Lymphocyte Abnormalities in Three Types of Hypertension in the Rat

PHILIP B. FURSPAN AND DAVID F. BOHR

SUMMARY Lymphocyte number and weight and their sodium and potassium contents and net passive fluxes were measured in spontaneously hypertensive stroke-prone rats, deoxycorticosterone acetate-treated rats, and two-kidney, one-clip renal hypertensive rats. Wistar-Kyoto rats were used as controls for the spontaneously hypertensive stroke-prone rats, and normal intact Sprague-Dawley rats were used as controls for the others. Blood lymphocyte count was higher and lymphocyte weight was lower in the hypertensive rats. Intralymphocytic sodium content (millimoles per kilogram of dry weight) was elevated in the three forms of hypertension as compared with control values (spontaneously hypertensive stroke-prone rats, 43.0 ± 1.7 vs Wistar-Kyoto rats, 37.3 ± 1.3; deoxycorticosterone acetate-treated rats, 44.4 ± 3.1 vs Sprague-Dawley rats, 36.1 ± 1.7; one-kidney, one-clip rats, 50.5 ± 3.7 vs Sprague-Dawley rats, 38.9 ± 2.0). Intralymphocytic potassium content was not significantly altered in any of the forms of hypertension. Lymphocytes from spontaneously hypertensive stroke-prone rats and deoxycorticosterone acetate-treated rats exhibited elevated net sodium fluxes (millimoles per kilogram of dry weight per hour) as compared with those of controls (spontaneously hypertensive stroke-prone rats, 7.00 ± 0.99 vs Wistar-Kyoto rats, 4.89 ± 0.63; deoxycorticosterone acetate-treated rats, 7.58 ± 0.97 vs Sprague-Dawley rats, 5.6 ± 0.64). Net potassium fluxes were significantly elevated only in the spontaneously hypertensive stroke-prone rats (14.07 ± 1.70 vs 8.23 ± 1.04 in Wistar-Kyoto rats). Sodium and potassium fluxes in lymphocytes from two-kidney, one-clip rats and Sprague-Dawley rats were not significantly different. The lymphocyte may provide a convenient and suitable system for the study and characterization of a generalized cell membrane abnormality that may be involved in the pathogenesis of hypertension. (Hypertension 7: 860-866, 1985)

KEY WORDS • membrane abnormality • sodium flux • potassium flux • deoxycorticosterone acetate • renal hypertension • genetic hypertension

In the past decade extensive research in experimental and clinical hypertension has indicated that there are cell membrane abnormalities associated with chronically elevated arterial pressure resulting from various initiating factors. Many aspects of membrane function appear to be altered: cation permeability, active transport, cotransport and countertransport, and calcium binding are some processes that have been examined and recently reviewed by Friedman, Postnov and Orlov, and Blaustein.

Although many types of cells have been studied and found to possess some type of membrane abnormality, most of this work has been done on the red blood cell. The ready availability, ease of handling, and extensive base of fundamental knowledge account for much of this preference. Of course, it is recognized that an abnormality in the membrane of this cell may not play a role in the pathogenesis of an elevated arterial pressure. This cell membrane is studied because of its possible use as a marker for the hypertensive process and because it may reflect similar abnormalities in vascular smooth muscle and regulatory centers in the central nervous system that do influence blood pressure. The advantages of the red blood cell, however, must be weighed against the nontypical characteristics of this cell (i.e., no nucleus and absence of protein synthesis). The lack of a nucleus would be especially critical in any study of mineralocorticoid hypertension in view of the nucleus-mediated effects of steroids. In addition, reports of changes in red blood cell sodium content and transport associated with hypertension have not been consistent.

Other blood cells have also been studied. Edmondson et al. reported abnormalities in cell sodium transport in leukocytes from patients with essential hyper-
tension. This abnormality manifested itself as a higher cell sodium content and a lower rate constant (fraction of ion transported per unit time) for active sodium efflux. A similar elevation of leukocyte sodium content in patients with essential hypertension was described by Araoye et al.12 Poston et al.13 reported that the incubation of leukocytes from normotensive patients in plasma from patients with essential hypertension results in an alteration of sodium transport similar to that seen in the leukocytes of the hypertensive patients. One disadvantage of leukocytes, however, is that they represent a mixed population of cells consisting of neutrophils, eosinophils, lymphocytes, and monocytes. Such a mixture presents difficulties in the interpretation of data.

Peripheral blood lymphocytes are relatively homogeneous, possess a nucleus, and are capable of protein synthesis. Evidence for the existence of the membrane abnormality in these cells comes mainly from studies of human essential hypertension. Ambrosioni et al.14 have reported an increased lymphocyte sodium content in patients with essential hypertension as compared with that in normotensive controls. Pedersen et al.15 ascribe a higher sodium influx in the lymphocytes from normotensive patients with a family history of hypertension to enhanced sodium-lithium countertransport and unidirectional net sodium flux. Jones et al.16 have examined sodium content and transport in thymocytes (T-lymphocytes) derived from the rat thymus gland. The T-lymphocytes from spontaneously hypertensive rats (SHR) exhibited higher sodium content and a decrease in the rate constant for total sodium efflux as compared with that in normotensive controls.

The present study attempted to define specific membrane properties and lymphocyte characteristics in three different types of hypertension in rats to determine whether the peripheral blood lymphocyte can be used as a suitable system for the study of abnormal membrane properties associated with hypertension.

Materials and Methods

Three forms of hypertension were studied: genetic, mineralocorticoid, and renal. For studies of genetic hypertension, spontaneously hypertensive stroke-prone rats (SHRSP) and control Wistar-Kyoto rats (WKY), approximately 7 to 10 months of age, were obtained from a large breeding colony maintained at the University of Michigan Medical School (Ann Arbor, MI, USA). Original breeders for this colony were obtained from the National Institutes of Health (Bethesda, MD, USA).

To study mineralocorticoid hypertension, Silastic strips impregnated with doxycorticosterone acetate (DOCA) were implanted subcutaneously (200 mg DOCA/kg) in unilaterally nephrectomized Sprague-Dawley rats (SD). Their drinking water contained 1% NaCl and 0.2% KCl (to minimize hypokalemia). Terminal blood pressure was measured, and lymphocytes were used 5 to 6 weeks after implantation. Intact age-matched, sex-matched, and weight-matched SD were used as controls.

To study renal hypertension, the two-kidney, one clip (2K1C) form of renal hypertension was induced by partial constriction of the left renal artery of SD. Intact SD, matched for age, sex, and weight, were used as controls.

Approximately 11 ml of blood was withdrawn within 60 seconds by means of aortic cannulation from rats anesthetized with pentobarbital. One milliliter of whole blood was set aside for a lymphocyte count (standard hemocytometry) and a determination of plasma electrolytes. Aliquots (2.5 ml) of the remaining blood were mixed with an equal volume of a physiological salt solution containing (mM) NaCl, 125; KCl, 5; NaHPO₄, 16; glucose, 10; pH adjusted with 0.1 N HCl to 7.4 at 25 °C. A density gradient was made up in the following manner: of the bottom layer 66.6% was 12% Ficoll (400,000 molecular weight polysaccharide; Sigma Chemical Co., St. Louis, MO, USA) and 33.3% was 50% sodium diatrizoate solution (Hypaque, Winthrop Labs, New York, NY, USA). Over this solution was layered a 70% Ficoll, 20% Hypaque, 10% water mixture. The 5-ml blood–physiological salt solution mixture was then placed on top of this density gradient. The tubes containing the blood mixture and density gradient were then centrifuged at 900 g for 18 minutes. The red blood cells go to the bottom of the tube, and the lymphocytes form a distinct band (between the plasma and gradient mixture), which renders them easily removed by pipette and combined in a 12-ml polycarbonate test tube. The polycarbonate tube containing the lymphocytes plus some of the gradient mixture and plasma was then centrifuged at 12,000 g for 3 minutes. The supernatant fluid was removed by aspiration. The remaining pellet of lymphocytes was resuspended in 6 to 8 ml of a different physiological salt solution containing (mM) NaCl, 140; KCl, 5; MgSO₄, 1.19 (morpholine)propanesulfonic acid, 2.0; CaCl₂·H₂O, 1.6; dextrose, 11.1; albumin (0.25%); adjusted with 0.1 N NaOH to pH 7.4 at 25 °C. We refer to this lymphocyte preparation as the lymphocyte–physiological salt solution suspension. A lymphocyte count was performed on this suspension using standard clinical hemocytometry. Cell density was found to be equivalent (± 15%) to that measured in whole blood.

The lymphocyte–physiological salt solution suspension was centrifuged at 12,000 g for 3 minutes. The supernatant was removed and saved for measurement of extracellular sodium and potassium concentrations. The cell pellet was washed twice in cold 0.3 M sucrose. The entire isolation procedure to this point was performed at room temperature and required approximately 90 minutes. The pellet was then dried at 80 °C for 2 hours. The dried pellet was then weighed on a Cahn electrobalance (Paramount, CA, USA), after which it was digested in 100 µl of 70% HNO₃ at 80 °C for 2 hours. The volume was brought up to 1 ml with 15 mM LiNO₃ for determination of sodium and potassium on a flame photometer (Model IL 443; Instrumentation Laboratories, Lexington, MA, USA). Values for sodium and potassium were expressed as millimoles per kilogram of dry cell weight.
The lymphocyte-physiological salt solution suspension was divided into two equal aliquots; half was used to determine initial intracellular and extracellular sodium and potassium, and half was placed in a 25-ml siliconized Erlenmeyer flask for incubation at 4 °C for 4 hours. This incubation time was chosen after preliminary experiments indicated that the flux rates for sodium and potassium were still exponential at 4 hours. Net fluxes commenced to slow down in 6 hours and reached virtual equilibrium at 9 hours. The flask was gently shaken in a wrist action shaker to prevent settling of the cells. Fluxes were determined by the following calculation:

$$J_{ml} = \frac{(C_f - C_i)}{t}$$

where $$J_{ml}$$ equals the net cation flux in millimoles per kilogram of dry cell weight per hour, $$C_f$$ equals the final intracellular cation concentration in millimoles per kilogram of dry cell weight, $$C_i$$ equals the initial intracellular cation concentration in millimoles per kilogram of dry cell weight, and $$t$$ equals time in hours.

Cell volume was determined using a concentrated lymphocyte-physiological salt solution mixture and the following formula:

$$V_i = \frac{Hct \times N}{t}$$

where $$V_i$$ equals lymphocyte volume in cubic millimeters, $$Hct$$ equals hematocrit of the lymphocyte-physiological salt solution mixture, and $$N$$ equals the number of lymphocytes per cubic millimeter of the lymphocyte-physiological salt solution mixture.

Since all the lymphocytes in the blood of a rat are necessary for a measurable hematocrit, only a limited number of volume measurements were performed on lymphocytes from SHRSP and WKY to establish the relation between cell volume and dry cell weight.

Blood pressure in the conscious rat was measured by the method of tail cuff plethysmography. Differences between any two groups of rats were tested for significance by means of the unpaired $$t$$ test. Differences between groups were considered significant when the $$p$$ value was 0.05 or less.

**Results**

Blood pressures of the three groups of hypertensive rats were quite similar, and each was significantly greater than that of its appropriate control (Figure 1).

Routine lymphocyte counts revealed a distinct difference between those from control and hypertensive rats (Figure 2). Blood lymphocyte count was higher in the hypertensive rats, and the greatest difference occurred between SHRSP (3130 ± 170/mm$^3$) and WKY (1680 ± 100/mm$^3$).

As shown in Figure 3, the dry cell weights (pellet weight/number of cells in pellet) of the lymphocytes from the SHRSP (0.26 ± 0.02 ng), DOCA-treated rats (0.17 ± 0.02 ng), and 2K1C rats (0.17 ± 0.02 ng) were significantly lower than those of their appropriate controls (WKY, 0.50 ± 0.06; SD, 0.26 ± 0.02; SD, 0.26 ± 0.02).

As shown in Figure 4, intralymphocytic sodium content (expressed as millimoles per kilogram of dry cell weight) was elevated in the three forms of hypertension as compared with control values (SHRSP, 43.0 ± 1.7 vs WKY, 37.3 ± 1.3; DOCA-treated rats, 44.4 ± 3.1 vs SD, 36.1 ± 1.7; 2K1C rats, 50.5 ± 3.7 vs SD, 38.9 ± 2.0). Intralymphocytic potassium content was not significantly altered in any of the forms of hypertension (SHRSP, 140.2 ± 6.0 vs WKY, 157.6 ± 7.4; DOCA-treated rats, 173.8 ± 19.2 vs SD, 146.4 ± 13.7; 2K1C rats, 169.4 ± 3.7 vs SD, 162.6 ± 7.1), as shown in Figure 5.

The flux values are presented in Table 1 both as unadjusted values and as values corrected to take into account the smaller size, and consequent greater surface area to volume ratio, of the lymphocytes from the hypertensive rats. The adjustments were based on the ratio of the surface area (4$$\pi r^2$$) to the volume of a sphere ($$4/3\pi r^3$$) for the different cells. The adjustment assumes that volume differences parallel differences in dry cell weight, that lymphocytes are spherical, and so on.
that surface area does not change independently of volume. The parallel relation between dry cell weight and volume is supported by results from measurements of cell volume of lymphocytes from two WKY and two SHRSP (WKY, $1.01 \times 10^{-12}$ mm$^3$ and $1.14 \times 10^{-12}$ mm$^3$; SHRSP, $0.49 \times 10^{-12}$ mm$^3$ and $0.64 \times 10^{-12}$ mm$^3$). The measured flux values for the lymphocytes of the hypertensive rats were adjusted according to the percent difference in surface area to volume ratio between lymphocytes from control and hypertensive rats. Calculations were based on ratios of cell weights. Lymphocyte cell weights were calculated for each rat based on the weight of the lymphocyte pellet and the number of lymphocytes in the pellet. The surface area to volume ratio of the lymphocytes from WKY was calculated to be 796, that of lymphocytes from the SHRSP was 965. This ratio for the lymphocytes from the SHRSP was 21% greater than that of the lymphocytes from the control rats. Hence, if the ratio of the control is considered to be 1, the flux for those from the hypertensive rat was adjusted by dividing the measured flux of their lymphocytes by 1.21. Because the surface area to volume ratios of lymphocytes from both DOCA-treated rats and 2K1C rats were 13.7% higher than control, their measured fluxes were adjusted by dividing by 1.137.

Unadjusted and adjusted values for sodium and potassium fluxes are presented in Table 1. Lymphocytes from SHRSP and DOCA-treated rats exhibited similarly elevated net sodium fluxes (adjusted) as com-
Figure 4. Average intracellular sodium contents (± SEM) of lymphocytes from control and hypertensive rats. Abbreviations as in Figure 1. *p < 0.05; **p < 0.01.

Figure 5. Average intracellular potassium contents (± SEM) of lymphocytes from control and hypertensive rats. Abbreviations as in Figure 1.

Table 1. Average Net Passive Fluxes of Sodium and Potassium Across the Membrane of Lymphocytes from Control and Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHRSP</th>
<th>SD</th>
<th>DOCA</th>
<th>SD</th>
<th>2K1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium influx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>4.89 ± 0.6 (14)</td>
<td>8.47 ± 1.2 (16)*</td>
<td>5.6 ± 0.6 (7)</td>
<td>8.62 ± 1.1 (7)†</td>
<td>4.62 ± 0.7 (6)</td>
<td>4.18 ± 0.7 (8)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>—</td>
<td>7.00 ± 1.0 (16)†</td>
<td>—</td>
<td>7.58 ± 1.0 (7)†</td>
<td>—</td>
<td>3.68 ± 0.6 (8)</td>
</tr>
<tr>
<td>Potassium efflux</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>8.23 ± 1.0 (14)</td>
<td>17.02 ± 2.0 (16)*</td>
<td>9.76 ± 1.2 (7)</td>
<td>12.96 ± 1.3 (8)†</td>
<td>9.25 ± 0.9 (8)</td>
<td>8.3 ± 1.2 (7)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>—</td>
<td>14.02 ± 1.7 (16)†</td>
<td>—</td>
<td>11.4 ± 1.1 (8)</td>
<td>—</td>
<td>7.3 ± 1.0 (7)</td>
</tr>
</tbody>
</table>

Adjusted values reflect correction for differences in surface area to volume ratios between lymphocytes from control and hypertensive animals (see text).

Number of rats is in parentheses.

WKY = Wistar-Kyoto rats; SHRSP = spontaneously hypertensive stroke-prone rats; SD = Sprague-Dawley rats; DOCA = deoxycorticosterone acetate–treated rats; 2K1C = two-kidney, one clip rats.

*p < 0.001, †p < 0.05, difference from control value.
pared with those in their controls (43% and 35% respectively). Net potassium flux in lymphocytes from SHRSP was also elevated compared with that in controls (70%). Sodium and potassium fluxes in lymphocytes from SD and 2K1C rats were not significantly different.

Discussion

Cell membrane properties and cell characteristics of peripheral blood lymphocytes from control and three different types of hypertensive rats were characterized and compared. The results indicate the occurrence of striking and important alterations of membrane permeability, cell cation content, lymphocyte count, and lymphocyte weight in the hypertensive as compared with control animals. In addition, there were interesting differences in the occurrence and magnitude of these alterations among the different types of hypertension.

An elevation of intralymphocytic sodium was associated with the three forms of hypertension studied. Increased sodium influx at 4 °C, however, was observed only with lymphocytes from SHRSP and DOCA-treated rats, and increased potassium efflux was observed only with lymphocytes from SHRSP. These findings are consistent with what has been reported in the literature for human lymphocytes and other cells (see introductory section). An elevation in intralymphocytic sodium concentration has been observed in clinical essential hypertension. Although, as far as we know, there have not been any reported studies of peripheral blood lymphocytes of rats, Jones et al. and Bradlaugh et al. have examined sodium content and transport in thymocytes (T lymphocytes) derived from the rat thymus gland. Jones et al. reported that T lymphocytes from spontaneously hypertensive rats exhibited higher sodium content and a decrease in the rate constant for total sodium efflux (active and passive combined) as compared with normotensive controls. Bradlaugh et al., however, did not find a significant difference in sodium rate constants (active or passive) of lymphocytes from spontaneously hypertensive rats and normotensive rats. These investigators have presented evidence that suggests that the decreased sodium efflux rate constant observed by Jones et al. is attributable to age of the rat rather than to the blood pressure elevation of spontaneously hypertensive rats. Differences in potassium and sodium contents that are dependent on the age of the cell have been reported in the red blood cell. However, we do not know if such age dependencies occur in the lymphocyte. We have no evidence bearing on the possibility that the differences we observed between lymphocytes from the different groups of rats were dependent on age differences of the lymphocytes.

An increased sodium and potassium permeability has also been reported for red blood cells and vascular smooth muscle from SHR and DOCA-treated rats. Friedman, however, did not observe an elevated sodium permeability in the tail artery from rats with established 2K1C renal hypertension.

Differences in the size of cells from control and hypertensive rats have been reported for red blood cells from spontaneously hypertensive rats and renomedullary interstitial cells from hypertensive salt-sensitive Dahl rats. Feig et al. suggest that the observed increased sodium flux (16%) of red blood cells from spontaneously hypertensive rats is mainly due to an increased surface area to volume ratio (9%) resulting from a lesser cell volume. We found, however, that the adjustment in surface area to volume ratio of the lymphocytes (e.g., SHRSP vs WKY, 21%) would only account for a relatively small fraction of the observed flux differences (e.g., SHRSP vs WKY, 73%).

The results of this study indicate that lymphocytes from SHRSP and DOCA-treated rats possess a cell membrane abnormality that results in an increased leakiness to sodium. Flux of potassium was increased in lymphocytes from SHRSP but not in those from DOCA-treated rats. Since these studies were performed at 4 °C, the flux values represent passive leak rather than active transport of sodium and potassium. The flux was measured only in the direction of the normal physiological gradients (i.e., sodium influx and potassium efflux). Similar studies on the red blood cell have demonstrated that this greater leak in the cell from the hypertensive animal occurs in both directions. Previous investigations suggest that increased cation permeability of cell membranes of hypertensive animals is associated with a reduced number of calcium binding sites. The lymphocytes from 2K1C rats did not exhibit increased membrane permeability to either sodium or potassium but did show an elevated intracellular sodium concentration. The presence of a circulating ouabainlike factor might account for this elevation in intralymphocytic sodium concentration. Haddy reviews the evidence for the existence of such a factor associated with hypertension.

A membrane abnormality in lymphocytes probably is not causally associated with hypertension. However, Norman et al. have presented evidence suggesting that a defect in the immune system plays at least a partial role in the onset and maintenance of hypertension in spontaneously hypertensive rats. The existence of a similar membrane abnormality in the cells of an area of the brain involved in the regulation of blood pressure has been postulated to be a primal factor in the pathogenesis of hypertension. Evidence of a reduced number of calcium binding sites on synaptosomal membranes from spontaneously hypertensive rats supports this theory.

The results presented in this study indicate that there are abnormalities in the lymphocyte membrane in hypertension. Direct evidence by others has indicated that similar abnormalities occur in the membrane of the vascular smooth muscle cell and have been inferred to occur in pressure regulating centers of the brain. For these reasons the lymphocyte may prove to be a convenient and suitable system for the study and characterization of a generalized cell membrane abnormality that may be involved in the pathogenesis of hypertension.
Acknowledgments
We thank Dr. John Jacquez for his insight into the problem of changing surface area to volume ratios.

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Lymphocyte abnormalities in three types of hypertension in the rat.

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Hypertension. 1985;7:860-866
doi: 10.1161/01.HYP.7.6.860

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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