various types of cells\(^{40}\) indicate in principle the involvement of membrane transport systems of univalent cations in the subsequent modification of the voltage-dependent calcium influx, as well as in the increase of its concentration via sodium-calcium exchange.

Characteristic of membrane alterations in primary hypertension is their restriction to the plasma membrane per se, and the fact that they are present in many different types of cells. This began to acquire a special importance in the light of data obtained in studies on erythrocytes concerning both the involvement of the membrane skeleton and the role of PKC in the development of these membrane alterations.\(^{41,42}\) Indirect indications of an involvement of the cytoskeleton can already be traced to the study by Sen et al\(^{43}\) in which a reduced erythrocyte volume in SHR was described. Data indicating decreased deformability of these cells in EH patients were also presented\(^{44}\); this could indicate an enhanced rigidity of the cytoskeleton. Our own study showed a reduced erythrocyte volume in EH patients similar to that in SHR, a feature independently pointed out by Bruschi et al.\(^{45}\) We suggested that the appearance of unusual cup-shaped erythrocytes was due to cell shrinking, as caused by an alteration of the membrane skeleton or increased PKC activity, or both.\(^{42}\)

Analyzing the changes in erythrocyte shape and volume, it was logical to conclude that these cytoskeleton-dependent performances might indicate the involvement of the phosphatidylinositol transmembrane signaling systems\(^{46–48}\) in membrane alteration and particularly of the part of this system that mediates signal transduction via diacylglycerol (DAG), resulting in PKC activation (the DAG/C-kinase pathway, according to Berridge).\(^{47}\) It is known that substrates for PKC are membrane skeleton proteins, phosphorylated by this kinase (see Figure 1).

This conclusion was also supported by the study that demonstrated, more than 10 years ago, an increase of the phosphoinositide (PI) metabolism in SHR erythrocytes.\(^{50}\) Alterations in PI metabolism were later also revealed in erythrocytes\(^{51}\) and platelets\(^{52}\) from EH patients and in SHR vascular smooth muscles.\(^{53}\) In short, this feature also appeared to be widespread. All this gave reason either to suspect a connection between increased PI metabolism and both the cell shape changes and the alterations of ion transport systems mentioned above or to suggest other ways of PKC activation.\(^{41,42}\)

In developing this topic, we carried out a series of studies on erythrocytes of healthy individuals to determine the influence of direct in vitro action of PKC on the cytoskeleton and on the ion transport characteristics of these cells (see Figure 1). For this purpose, we used the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), which imitates the natural activation of this kinase by DAG. In this way, we were able to reproduce a substantial part of the alterations typical of the membrane defect in hypertension. It was shown, in particular, that the activation of PKC (but not protein kinase A) reproduces the changes in erythrocyte cell shape and volume as well as cytoskeleton protein phosphorylation (bands 4.1 and 4.9), and these changes were essentially similar to those observed in primary hypertension. Further, PKC activation by TPA leads to an increase of rates in the Na\(^+\)-Na\(^+\) and Na\(^+\)-H\(^+\) transport systems to levels comparable with those observed in primary hypertension, as well as to an increased Ca\(^{2+}\) influx rate in erythrocytes.\(^{45,49}\) The next step was the direct measurement of PKC activity in erythrocytes, which revealed a nearly twofold increase, both in EH patients and in SHR, as com-
pared with controls; this increase does not appear in secondary forms of hypertension.54

The PkC activity measured in brain tissue also turned out to be significantly higher in SHR than in Wistar-Kyoto rats (WKY).55 Earlier a similar difference between SHR and WKY in PkC activity had been observed in platelets of SHR.56 Thus, activation of PkC is able to bring about changes in the erythrocyte skeleton, as well as in the function of ion carriers, and these changes are in principle similar to those observed in primary hypertension. This led us to suggest that, both in the case of hypertension and under in vitro TPA action, the same pathogenetic mechanism is involved and that it is precisely enhanced PkC activity that plays the key role in the development of membrane abnormalities in primary hypertension, or at least part of them. The use of erythrocytes as the object of study in the work described above made it impossible to analyze the significance in the development of membrane alteration of the other part of the PI signaling system, mediating signal transduction via inositoltriphosphate (IP3), followed by Ca2+ liberation from the cytoplasmic reticulum (the IP3/Ca2+ signal pathway, according to Berridge47) (see Figure 1). The fact that the phosphoinositol metabolism in the plasma membrane in cells of various types is enhanced in primary hypertension gives grounds to suggest that the activation of this part of the PI signaling system may give a substantial contribution to the increase of the cytoplasmic Ca2+ pool.

FIGURE 1. Simplified outline of phosphoinositol signaling system. (Adapted and modified from Reference 47.) A growth factor or some other agonist (A) binds with a receptor (R) that, in conjunction with a G protein (Gp), activates phospholipase C (PL C), resulting in the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2). This hydrolysis leads to the formation of two types of intracellular messengers: inositoltriphosphate (IP3) and diacylglycerol (DG). IP3 migrates into the cytoplasm, causing Ca2+ release from the cytoplasmic reticulum (CR). DG remaining in the plasma membrane (PM), activates protein kinase C (PkC), which in turn phosphorylates certain cytoskeleton proteins, initiating, along with other protein kinases, certain cytoskeleton-depending reactions (e.g., vesicle formation, cell shrinking) and activating Na+-H+ exchange (probably other ion carriers), thus inducing increased concentration of cytoplasmic Ca2+ and pH. Bold, dotted arrow shows direct activation of PkC by 12-O-tetradecanoylphorbol-13-acetate (TPA) in our study.59

All the above definitely places the PI transmembrane signaling system at the center of the search for the cause of membrane alterations as well as for the etiologic basis of this pathology.

This suggestion is in agreement with a series of known facts whose true significance becomes clear in the context of the topic developed here. These include phenomena, repeatedly demonstrated in SHR and less often in EH patients, that can be described as the result of the enhanced influence of tissue growth factors or factors mimicking their action. In particular, for SHR, these include the hyperplasia and hypertrophy of vascular smooth muscle cells in SHR (for review, see Reference 57) and the proliferation of sympathetic terminals supplying the arterial bed.58 Erythrocytosis, revealed in SHR and in EH patients53,54 as well the increased number of platelets in SHR (unpublished data obtained in our laboratory), should also be placed in the same category.

The presence of circulating humoral agents in primary hypertension, sufficiently powerful to initiate by themselves the action of tissue growth factors, could hardly have remained unnoticed. On the other hand, before these facts were observed, Folkow59 demonstrated the hemodynamic importance of resistance vessels in structural adaptation in chronic hypertension, which suggested that the underlying process of vascular smooth muscle hypertrophy might
be genetically reinforced. There is now very strong evidence of this, especially in SHR. The results of studies by Yamori et al.,\(^6\) which showed the enhanced ability of vascular smooth muscle cells of SHR to proliferate in a cell culture and possess increased sodium influx rate through the plasma membrane, clearly indicated that these properties are not due to extracellular influences but are intrinsic to the cells themselves. The source of these properties should be traced back to the cell genome.

By returning to the special traits of altered membrane characteristics in primary hypertension and combining them with the newly discovered role of PkC in their origin, we can recognize features indicating the possible involvement of cellular oncogenes (proto-oncogenes), whose altered expression in the case of hypertension could in principle explain at least a part of the membrane abnormalities described up to now.\(^5\),\(^6\)

Indeed, the plasma membrane could be considered as a kind of "screen," in which one can observe the activity of most of the known cell oncogene products. They are represented in the plasma membrane by systems carrying out reception and signal transduction, in the form of enzymes monitoring the membrane skeleton function and the rate of ion carrier translocation (for instance Na\(^+\)-H\(^+\) exchange), as well as in the form of growth-promoting factors. Abnormally operating proto-oncogenes may directly or indirectly intervene in the PI signal transduction pathway, thus activating PkC.

Conjecturing the participation of proto-oncogenes in the mechanism of plasma membrane alterations, we undertook (in collaboration with the laboratory of molecular biology of oncogene viruses, All-Union Oncological Centre, Moscow), the study of the restriction fragment length polymorphism (RFLP) of DNA on certain oncogene loci, comparing SHR and control WKY rats. This study established, in partic-

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>(\text{Na-K cotransport} (\mu\text{mol/l cells/hr}))</th>
<th>(\text{Ca}^{2+} \text{ content (in the presence of } 5\text{ mM Na}_2\text{VO}_4) (\mu\text{mol/l cells}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. WKY</td>
<td>5</td>
<td>298±36</td>
<td>21.1±1.9</td>
</tr>
<tr>
<td>2. (c\text{-src}^{+}) (homozygous)</td>
<td>8</td>
<td>251±24</td>
<td>19.1±0.8</td>
</tr>
<tr>
<td>3. (c\text{-src}^{+}) (heterozygous)</td>
<td>14</td>
<td>379±22</td>
<td>22.2±1.5</td>
</tr>
<tr>
<td>4. (c\text{-src}^{+}) (homozygous)</td>
<td>4</td>
<td>449±96</td>
<td>24.1±1.8</td>
</tr>
<tr>
<td>5. SHR</td>
<td>5</td>
<td>431±50</td>
<td>40.7±3.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \(n\) is the number of rats; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Reprinted with permission from Kotelevtsev et al.\(^6\)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>(%)</th>
<th>(\text{Ca}^{2+} \text{ content} (\mu\text{mol/l cells}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(s\text{rc}^{+})</td>
<td>50</td>
<td>40.7±3.1</td>
</tr>
<tr>
<td>(s\text{rc}^{+})</td>
<td>30</td>
<td>30.7±2.1</td>
</tr>
<tr>
<td>(s\text{rc}^{+})</td>
<td>20</td>
<td>20.7±1.1</td>
</tr>
<tr>
<td>(s\text{rc}^{+})</td>
<td>10</td>
<td>10.7±0.1</td>
</tr>
</tbody>
</table>

These data demonstrate that \(c\text{-src}^{+}\) locus cosegregates with the genome locus that determines the value of sodium-potassium cotransport.

Calcium content in erythrocytes of the homozygous \(c\text{-src}^{+}\) group was equal to that of WKY rats and differs from the heterozygous \(c\text{-src}^{+}\) group (which has an intermediate value) and from the homozygous \(c\text{-src}^{+}\) group, in which the rate of sodium-potassium cotransport was equal to that of SHR. These data demonstrate that \(c\text{-src}^{+}\) locus cosegregates with the genome locus that determines the value of sodium-potassium cotransport.

Continuing this study, our group,\(^6\) undertook an investigation to determine the cosegregation of \(c\text{-src}^{+}\) alleles in second generation hybrids (SHR×WKY)\(^2\), with both the rate of sodium-potassium cotransport and \(Ca^{2+}\) accumulation (in the presence of the calcium ATPase inhibitor orthovanadate) in erythrocytes. In our previous study, a positive correlation of these parameters with the level of blood pressure was demonstrated on (SHR×WKY)\(^2\) hybrids.\(^5\) Second generation hybrids (SHR×WKY)\(^2\) were segregated into three groups according to their \(c\text{-src}^{+}\) genotypes: two homozygous groups, corresponding to SHR or WKY rats (denoted by \(s\text{rc}^{+}\) and \(s\text{rc}^{+}\)) and one heterozygous group (denoted by \(s\text{rc}^{+}\)). Genotypes were determined in restriction fragments of DNA (HindIII and \(PstI\) restrictases were used), hybridized with \(v\text{-src}\). As can be seen from Table 1, the rate of sodium-potassium cotransport in the homozygous \(s\text{rc}^{+}\) group was equal to that of WKY rats and differs from the heterozygous \(s\text{rc}^{+}\) group (which has an intermediate value) and from the homozygous \(s\text{rc}^{+}\) group, in which the rate of sodium-potassium cotransport was equal to that of SHR. These data demonstrate that \(c\text{-src}^{+}\) locus cosegregates with the genome locus that determines the value of sodium-potassium cotransport.

Although the specific mechanism of \(c\text{-src}^{+}\) participation in membrane performances under discussion is obscure and requires further investigation, the results already obtained at this stage of the study confirm our conjecture of proto-oncogene involvement in the development of the widespread membrane alteration in primary hypertension.

A product of the proto-oncogenes \(c\text{-src}^{+}\) tyrosine kinase \(p60^{c\text{-src}^{+}}\), localized in the plasma membrane, interacting with PkC and effecting growth-promoting action in cells (for details, see References 66, 67), \(p60^{c\text{-src}^{+}}\) is expressed in a large amount of highly differentiated cells, which could indicate an important role of this oncoprotein in the normal functioning of the plasma membrane and of the cells as a whole.
study of cell membrane properties in hypertension is in agreement with the opinion that both its forms discussed here have deep pathogenetic similarity, although it does not mean we view them as identical. How far can we go in assessing the pathogenetic similarity on the genome level between spontaneous hypertension in rats and essential hypertension in humans? This should be resolved by further research.

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