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ARTERIAL HYPERTENSION

ARTERIAL HYPERTENSION THE RED CELL IN HUMAN

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SUMMARY

A survey of the current state of some haemodynamic factors and red cell involvement in arterial hypertension is presented. Blood viscosity, as one of the major determinants of flow resistance, may play a significant role in blood pressure regulation. Red cell rigidity, in addition to some other rheologic abnormalities, might contribute to the elevation of blood viscosity and consequently to worsening flow conditions, either in hypertensive human or experimental animal models. Structural disturbances in red cell membrane and abnormalities in transmembrane ionic fluxes may assume great pathophysiological significance, where affecting red cell deformability and consequently blood viscosity.

INTRODUCTION

In the light of the classic pathophysiological concepts, any tentative to associate the pathogenic mechanisms of arterial hypertension to some red cell features would be a little less than heretic.

According to far distant viewpoints, the increase in blood pressure was claimed to result from an isolated dysfunction that, centred on the kidney, would determine an increase on the vascular resistance.[1] This apparently convincing relationship could be linked by the renin-angiotensine system and its functional interaction with aldosterone secretion and the sympathetic nervous system. Due to some contradictory results, where emerged the individualization of several types of arterial hypertension, it was realized that the increase on blood pressure could not be the consequence of hyperactivity of the renin-angiotensine alone (for reviews, see [2-4]).

In the present, the mosaic theory of hypertension of Page [5, 6] is no more the fuzzy thinking as was looked some years ago. According to this concept, a number of regulatory components may be interrelated to control blood pressure and tissue perfusion.

However, the cause for the elevated arterial pressure in 90-95 % of patients cannot be determined. These patients have essential (or primary) hypertension (EH), in opposition to the secondary hypertension of a well-known established cause. Since EH is not an homogeneous entity (where some inherited defects seems to be involved), the mechanisms underlying blood control disregulation may differ between clinically similar patients. Whatever the number of factors involved in EH, this pathologic disorder must be recognized as a chronically consequence of vascular changes (Fig. 1), that are associated to increased blood pressure (for reviews, see [4, 7]).

Haemodynamic factors are amongst these possible regulatory participants, being the total peripheral resistance and cardiac output believed as the major physiologic determinants of arterial pressure (for reviews, see [7,8]). The resistance to blood flow in turn depends on the product of blood viscosity and vascular hindrance.

Although the significance of changes in the blood rheology has been generally negleted, there is strong evidence that such disturbances might increase flow resistance (for review, see [9]). In part, those abnormalities could reflect a decrease in red cell deformability.

A great deal of interest about the possible importance of red cell in essential hypertension was derived since abnormalities in sodium transport were first reported in 1960, in erythrocytes from hypertensive subjects.[10] The consequence was a flood of reports (and a great deal of controversy) showing marked changes in transmembrane fluxes of Na+, K+ and Li+ in erythrocytes from both essential hypertension humans (EH) and genetically spontaneous hypertensive rats (SHR, for review see 11). Comparable alterations have been also detected in other circulating cells or tissue (e.g. vascular smooth muscle).[11, 12]

The pathophysiological meaning of these changes is still poorly understood. As some of them appear frequently in families with a high propensity to EH, they might be used to identify subjects that have or are destined to have EH. This perspective, still questionable, would be in line with recent epidemiological observations that have demonstrated a relatively strong genetic component on the determination of the blood pressure.[13]

Generally, the alterations observed in red cell transport systems would represent part of a more widespread abnormality involving different cell membranes.[14] A series of facts suggest that cell membranes may be considered a basic determinant affecting the regulation of blood pressure.[12] The availability and ease of manipulation

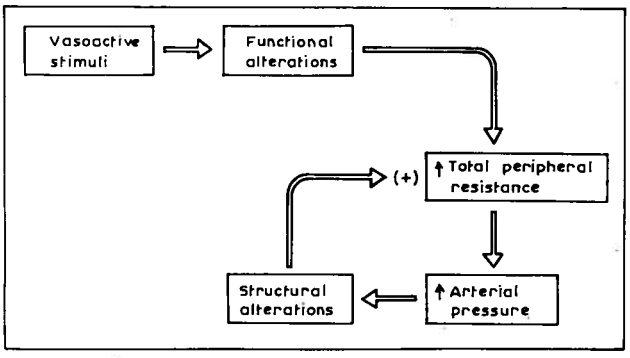

Fig. 1: Proposed common pathway that leads to increased arterial pressure in most forms of hypertension. According to recent views, a complex interactions of multiple factors may account to the increased total peripheral resistance that results in established hypertension. Vasoactive stimuli (e.g. neurogenic, hormonal and/or miogenic changes) might be the cause of functional alterations in vascular smooth muscle (pressure independent) that preceedes the development of high blood pressure. The maintenance of increased wall stress and/or lesions in the cellular components in the vessel wall might result in structural abnormalities in vasculature and subsequent impairement of pathological conditions which account for sustained hypertension.

would make the cells an excellent representative of more difficulty accessible tissues.

Although a direct causal relationship between red cell ion transport and hypertension seems unlikely, there are reasons to believe that an increase in arteriolar tone, and consequentely the development of hypertension, might be linked to previous alterations in cation involvements (Na^+-Ca^{++} exchange) or vascular smooth muscle.[15] In any case, those ion transport abnormalities might indicate localized changes in membrane organization (possibly involving lipid-protein interactions) [14] or the effect of circulating factors.

The ability of circulating red blood cells to deform is determined by the physical properties of the cell membrane, in addition to the internal viscosity of the cell and its shape/volume relationship.[9, 16] Therefore, changes in membrane fluidity could theoretically have some influence on the rheologic properties of the erythrocytes in hypertension. Whether those rheologic alterations will contribute to the development of hypertension is still open to further discussion.

In this overview some essential features of the pathogenic mechanisms of hypertension are outlined, an attempt is made to correlate blood viscosity with vascular resistance, and the rheologic behavior of red blood cells is emphasized in connection with structural disturbances in red cell membrane and transmembrane ionic fluxes.

HAEMODYNAMICS AND BLOOD VISCOSITY FACTORS

Beyond a diversity of pathogenic factors, elevated pressure in EH patients or its best experimental animal model, the spontaneously hypertensive rat (SHR), is mostly associated with haemodynamic disturbances. In this terms, the abnormal and progressive increase in arterial pressure must be dependent by either an elevated total peripheral resistance and/or increased cardiac output.

Although in most forms of hypertension (including the restrict group of secondary forms) cardiac output remains normal, all show increased total peripheral resistance to blood flow (for reviews, see [2,3]).

In macrocirculation, the overall vascular resistance (R) is primarily determined by a relation between the arterial-venous pressure drop (ΔP, equivalent to the arterial pressure) and the forward flow, of blood, given by the cardiac output ($\dot{Q}$), as follows: $R = \Delta P/\dot{Q}$. As blood flows in the microcirculation (consisting of arterioles, capillaries and venules) a different species of restriction appears, affecting the delivery of oxygen and nutritive substances to tissues. At this vascular territory, when the major portion of the pressure drop occurs, the resistance through a simple vessel or vascular network is equal to the product of vascular hindrance (Z) and blood viscosity (η).

Vascular hindrance, representing the contribution of vessel geometric factors (without blood viscosity interferance) is expressed by the Poiseuille-Hagen law, where Z is proportional to the inverse fourth power of the vessel radius: $Z = \frac{1}{r^4}$ Therefore vasoconstriction causes an increase in Z whereas vasodilation leads to lower Z (for review, see [16]).

Although the effects of slight modifications in blood viscosity have been considered insufficient to explain the elevated pressure in certain forms of hypertension [3], significant variations in blood viscosity have been described in cardiovascular disorders [17-19] including sustained and borderline EH [17, 20-25]. Similar increases in blood viscosity was detected in SHR [26, 27]; in these experiments the reduction of blood viscosity by isovolemic hemodilution was followed by a significant increase of high blood pressure in hypertensive rats[28]. Moreover, blood viscosity in parallel with arterial pressure are reduced by some non-diuretic antihypertensive drugs, either in hypertensive patients [23, 29-31] or experimental animal models [32]; a direct correlation between whole blood viscosity and mean arterial pressure could be observed [25, 30,31].

These findings might indicate that rheological changes are associated with to increased blood pressure, even at the stage preceding established hypertension.

The haemodynamic role of increased blood viscosity in the elevation of blood pressure was also attributed to a malfunction of hipothetical vascular viscosity receptors [33]. However, there is presently no clearly defined pathogenic relationship between such changes.

Blood viscosity is a function of haematocrit, plasma viscosity, red cell aggregation and red cell deformability [9]. The interplay between these variables and the extent of their contribution to overall blood viscosity depends on the local conditions of blood flow.[34] Although some conflicting results have occasionally emerged, significant changes in all these major factors of blood viscosity have been described in EH patients.

In the early study of Tibblin and co-workers,[21] the increase in haematocrit of hypertensive patients was considered as the primary contributing factors to elevated blood viscosity; a possible role could be also suspected for fibrinogen. More recently, rheologic changes other than increased haematocrit were shown clearly responsable fot the elevared blood viscosity in sustained [24, 30, 31, 35-38] and borderline [25, 31, 33] EH.

Blood viscosity remained higher even when evaluated in hypertensive patients with matched haematocrit values; thus the increase in haematocrit only partly could explain the observed blood hyperviscosity in all patients.[25] Moreover, the association of higher haematocrit with EH has hot been confirmed in some other clinical studies,[29, 35, 37] where increased blood viscosity was also not detected,[29, 35] unless corrected to a standard haematocrit.[37]

Either in borderline or established EH, the magnitude of blood viscosity elevation, as well as the factors determining such increase, were dependent upon the shear rates normally existing in the blood vessels. At high shear rates approximately equivalent to blood flow conditions in the microcirculation,[34] the increase of blood viscosity was between 1/2 and 1/3 less than at lower shear rates prevailing in large vessels.[24, 25] Whereas at these low shear rates the viscosity of blood was largely determined by increased haematocrit plus higher plasma viscosity and red-cell aggregation, at higher shear rates the elevation of blood viscosity was due to higher haematocrit and plasma viscosity alone.[17, 20, 24, 25]

The increase in plasma viscosity and red cell aggregation was apparently due to the elevation of fibrinogen concentration both in borderline or established EH [24, 25, 30, 31] and SHR.[26, 27] In the sequence to these studies, it might be concluded that elevated haematocrit, plasma viscosity and red cell aggregation, by increasing blood viscosity, are potencial determinants of higher blood pressure.

These findings might be supported by some clinical [35] and experimental studies,[27, 28] although being contested by others.[29, 37] The reasons for such discrepancies are not clear but could reflect the diversity of the patients studied or, more generaly, the heterogenous pathogenic nature of the hypertensive disorder.

Whereas there is general agreement on the occurrence of increased plasma viscosity and fibrinogen concentration in association with arterial hypertension, conflicting reports have appeared upon the contribution of red cell deformability. A clear reduction of whole blood filterability, worsing with time, was observed in patients with malignant phase hypertension,[37] in apparent dependence of plasma proteins and other factors. Such findings in blood filterability are in accord with our previous results on whole blood filterability in moderate EH patients [36] and studies of other groups.[35, 38, 39]

As whole blood filterability depends on the external fluid viscosity acting on cell surface, haematocrit and red cell aggregation, in addition to the degree of erythrocyte deformation,[9] filterability tests where recently assessed by us on red cell suspensions; even so, a clear decrease in erythrocyte filterability was observed in a group of hypertensive subjects.[40]

In other report, using the measurement of erythrocyte internal viscosity (Tk), the presence of low deformable red cells was suggested as an associated cause of hypertension.[33] Red cell deformability, as measured by a filtration technique, is also impaired in experimentally hypertensive dogs.[32]

In contrast to these findings some reports on EH [29] and SHR [26, 27] did not show significant differences in red cell deformability between hypertensive and normotensive groups.

Beyond the contribution of decreased cell deformability to the viscous resistance of blood to flow, an elevation of red cell 2,3-diphosphoglycerate concentration is claimed for hypertensive patients;[36, 41, 43] 2,3-diphosphoglycerate levels were significantly, correlated with systolic, mean and diastolic blood pressures.[42]

If such increase of 2,3-diphosphoglycerate reflects a compensation [44] for tissue underperfusion associated with estabili-

shed hypertension, a parallel right-shifted deviation of the oxyhemoglobin curve (increased P50) should be expect. Such relationship would aggree with the increased oxygen consumption that has been observed in human hypertension [45] and SHR,[46] although with unchanged 2,3-diphosphoglycerate levels.[46] However, the absence of significant increase of P50 in the hypertensive patients, as shown in our studies,[36] might suggest some other physiological role for increased 2,3-diphosphoglycerate in rigid erythrocytes.

SODIUM TRANSPORT ABNORMALITIES

In the sequence of the original demonstration of increased red cell sodium concentration and sodium-to-potassium ratio in patients with essential hypertension,[10] similar abnormalities in sodium transport were detected in white blood cells [47] and proposed to other tissues for this type of pathology.[15, 48, 49]

Since then, and in despite to some recent uncertainty and doubts about methodology and adequacy of matching hypertensive to normotensive subjects,[50] a variety of abnormalities on red (and white) blood cells have been associated to EH, and also partly sustained in SHR.[11] Similar alterations have been also observed in the normotensive relatives of such patients.[11]

The three pathways for sodium transport that have received the greatest attention are the sodium pump[51], sodium-potassium cotransport[52] and sodium-lithium countertransport[53]. The mechanisms of these different Na+ transport pathways in human erythrocytes (represented in Fig. 2) are briefly resumed in following.

As in all other cells, most of the extrusion of Na^+ from the red cell is carried out by a ouabain-sensitive complex functional protein, the Na^+, K^+-ATPase. This active transport mechanisms of Na^+, closely coupled with K^+, generates a Na^+ and K^+ electrochemical gradient across the cell membrane, in dependence of the energy supplied by the metabolism of glucose; the same pump unit that extrudes Na^+ carries K^+ inward, in a approximate ratio of $3Na^+/2K^+$; in the stationary state, the K^+ gradient is about 30/1 (intracellular/external), being the intracellular Na^+ concentration about 15 times lower than the external Na^+ concentration. Failure in red cell ATP concentration impairs the transport of Na^+ and so diminishes the gradients.

The Na^+, K^+ pump-activitty is also dependent on the concentration of both cations: an increase on Na^+ internally or an excess of external K^+ stimulates pump activity until a maximum is reached. *In vivo* and under normal conditions, the pump is in a steady-state, determined by the extra — and intracellular Na^+ and K^+ concentrations, that corresponding to a submaximum estimulation. Due to the continuous Na^+ and K^+ leaks through the lipid bilayer (passive permeability) both cation gradients tend to be dissipated unless the pump activity is preserved.[51]

The remaining two Na^+ transport systems do not derive energy from the hydrolysis of ATP but rather from the electrochemical gradient of another participating ion. Both pathways are responsible for 10 to 20 percent of the total residual fraction of the Na^+ and K^+ fluxes. If the participating ion must be situated on the opposite side of the membrane, the system is called countertransport; if it is in the same side of the membrane, it is called cotransport.

The Na^+-cotransport system (ouabain-resistant and furosemide-sensitive) promotes a coupled efflux or influx of both Na^+ and K^+ ions, driven by an appropriate transmembrane electrochemical gradient of the co-ion. Thus the net-outward uphill Na^+ transport is stimulated by internal Na^+, whereas the inward cotransport is stimulated by external K^+. Under basal conditions, the inward and outward ion cotransport movements compensate for each other.[52]

In human erythrocyte the cotransport fluxes are about 10 times lower than those dependent from the Na^+, K^+ pump. It has been suggested that the pathway remains inactive whereas the intracellular sodium concentration is kept at acceptable levels by the pump; above a critical level, when the Na^+ pump is maximally stimulated, any increase on intracellular Na^+ concentration activates the Na^+, K^+-cotransport. More generally, these systems might participate as a gradient regulator, where any change in internal Na^+ or external K^+ levels activates a cotransport flux to the opposite membrane side of the ionic alteration.

The Na^+-K^+ (Li^+) coutertransport pathways involves a 1:1 exchange of internal for external Na^+ ion. This system (ouabain-resistant and phloretin-sensitive) may accept Li^+ ions to exchange for Na^+ or other Li^+ cations. As the countertransport pathway hardly effects the overall distribution of Na^+ across the cell membrane, it seems unlikely to be of any physiological relevance.[53].

Flux changes across the red cell membranes and involving all

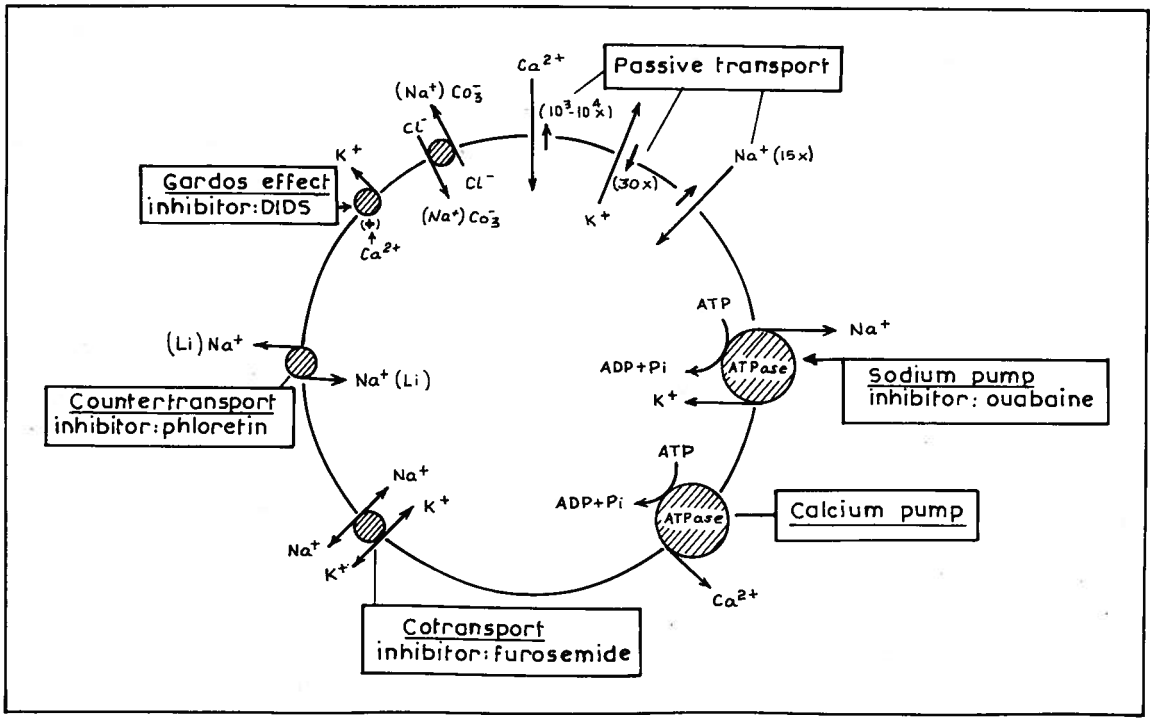

Fig. 2: Cation transport systems in normal erythrocyte.

described systems have been reported in human hypertensives and SHR. These abnormalities would aggree with the described increase in intraerythrocyte Na^+ concentration,[10] if recent studies have not questionated such findings.[11, 50]

In contrast to the decreased pump activity described in leucocytes of patients with EH,[47, 54] conflicting results emerge from studies on red cells (Fig. 3)

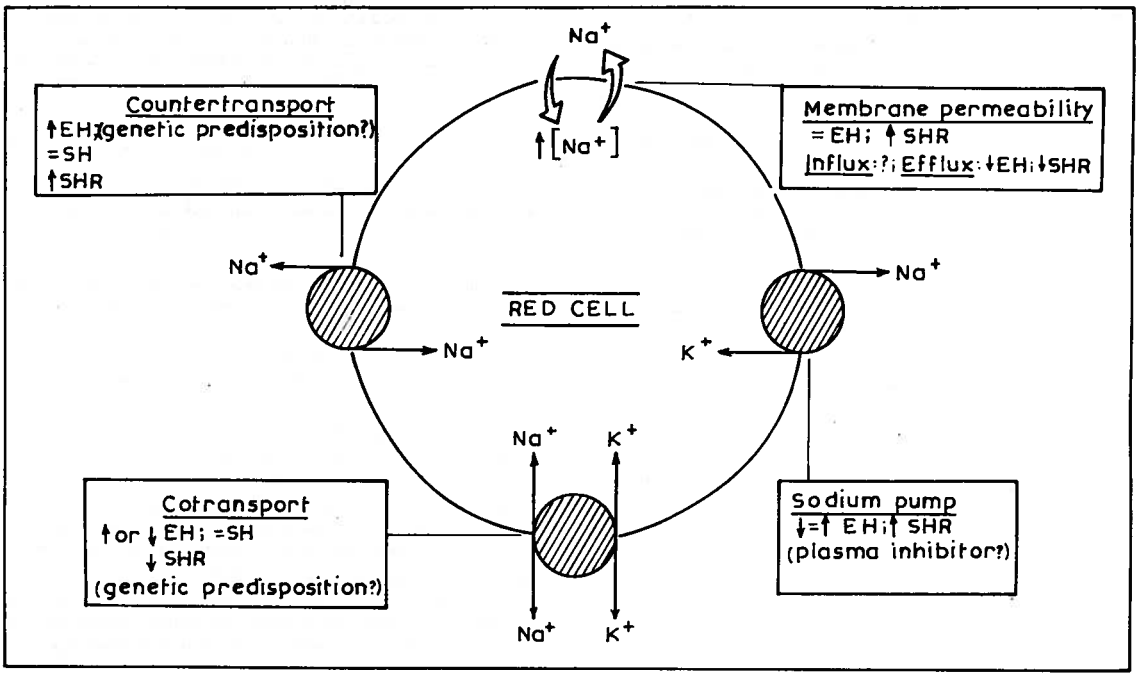

Fig. 3: Sodium transport systems in human and experimental hypertension. Legend — EH: essential hypertension; SH: secondary hypertension; SHR: spontaneously hypertensive rats; ↑ = ↓: values higher, equal or lower than normal controls.

Preliminary results in erythrocytes of EH patients showed that the Na+, K+-ATPase activity was normal or slightly increased. [55] No differences on the red cell ouabain-sensitive pump were also detected between hypertensives (with and without medication) and normotensive subjects.[56-58] Such observations are in conflict with the data of Postnov et al[59] and some of our results.[60, 61]

According to Postnov et al,[59] the decrease of Na^+, K^+-ATPase activity in hypertensive erythrocytes (mostly from subjects with recently stopped medication) could be due to a decrease of the binding ability of the inner part of the red cell membrane for calcium. This membrane defect was suggested as a local manifestation of a more general cell membrane alteration affecting the control mechanisms of EH.[12]

In a previous study of our laboratory, the decrease in red cell ouabaine-sensitive Na^+, K+-ATPase activity was shown in a group of hypertensive subjects, with and without medication.[60] More recently, a significant decrease in that ATPase activity was limited to the untreated patients.[61]

Furthermore, in the plasma of patients with EH, an inhibitor of the Na+, K+ ATPase was observed. [62] However, it remains to be convincingly demonstrated that this inhibitor influences ion fluxes or Na^+, K^+-pump activity in the erythrocyte membrane.

According to the initial studies of Garay et al,[55, 63] the outward furosemide-sensitive Na^+, K^+ cotransport was decreased in erythrocytes of patients with EH, as compared to normotensives or patients with renovascular hypertension. These observations, initially confirmed by other groups,[47, 64] would suggest that the absence of the cotransport pathway in EH played an aetiological role in the development of the high intracellular Na^+ concentration, and might represent a genetic marker for the disease.[55, 58, 63] Other studies have not confirmed those findings, with some groups reporting an increase in Na^+-K^+cotransport and others observing no change.[56, 65-67]

Further investigation have shown that the Na^+-Na^+/Li^+ countertransport across erythrocyte membranes is also increased in patients with EH but appears normal in secondary hypertension.[65, 68, 70] This abnormality seems to be most pronounced in patients with a genetic predisposition to EH [65, 71] and shows an apparent dependence on a dialysable plasma factor.[72]

In contrast, other studies have not confirmed any alterations in the countertransport pathway between essential hypertensive and normotensive subjects.[67]

Therefore, the interaction of the various components of sodium transport in the red cell membrane from essential hypertensive subjects are complex and yet poorly understood. The effect of treatment of hypertension on these systems remains to be clarified, although some observations have already suggested that some medication in use might reverse the underlying changes in membrane transport.

FUNCTIONAL AND STRUCTURAL ALTERATIONS OF THE MEMBRANE

A common membrane defect has been proposed to explain some functional alterations demonstrated in erythrocytes and a variety of cell types in essential hypertension and spontaneously hypertensive rats.[12] Such *membrane concept* may be considered the result of a vast accumulation of data, initially provoked by studies in the vascular smooth muscle.

In addition to the observed increase of water and sodium contents in the renal artery wall of hypertensive subjects,[48] further observations showed that the levels of water and some ions (Na^+, K^+, Cl^-, Ca^{2+}) were also elevated in experimental hypertension.[73-75] Proceeding from these observations, a relationship between vascular reactivity, vasoconstriction and ion balance changes was suggested.[49, 76]

Later on, increased K^+ and Cl^- turnover [77] and higher rate of

Na^+ efflux [78] were noted in arterial smooth muscle cells of hypertensive rats; a decrease in membrane calcium binding paralel to increased Ca^{2+}, Mg^{2+}-ATPase were also reported to normotensive rats.[79]

Although attractive, the derived hypothesis[81] that enhanced sodium concentration in vascular smooth muscle may increase intracellular calcium levels in hypertension, and so stimulte the vascular tone,[15] is still questionable. In alternative to the Na^+-Ca^{2+} exchange concept, the activation of the actomyosin complex in hypertension might due primarily to an alteration of membrane regulation over intracellular Ca^{2+} binding and concentration (for review, see 12).

Later studies have demonstrated that such ionic alterations are not limited to the cardiovascular contracting cells but are indeed extensive to other cells, either in human or experimental hypertension. In addition to the described alterations in countertransport, cotransport and sodium pump systems for univalent cations in erythrocytes of essential hypertensive patients (see Sodium Transport Abnormalities), similar membrane defects have been detected in SRH erythrocytes. Thus, erythrocyte membrane ouabain-independent permeability to Na^+ and K^+ is increased in SHR,[78, 82-84] apparently due to an intrinsic defect in cell membrane [85] genetically linked to the hypertensive process.[86, 87]

Further studies suggested that such altered cation transport in SHR erythrocytes, as well as in essential hypertensive patients, is associated with an abnormal distribution of intracellular calcium [58, 82] (Fig. 4). Furthermore, erythrocyte membrane of both the SHR [88, 89] and patients with EH [90] differ from those of normotensive controls by their higher passive permeability to calcium and increased Ca^{2+} concentration. No such alteration were observed in steroid-induced hypertensive rats.[88]

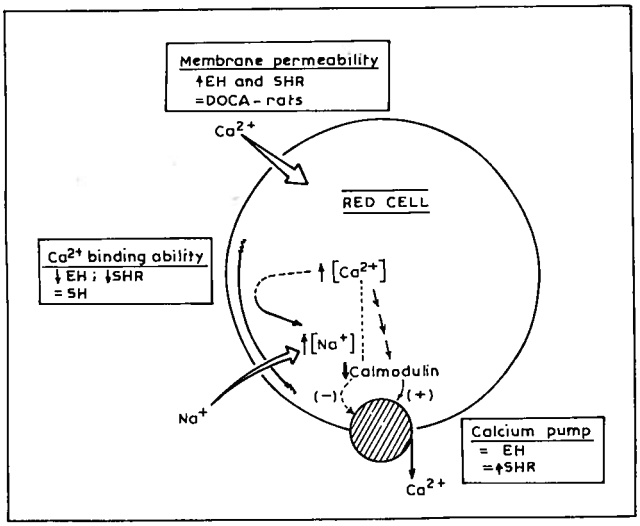

Fig. 4: Proposed abnormalities of red calcium transport and activity in human and experimental hypertension.
Legend — the same as in Fig. 3.

A decrease in Ca^{2+} binding by the cytoplasmic side of the membrane was shown, both in SHR and EH erythrocytes,[91-93] but not observed in erythrocytes of patients with chronic renal hypertension.[92] The decrease in Ca^{2+} binding ability, atributed to a reduction in the number of calcium binding sites on the inner surface of the membrane,[89, 91] might be directly related to the increased passive permeability of the erythrocyte membrane for Na^+ and K^+ [58, 82] and decreased Na^+, K^+-ATPase activity.[58] A similar decrease in sodium binding to the inner side of the membrane was observed in erythrocytes of essential hypertensive patients.[94] These data seem to indicate the presence of structural alterations in the erythrocyte membranes of EH patients and SHR, located in the inner part of the membrane that determines the permeability to univalent cations and probably enhance Ca^{2+} influx and intracellular concentration. In fact, the properties of erythrocyte membranes seem dependent on the effects of calcium on membrane conformation.[95]

The decrease in Ca^{2+} binding ability was also reported in plasma membrane of adipocytes,[96] cardiomyocytes, hepatocytes and synaptosomes,[89, 97] and in the microsomal fractions of arterial smooth muscle cells [98] from spontaneously hypertensive rats, compared with normotensive controls. This could suggest that defective calcium handling by membranes (as well as other ionic transport abnormalities) being extensive to different cell types, indeed present great pathogenic importance.

The possible role of calcium and other divalent cations in the pathogenesis of essential hypertension has been receiving increasing support.[99, 100] If such interaction do exist in excitable cells, as suggested for vascular smooth muscle of SHR,[101, 102] the resultant intracellular cationic changes (particularly calcium and sodium overload) could be involved in increased blood pressure.

The increased intracellular Ca^{2+} concentrations, as observed in erythrocytes of SHR [103] and patients with EH,[90] may be due to enhanced passive calcium influx and/or to a decreased rate of active calcium extrusion. A defective interaction between calmodulin and the Ca^{2+}-ATPase in erythrocyte membrane of SHR and patients with essential hypertension,[93, 104] also shown in plasma membranes of SHR brain nerve tissure,[104] would support an increase of the intracellular Ca^{2+} pools not limited to erythrocytes.

However, the observation [88] that the specific activity of Ca^{2+}-ATPase is already increased in erythrocyte membranes from SHR suggests an higher rate of Ca^{2+} extrusion, parallel to an increase in passive calcium influx. According with this report, the elevated intracellular calcium concentration would induce a compensatory activation of the Ca^{2+}-ATPase, as evidenced by a higher affinity of the enzyme for Ca^{2+}. Such alterations might be related to some detected abnormalities of the phospholipid composition in SHR erythrocytes membranes, but absent in DOCA rats.[88]

Although the Ca^{2+}-ATPase activity of erythrocyte membranes did not differ between SHR and normotensive controls,[89] there are cumulative data that support the involvement of membrane lipid alterations in the described abnormalities of calcium transport in SHR erythrocytes.

Recent fluorescence polarization studies showed that the microviscosity of erythrocyte membranes from SHR is higher than in normotensive controls.[105] These findings, confirmed in patients with EH but inexistent in patients with renal hypertension,[92] are in agreement with our results in erythrocytes of essential hypertensive patients.[61] Therefore, it seems reasonable to conclude that the erythrocyte membranes of SHR and essential hypertensive patients have increased rigidity of the lipid bilayer. This alteration seems to be also extensive to the plasma membrane of hepatocytes, cardiomyocytes and of synaptosomes from both SHR or hypertensive-prone rats.[97, 106] These last results would confirm a genetic abnormality in hypertensive rats leading to diffuse alterations of cell membrane structure.

In addition to increased microviscosity, the polarization studies showed a decreased rate of lateral diffusion, both in the lipid bilayer and in sites of lipid-protein interactions in the erythrocyte membrane of essential hypertensive patients.[92] Such abnormalities might be related to specific defects in lipid or protein components of erythrocyte membranes, as well as altered ionic fluxes and enzymatic activities reported in EH patients and SHR.

Although contradictory, it has been recently reported that the content and/or metabolism of inositol phospholipids are altered in erythrocyte membranes of SHR, even before they became hypertensives.[107, 108] This fact acquires a special dimension as one realizes that such minor class of phospholipids may be involved in the Ca^{2+}-gating system of plasma membrane that controls the transport of surface-bound Ca^{2+} ions into the cell.[109] Moreover, both the cation-transporting ATPases in the human erythrocyte membranes have been shown to depend upon the inner lipid monolayer, the Na^+, K^+-ATPase activity being governed by phosphatidylserine alone whereas the activity of Ca^{2+}, Mg^{2+}-ATPase is affected by the total glycophospholipids.[110-111] Therefore, changes in plasma membrane phospholipids affecting cell membrane fluidity could modify cation ionic fluxes, as demonstred in hypertension.

The exact functional repercussions of the well established interrelation of red cell AChE and phosphatidylinositol [112] also requires further studies. Apparently, both the substract and an inhibitor of

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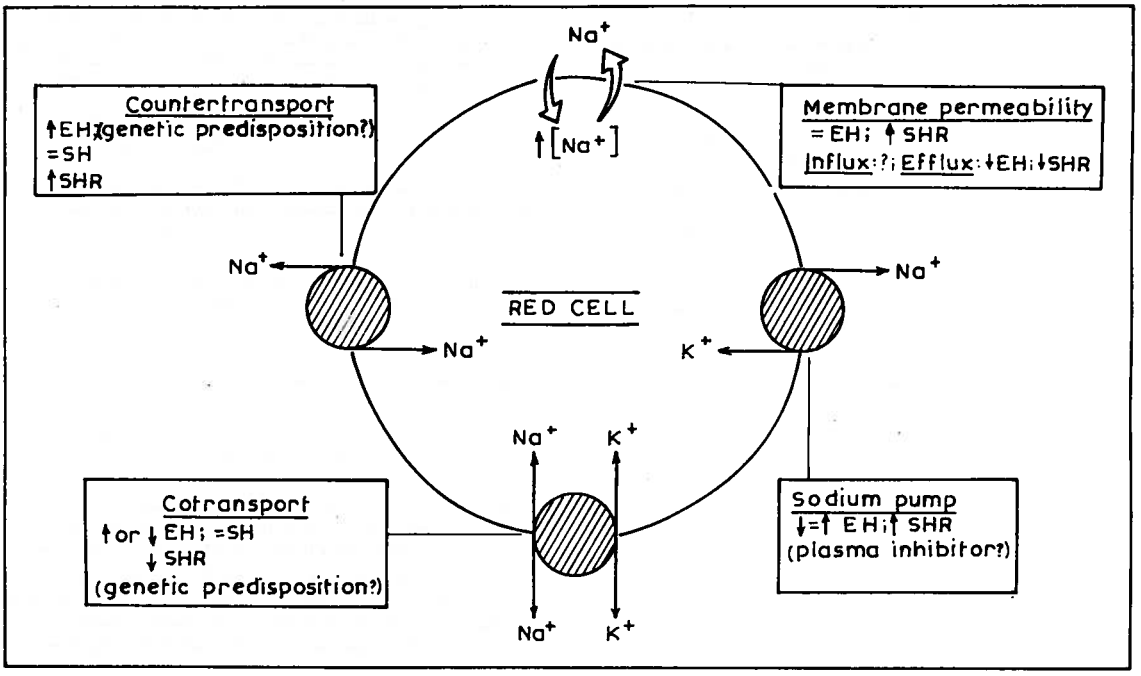

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Later on, increased K^+ and Cl^- turnover [77] and higher rate of

AChE influence Ca^{2+} binding to erythrocytes of SHR and essential hypertensive subjects.[113] If increased AChE activity in erythrocytes of EH patients [60, 61] is related to altered calcium transport can not be presently ruled, in or out.

The hypothesis suggesting the existence of localized changes in membrane organization, possibly involving lipid-protein interactions is in accord with results upon temperature-dependent cation fluxes in erythrocytes of patients with EH [114] and spontaneously hypertensive rats.[115]

Beyond previously described differences in lipid composition, recent studies have also pointed to localized structural modification in the membrane protein of SHR erythrocytes, one group showing a decrease [116] whereas another group claims for an increase of band 3 protein.[117] In a preliminary work of our laboratory,[40] also a decrease in band 3 protein in association with other minor abnormalities of the electrophoretogram pattern was evidenced in erythrocyte membranes of EH patients.

CONCLUSIONS

There in increasing evidence that changes in structural and functional properties of a diversity of cell membranes are associated with primary human and experimental hypertension and even observed at a prehypertensive stage.

In this concern, the disturbances observed in erythrocyte membranes would be acting as markers of such widespread abnormality, probably genetically determined. Such red cell membrane alterations may interfere with cell deformability during flow through capillary blood vessels; in association with some others modifications of blood composition, the resulting increase in whole blood viscosity would contribute to enhance vascular peripheral resistance.

However, the link between elevated arterial hypertension and occurring alterations in red cell membrane conformation and transport still wait further insight.

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BIBLIOGRAPHY

1. GLODBLATT, H.; LYNCH, J.; HANSAL, R. F.; SUMMERVILLE, W. W.: Studies on experimental hypertension. Production of persistant elevation of systemic blood pressure by means of renal ischaemia. *J. Exp. Med.*, 1934; 59: 347-379.
2. FROHLICH, E. D.: Hemodynamic factors in the pathogenesis and maintenance of hypertension. *Fed. Proc.*, 1982; 41: 2400-2408.
3. FROHLICH, E. D.: Mechanisms contributing to high blood pressure. *Ann. Int. Med.*, 1983; 98 (part 2): 709-714.
4. DAVIDMAN, M.; OPSAHL, J.: Mechanisms of elevated blood pressure in human essential hypertension. *Med. Clin. Nth. Am.*, 1984; 68: 301--320.
5. PAGE, I. H.: The nature of nature of arterial hypertension. *Arch. Intern. Med.*, 1963; 14: 103-115.
6. PAGE, J. H.: Pathogenesis of arterial hypertension. *J. Am. Med Ass.*, 1969; 140: 451-462.
7. WALLACE, J. M.: Hemodynamic lesions in hypertension. *Am. J. Card.*, 1975; 36: 670-675.
8. GUYTON, A. C.; HALL, J. E.; LOHMEIER, T. E.; JACKSON, T. E.; KASTNER, P. R.: Blood pressure regulation: basic concepts. *Fed. Proc.*, 1981; 40: 2252-2256.
9. CHIEN, S.: Determinants of blood viscosity and red cell deformability. *Scand. J. Clin. Lab. Invest.*, 1981; 41: (suppl. 156): 7-12.
10. LOSS, H.; WEHMEYER, H.; WESSELS, F.: The water and electrolyte content of erythrocytes in arterial hypertension. *Clin. Wochenschr.*, 1960; 38: 393-395.
11. MEYER, P.; GARAY, R. P.; MENDONÇA, M.: Ion transport systems in hypertension. In: J. Genest et al (eds), Hypertension, 2nd ed, McGraw-Hill, New York, 1983; 108-116.
12. POSTNOV, Y. V.; ORLOV, S. N.: Alteration of cell membrane in primary hypertension. In: J. Genest et al (eds), Hipertension 2nd ed, McGraw-Hill, New York, 1983; 95-107.
13. MIALL, W. E.; OLDHAM, P. D.: The hereditary factors in arterial blood pressure. *Br. Med. J.*, 1962; i: 75-80.
14. FRIEDMAN, S. M.: Cellular ionic perturbations in hypertension. *J. Hypertension*, 1983; 1: 109-113.
15. BLAUSTEIN, M. P.: Sodium ion, calcium, ion, blood pressure regulation, and hypertension; a reassessment and a hypothesis. *Am. J. Physiol.*, 1977; 232: C165-C173.
16. CHIEN, S.; LIPOWSKY, H. H.: Correlation of hemodynamics in macrocirculation and microcirculation. *Int. J. Microcir. Clin. Exp.*, 1982; 1: 351-365.
17. CHIEN, S.: Blood rheology in hypertension and cardiovascular disease. *Cardiovasc. Med.*, 1977; 2: 356-360.
18. JAN, K. M.; CHIEN, S.; BIGGER, J. T. JR.: Observation on blood viscosity changes after acute myocardial infarction. *Circulation*, 1977; 51: 1079-1084.
19. DORMANDY, J. A.: Influence of blood viscosity on blood flow and the effect of low molecular weight dextran. *Br. Med. J.*, 1971; 4: 716-719.
20. HARRIS, I.; MCLOUGHLIN, G.: The viscosity of blood in high blood pressure. *Q. J. Med.*, 1930; 23: 451-464.
21. TIBBLIN, G.; BERGENTZ, S. E.; BJURE, J.; WILHELMENSEN, L.: Hematocrit, plasma protein, plasma volume and viscosity in early hypertensive disease. *Am. Heart. J.*, 1966; 72: 165-176.
22. LETCHER, R. E.; CHIEN, S.; LARAGH, J. H.: Changes in blood viscosity accompanying the response to prazosin in patients with essential hypertension. *J. Cardiovasc. Pharmacol.*, 1979; 1 (suppl.): 8-20.
23. EMERY, A. C. JR.; WHITCOMB, W. H.; FROLICH, E. D.: Stress polycythemia and hypertension. *JAMA*, 1974; 229: 159-162.
24. LETCHER, R. L.; CHIEN, S.; PICKERING, T. G.; SEALEY, J. E.; LARAGH, J. H.: Direct relationship between blood pressure and blood viscosity in normal and hypertensive subjects. *Am. J. Med.*, 1981; 70: 1195-1202.
25. LETCHER, R. L.; CHIEN, S.; PICKERING, T. G.; LARAGH, J. H.: Elevated blood viscosity in patients with borderline essential hypertension. *Hypertension*, 1983; 5: 757-762.
26. SEIFFGE, D.: Hemorheologic studies in spontaneously hypertensive rats (EHR). In: J. F.; Stoltz, P. Drouin (eds), Hemorheology and Disease, Doin Editeurs, Paris, 1980; 487-494.
27. SEIFFGE, D.: Blood rheological studies in the spontaneously hypertensive rats (SHR). In: H. Henrich (ed), Microvascular Aspects of Spontaneous Hypertension, Hans Huber Publ. Bern-Stuttgart-Vienna, 1982; 55--71.
28. SEIFFGE, D.: Hemodilution in spontaneously hypertensive rats: its effect on blood pressure and blood rheology. *Bibl. Haematol.*, 1981; 47: 70-76.
29. CAIMI, G.; CATANIA, A.; FRAZZETTA, CAPOBIANCO, D.; SARNO, A.: Essential hypertension. The behaviour of the haemorheological determinants and their trends during beta-blocker treatment. *Jp. Heart. J.*, 1983; 24: 723-729.
30. CHIEN. S.; LETCHER, R. L.: Relation of blood pressure in essential hypertension. 2nd European Conference on Clinical Haemorheology, London, 1981; Sept. 30-Oct. 2.
31. CHIEN, S.; LETCHER, R. L.; PICKERING, T. G.; DEVEREAUX, R. B.: Blood rheology in hypertension. 3rd European Conference on Clinical Haemorheology, Baden-Baden, August, 1983; 24-27.
32. DE CLERCK, F.; JAGENEAV, A.; DOM, J.: Haemorheological effects of ketanserin, a selective 5-HT2 receptor antagonist, in aged spontaneously hypertensive dogs. *Arch. Int. Pharmacology*, 1982; 258: 100--115.
33. DINTENFASS, L.: Malfunction of viscosity receptors (viscoreceptors) as the cause of hypertension. *Am. Heart. J.*, 1976; 92: 260-263.
34. SCHMID-SCHONBEIN, H.: Interaction of vasomotion and blood rheology in haemodynamics. In: G. D. O. Lowe, J. C. Bardenel, C. D. Forbes (eds), Clinical Aspects of Blood Viscosity and Cell Deformability, Springer-Verlag, Berlin-Heidelberg — New York, 1981; 49-66.
35. PALARETI, G.; FABRITIIS, A.; POGGI, M.; TRICARICO, M. G.; BORGALTI, E.; COCCHERI, S.: Haemorheologic parameters in essen-

tial hypertension and early changes during antihypertensive treatments. 2nd European Conference on Clinical Haemorheology, London, 1981; Sept. 30-Oct 2.

36. FREITAS, J.; BRAZ-NOGUEIRA, J.; NOGUEIRA DA COSTA, J.; MARTINS, SILVA, J.: Whole blood filterability and blood oxygen transport in human hypertension. *Clin. Haemorheology*, 1983; 3: 367--373.

37. ISLES, C.; LOWE, G. D. O.; DRUMMOND, M, M.; FORBES, C. D.; KENNEDY, A. C.; LEVER, A. F.: Blood rheology in malignant phase hypertension. *Scand. J. Clin. Lab. Invest.*, 1981; 41: (suppl. 156): 175--176.

38. DE CREE, J.; LEEMPOELS, J.; GEUKENS, H.; COCK, W.; VERHAEGEN, H.: RBC deformability in patients with essential hypertension and myocardial infarction. Inprovement by treatment with a selective 5HT2 antagonist. 2nd European Conference on Clinical Haemorheology, London, 1981; Set. 30-Oct, 2.

39. CHIEN, S.: Blood rheology in hypertension and cardiovascular disease. In: J. H. Laragh (ed), Topics in Hypertension, Yorke Med. Brooks, New York, 1980; 159-156.

40. PROENÇA, M. C.; MARQUES, S. M.; BRAZ-NOGUEIRA, J.; SEGURADO, M. D.; NOGUEIRA DA COSTA, J.; MARTINS E SILVA, J.: Erythrocyte deformability and membrane protein pattern in essential hypertension. 3rd European Conference on Clinical Haemorheology, Baden-Baden 1983; 24-27.

41. MARTINS E SILVA, J.; BRAZ-NOGUEIRA, J.; BARROCA, J. P.; LEVY CRUZ, F.; GORJÃO CLARA, J.; FREITAS, J.; NOGUEIRA, DA COSTA, J.; MANSO, C.: Red cell metabolism in human arterial hypertension. Role of 2,3-diphosphoglycerate. *Rev. Cin. Méd.* (Lisbon), 1978; 142: 227-245.

42. HARPER, R. N. J.; JAMES, P. M. Jr.: Correlation of 2,3--diphosphoglycerate and essential hypertension. *J. Surg. Res.*, 1978; 25: 74-76.

43. KASPARIAN, A. K.; GOULIARIS, A.; PAPADOYANNIS, D. E.; TASSIOPOULOS, T.; KARATZAS, N. B.: Red cell 2,3--diphosphoglycerate in hypertension before and after therapy. *Eur. Heart. J.*, 1984; 5: (abstr. suppl. 1): 69.

44. FINCH, C. A.; LENFANT, C.: Oxygen transport man. *N. Engl. J. Med.*, 1972; 286: 407-415.

45. SANNERSTEDT, R.: Hemodynamic findings at rest and during exercise in mild arterial hypertension. *Am. J. Med. Sci.*, 1969; 258: 70-79.

46. WALSH, G.; TSUCHIYA, M.; COX, A. C.; TOBIA, A. J.; FROHLICH, E. D.: Altered haemodynamic responses to acute hypoxemia in spontaneously hypertensive rats. *Am. J. Physiol.*, 1978; 234: H275--H279.

47. EDMONDSON, A. P. S.; THOMAS, R. D.; HILTON, P. J.; PATRICK, J.; JONES, N. F.: Abnormal leucocyte composition and sodium transport in essential hypertension. *Lancet*, 1975; 1: 1023-1025.

48. TOBIAN, L.; BINION, J. T.: Tissue cation and water in arterial hypertension. *Circulation*, 1952; 5: 75-154.

49. SOMLYO, A. D.; SOMLYO, A. V.: Vascular smooth muscle II. Pharmacology of normal and hypertensive vessels. *Pharmacol. Rev.*, 1970; 22: 249-353.

50. SWALES, J. D.: Interpreting ion transport studies in hypertension: methods, myths and hypothesis. *J. Hypertension*, 1983; 1 (suppl. 2): 391--394.

51. CAVIARES, J. D.: The sodium pump in human red cells. In: J. C. Ellory, V. L. Lew (eds), Membrane Transport in Red Cells, Academic Press, London, 1977; 1-37.

52. WILEY, J. S.; COOPER, R. A.: A furosemide – sensitive cotransport of sodium plus potassium in the human red cell. *J. Clin. Invest.*, 1974; 53: 745-755.

53. HAAS, M.; SCHOOLER, J.; TOSTESON, D. C.: Coupling of lithium to sodium transport in human red cells. *Nature* 1975; 258: 424-427.

54. HEAGERTY, A. M.; MILNER, M.; BING, R. F.; TURSTON, H.; SWALES, J. D.: Leucocyte membrane sodium transport in normotensive population: dissociation of abnormalities of sodium efflux from raised blood pressure. *Lancet*, 1982; II: 894-896.

55. GARAY, R. P.; DAGHER, G.; PERNOLLET, M. G.; DEVYNCKE, M. A.; MEYER, P.: Inherited defect in a Na^+, K^+ cotransport system in erythrocytes from essential hypertensive patients. *Nature*, 1980; 284: 281-283.

56. SWARTS, H. G. P.; BONTING, S. L.; DE PONT, J. J. H. H. M.; STEKHOVEN, F. M. A. H. S.; THIEN, T. A.; VAN'T LAAR, A.: Cation fluxes and Na^+, K^+ activated ATPase activity in erythrocytes of patients with essential hypertension. *Clin. Exp. Hypert.*, 1981; 3: 64--67.

57. COLE, G. H.: Erythrocyte membrane sodium transport in patients with treated and untreated essential hypertension. Circulation, 1983; 68: 17--22.

58. LIGNEN, P. M.; BUYAMBA-KABANGU, J. R.; FAGARD, R. H.; GROESENEKEN, D. R.; STAENSEN, J. A.; AMERY, A. K.: Intracellular concentration and transmembrane fluxes of sodium and potassium in erythrocytes of white normal male subjects with and without a family history of hypertension. *J. Hypertension*, 1984; 2: 25-30.

59. POSTNOV, Y. U.; ORLOV, S. N.; SHEVCHENKO, A.; ADLER, A. M.: Altered sodium permeability, calcium binding and Na-K-AT Pase activity in the red blood cell membrane in essential hypertension. *Pflugers Arch.*, 1977; 317; 363-269.

60. PROENÇA, M. C.; BRAZ-NOGUEIRA, J.; RIBEIRO, M. H.; NOGUEIRA DA COSTA, J.; MARTINS E SILVA, J.: Actividade da acetilcolines terase e adenosina-trifosfatase Na^+, K^+-dependente na membrana eritrocitária de doentes com hipertensão arterial. *Rev. Port. Cardiol.*, 1982; 1: 188 (abstract).

61. PROENÇA, M. C.; BRAZ-NOGUEIRA, J.; NOGUEIRA DA COSTA, J.; MARTINS E SILVA, J.: Fluorescence studies, acetylocholinesterase and Na^+, K^+-dependent ATPase activities on erythrocyte membranes from normal and hypertensive subjects-3rd European Conference on Clinical Haemorheology, Baden-Baden, August 1983; 24-27.

62. HAMLYN, J. M.; RINGEL, R.; SCHAEFFER, J.; LEVINSON, P. D.; HAMILTON, B. P.; KOWARSKI, A. A.; BLAUSTEIN, M. P.: A circulatory inhibitor of (Na^+, K^+) ATPase associated with essential hypertension. *Nature* 1982; 300: 650-652.

63. GARAY, R. P.; ELGHOZI, J. L.; DAGHER, G.; MEYER, P.: Laboratory distintion between essential and secondary hypertension by measurement of erythrocyte cation fluxes. *N. Engl. J. Med.*, 1980; 302: 769-776.

64. DAVIDSON, J. S.; OPIE, L. H.; KEDING, B.: Sodium-potassium cotransport activity as genetic marker in essential hypertension. *Br. Med. J.* 1982; 284: 539-541.

65. CANESSA, M.; ADRAGNA, N.; SOLOMON, H.; CONNOLLY, T.; TOSTESON, D. C.: Measured sodium-lithium countertransport in red cells of patients with essential hypertension. *N. Engl. J. Med.*, 1980; 302: 772-776.

66. MAHONEY, J. R.; ETKIN, N. L.; MCSWIGAN, J. D.; EATON, J. W.: Assessement of red cell sodium transport in essential hypertension. *Blood*, 1982; 59: 439-442.

67. DUHM, J.; GOBEL, B. O.; LORENZ, R.; WEBER, P. C.: Sodium-lithium exchange and sodium-potassium cotransport in human erythrocytes. A simple uptake test applied to normotensive and essential hypertensive individuals. *Hypertension*, 1982; 4:447-482.

68. TREVISAN, M.; OSTROW, D.; COOPER, R.; LIU, K.; SPARK, S.; OKONER, A.; STEVENS, E.; MARQUARDT, J.; STAMLER, J.: Abnormal red blood cell ion transport and hypertension. *Hypertension*, 1983; 5: 363-367.

69. ADRAGNA, N. C.; CANESSA, M. L.; SOLOMON, H.; SLATER, E.; TOSTESON, D. C.: Red cell lithium-sodium countertransport and sodium-potassium cotransport in patients with essential hypertension. *Hypertension*, 1982; 4: 795-804.

70. BRUGNARA, C.; CORROCHER, R.; FORONIL, L.; STEINMAYER, M.; BONFANTI, F.; DESANDRE, G.: Lithium-sodium countertransport in erythrocytes of normal and hypertensive subjects. Relationship with age and plasma renin activity. *Hypertension*, 1983; 5: 529-534.

71. COOPER, R.; LIGRADY, D.; NANAS, S.; TREVISAN, M.; MANSONR, M.; HISTAND, P.; OSTROW, D.; STANLER, J.: Increased sodium-lithium countertransport in college students with elevated blood pressure. *JAMA*, 1983; 249: 1030-1034.

72. WOODS, J. W.; PARKER, J. C.; WATSON, B. S.: Perturbation of sodium-lithium countertransport in red cells. *N. Engl. J. Med.*, 1983; 308: 1258-1261.

73. TOBIAN, L.; BINION, J.: Artery wall electrolytes in renal and DCOA--hypertension. *Am. J. Physiol.*, 1952; 171: 733-735.

74. JONES, A. W.; FEIGE, E. O.; PETERSON, L. H.: Water and electrolyte content normal and hypertensive arteries in dogs. *Circ. Res.*, 1964; 15: 386-392.

75. TOBIAN, L.; CHESLEY, G.: Calcium content of arteriolar walls in normotensive and hypertensive rats. *Proc. Soc. Exp. Biol. Med.*, 1966; 121: 340-343.

76. FRIEDMAN, S. M.; FRIEDMAN, C. L.: The ionic matrix of vasconstriction. *Circ. Res.*, 1967; 24 (suppl. II): II-147-II-155.

77. JONES, A. W.: Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats: influence of aldosterone, norepinefrine and angiotensin. *Circ. Res.*, 1973; 33: 563-572.

78. FRIEDMAN, S. M.; FRIEDMAN, C. L.: Cell permeability, sodium transport and the hypertensive process in the rat. *Circ. Res.*, 1979; 39: 433-440.

79. AOKI, K. SKEDO, M.; YAMASHITA, K.; TAZUMI, K.; JATO, I.; HOTTA, R.: Cardiovascular contraction in spontaneously hypertensive rats: Ca^{2+} interactions of myofibrils and subcellular membrane of heart and arterial smooth muscle. *Jpn. Circ. J.*, 1974; 38: 1115-1119.

80. SHIBATA, S.; KUCHII, M.; TANIGUICHI, T.: Calcium fluxes and binding in the aortic smooth muscle from spontaneously hypertensive rats. *Blood Cells*, 1975; 12: 279-289.

81. BOHR, D. F; SEIDEL, R.; SOBIESKI, J.: Possible role of sodium-calcium pumps in tension development of vascular smooth muscle. *Microvasc. Res.*, 1969; 1: 333-343.

82. POSTNOV, Y. U.; ORLOV, S.; GULAK, P.; SHEVCHENKO, A.: Altered permeability of the erythrocyte membrane of sodium and potassium in spontaneously hypertensive rats. *Pflugers Arch.*, 1976; 365: 257--263.

83. BEN-ISHAY, D.; AVIRAM, A.; VISKOPER, R.: Increased erythrocyte sodium efflux in genetic hypertensive rats of the Hebrew University strain. *Experientia*, 1975; 31: 660-662.

84. FRIEDMAN, S. M.; NAKASHIMA, M.; MCINDOE, R. A.; FRIEDMAN, C. L.: Increased erythrocyte permeability to Li and Na in the spontaneously hypertensive rats. *Experientia*, 1976; 32: 476-478.

85. VAN DE VEN, C. J. M.; BOHR, D. F.: Intrinsic difference in erythrocyte membrane in spontaneously hypertensive rats characterized by Na^+ and K^+ fluxes. *Plugers Archiv.*, 1983; 399: 74-78.

86. TRIZIO, D.; FERRARI, P.; FERRANDI, M.; TORIELLI, L.; BIANCHI, G.: Expression at the haemapoietic stem cell level of the genetically determined erythrocyte membrane defects in the milan hypertensive rat strain. First European Meeting in Hypertension, Milan, 1983, June, 29th--1st May.

87. MENDONÇA, M.; GRICHOIS, M. L.; GARAY, R. P.; SANARD, J.; BEN-ISHAY, D.; MEYER, P.: Abnormal net Na^+ and K^+ fluxes in erythrocytes of three varieties of genetically hypertensive rats. *Proc. Natl. Acad. Sc. USA*, 1980; 77: 4283-4286.

88. CHAN, T. C. K.; GODIN, D. V.; SUTTER, M. C.: Erythrocyte membrane abnormalities in hypertension: a comparison between two animal models. *Clin. Exper. Hyper.*, 1983; A5: 691-719.

89. DEVYNCK, N. A.; PERNOLLET, M. G.; NUNEZ, A. M.; MEYER, P.: Analysis of calcium binding handling in erythrocyte membranes of genetically hypertensive rats. *Hypertension*, 1981; 3: 397-403.

90. WEHLING, M.; VETTER, W.; NEYSER, L.; GROTH, H.; BOERLIN, H. J.; LOCHER, R.; SIEGENTHALER, W.; KUHLMANN, U.: Altered calcium and sodium metabolism in red blood cells of hypertensive man: assessment by ion-selective electrodes. *J. Hypertension*, 1983; 1: 171-176.

91. POSTNOV, Y. V.; ORLOV, S. B.; POKUDIN, N.: Decrease calcium binding by the red blood cell membrane in spontaneously hypertensive rats and in essential hypertension. *Pflugers Arch.*, 1979; 379: 191-195.

92. ORLOV, S. N.; POSTNOV, Y. V.: Ca^{2+} binding and membrane fluidity in essential and renal hypertension. *Clin. Sci.*, 1982; 63: 281-284.

93. ORLOV, S. N.; POKUDIN, N. I.; POSTNOV, Y. V.: Calmodulin-dependent Ca^{2+} transport in erythrocytes of spontaneously hypertensive rats. *Pflugers Arch.*, 1983; 397: 54-56.

94. URRY, D. W.; TRAPANE, T. L.; ANDREWS, S. K.; LONG, M. M.; OVERBECK, H. W.; OPARIL, S.: NMR observations of altered sodium interations with human erythrocyte membrane of essential hypertensives. *Biochem Biophys. Res. Commun.*, 1980; 90: 514-521.

95. ROMERO, P. J.: Role of membrane bound Ca in ghost permeability to Na and K. *J. Membr. Biol.*, 1976; 29: 329-335.

96. POSTNOV, Y. V.; ORLOV, S. N.: Evidence of altered calcium accummulation and calcium binding by the membrane of adipocytes in spontaneously hypertenssive rats. *Pflugers Arch.*, 1980; 385: 85-89.

97. DEVYNCK, M. A.; PERNOLLET, M. G.; NUNEZ, A. M.; ARAGON, I.; MONTENAY, T.; HELENE, C.; MEYER, P.: Biophysical and biochemical demonstration of a difference alteration of plasma membrane of SHR's. Eight Meeting of the International Society of Hypertension, Milan, 1981, May 31-June 3.

98. ORLOV, S. N.; POSTNOV, Y. V.: Calcium accummulation and calcium binding by the cell membranes of cardiomyocytes and smooth muscle of aorta in spontaneously hypertensive rats. *Clin. Sci.*, 1980; 59 (suppl): 207s-209s.

99. MCCARRON, D. A.: Low serum concentration of ionized calcium in patients with hypertension. *N. Engl. J. Med.*, 1982; 307: 226-228.

100. RESNICK, S. M.; LARAGH, J. H.; SEALEY, J. E.; ALDERMAN, M. H.: Divalent cations in essential hypertension. *N. Engl. J. Med.*, 1983; 309: 888-891.

101. JONES, A. W.: Altered ion transport in large and small arteries from spontaneously hypertensive rats and the influence of calcium. *Circ. Res.*, 1974: 34 (suppl 1): I-117-I-122.

102. HERMSMEYER, K.: Cellular basis for increased sensitivity of vascular smooth muscle in spontaneously hypentensive rats. *Circ. Res.*, 1976; 38 (suppl. II): II-53-II-57.

103. ZYDEK, W.; VETTER, H.; ZUMKLEY, H.: Intracellular cation activities and concentration in spontaneously hypertensive and normotensive rats. *Clin. Sci.*, 1981; 61 (suppl.): 41s-43s.

104. POSTNOV, Y. V.; ORLOV, S. N.: Calmodulin dependent Ca^{2+} transport across plasma membrane in primary hypertension. First European Meeting on Hypertension, Milan, 1983, June, 29 th May-1st .

105. MONTENAY-GARESTIER, T.; ARAGON, I.; DEVYNCK, M.; MEYER, P.; HELENE, C.: Evidence for structural changes in erythrocyte membranes of spontaneously hypertensive rats. A fluorescence polarization study. *Biochem. Biophys. Res. Commun.*, 1981; 100: 660-665.

106. ARAGON, J.; MONTENAY-GARESTIER, T.; DEVYNCK, M. A.; PERNOLLET, M. G.; NUNEZ, A. M.; BEN-ISHAY, D.; MEYER, P.; HELENE, C.: Diffuse structural abnormalities in cell membranes from genetically hypertensive rats: a fluorescence polarization study. Ninth Scientific Meeting of the International Society of Hypertension, México, *Feb.*, 1982; 21-24.

107. BORISKINE, G. M.; GULAK, P. V.; POSTNOV, Y. V.: Phosphoinositide content in the erythrocyte membrane of rats with spontaneously renal hypertension. *Experientia* 1978; 34: 744.

108. KOUTONZOV, S.; MARCHE, P.; CLOIX, J. F.; MEYER, P.: Phospholipid phosphorylation in erythrocyte of spontaneously hypertensive rats. *Am. J. Physiol.*, 1982; 243: H590-H597.

109. MICHELL, R.H.: Inositol phospholipids in membrane function. *TIBS*. 1979; 4: 128-131.

110. SKOU, J. C.: The (Na^+, K^+)-activated enzyme system and its relationship to transport of sodium and potassium. *Q. Rev. Biophys*, 1975; 7: 401-436.

111. ROELOFSEN, B.; SCHATZMANN, H. J.: The lipid requirement of the (Ca^{2+}, Mg^{2+})-ATPase in the human erythrocyte membrane, as studied by various highly purified phospholipases. *Biochem. Biophys.*, Acta 1977; 464: 17-25.

112. LOW, M. G.; FINEAN, J. B.: Non lytic release of acetylcholinesterase from erythrocytes by a phosphodylinositol-specific phospholipase C. *FEBS Letters*, 1977; 82: 143-146.

113. GULAK, P. V.; BORISKINE, G. M.; POSTNOV, Y. V.: Ca^+ binding to erythrocyte membrane of hypertensive men and rats: effects of acetylcholine and eserine. *Experientia*, 1979; 35: 1471.

114. LEVY, R.; ZIMLICHMAN, R.; KEYNAN, A.; LIVNE, A.: The erythrocyte membrane in essential hypertension. Modified temperature – dependence of Li^+ efflux. *Biochim. Biophys. Acta*, 1982; 685: 214-218.

115. HARRIS, A. L.; GUTHE, C. C.; VAN'T VEER, F.; BOHR, D. F.: Temperature dependence and bidirectional cation fluxes in red blood cells from spontaneously rats. *Hypertension*, 1984; 6: 42-48.

116. CLOIX, J. F.; DEVYNCK, M. A.; FUNCK-BRENTANO, J. L.; MEYER, P.: Particularités protéiques des membranes et du plasma chez le rat spontanément hypertendue. *C. R. Acad. Sc. Paris*, 1983; 294 (série III): 661-663.

117. GULAK, P. V.; ORLOV, S. N.; POKUDIN, N. I.; POSTNOV, Y. V.; LITVINOV, I. S.; ORLOV. N. J.; SHNYROV, V. L.: Microcalorimetry and electrophoresis of the erythrocyte membrane of spontaneously hypertensive rats. *J. Hypertension.*, 1984; 2: 81-84.