It is significant that the main mechanism of the cross-protective effect of adaptation revealed by us is an increased tissue capacity for utilizing the oxygen supplied by the blood. This important shift may be determined by at least two factors: 1) better preservation of the capillary-cell membrane barrier in adapted animals and, accordingly, accelerated oxygen diffusion across them; 2) more intense oxygen consumption in adapted animals, possibly due to better oxygen utilization and oxidative phosphorylation in the mitochondria. Of course, these conclusions should be experimentally verified. However, it has been recently revealed that adaptation to repeated restrained stress results in the development of the phenomenon of adaptive stabilization of structures (PASS). PASS manifests itself in an increased stability of the cytoplasmic membrane and sarcoplasmic reticulum structures of the mitochondria [5] and cell nuclei [6] to autolysis and lipid peroxidation. It may be asserted that under the conditions of our investigation PASS development played a certain role in the preservation of cellular structures under severe hypoxia and thereby in the maintenance of a high rate of oxygen diffusion and consumption in the cells.

REFERENCES

1. S. V. Branovitskaya, P. B. Medvedev, and Yu. A. Fialkov, *Computer Mathematics in Chemistry and Chemical Technology* [in Russian], Vysha Shkola, Kiev (1986), p. 216.
2. Yu. N. Kopylov, L. Yu. Golubeva, V. A. Saltykova, *et al.*, *Byull.Eksp.Biol.*, №9., 244-246 (1990).
3. V. P. Pozharov, *Fiziol.Zh.*, **35**, №4, 119-121 (1989).
4. A. Kuroshiva, G. Habara, A. Uehara, *et al.*, *Pflug.Arch.*, 402, №5, 402-408 (1984).
5. F. Z. Meerson, I. Yu. Malyshev, and A. V. Zamotrinsky, *Basic Res.Cardiol.*, **86**, №1, 87-98 (1991).
6. F. Z. Meerson, I. Yu. Malyshev, and A. B. Shneider, *Basic Res.Cardiol.*, **86**, Suppl.3, 205-214 (1991).
7. F. Z. Meerson, *Adaptive protection of the Heart; Protecting against Stress and Ischemic Damage*, Boca Raton, Florida (1991).
8. L. Mikulaj, R. Kvetnansky, and K. Murgas, *Rev. Czechosl.Med.*, **20**, №3, 162-169 (1974).
9. T. Minyailenko, V. Pozharov, and M. Seredenko, Abstr.2nd Intern.Symp. on Hypoxia, Berlin, (1991) p.22-22a.
10. V.Pozharov, *Ibid.*
11. J.Wallace and M.Cohen, *Amer.J.Physiol.*, **247**, 6127-6132 (1984).

Na^+ and K^+ Permeability of Erythrocyte Membranes and their Phospholipid Composition in Patients with Essential Hypertension

N. I. Nadiradze, A. N. Grekulova, and V. G. Kavtaradze

UDC 616.12−008.331.1.−07:616.155.1−008.6

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 135−137, February, 1993
Original article submitted August 10, 1992

Key Words: *hypertension; erythrocyte permeability; phospholipids; total phosphorus*

Recent investigations have provided substantial evidence for the existence of membrane cation transport disturbances in patients with essential hypertension (EH), either in electrically excitable or nonexcitable cell types [4-6]. These facts produce new insight into this pathology which allow us to consider EH as a peculiar kind of membrane pathology. Cell membrane permeability depends strictly on structure. The shape of erythrocytes is determined mainly by the membrane proteins as well as membrane lipid composition. The phospholipid composition of the erythrocyte membrane is of interest, since phospholipids (PL) are the main component

Research Institute of Clinical and Experimental Cardiology, Republic of Georgia, Tbilisi. (Presented by M. M. Khananashvili, Member of the Russian Academy of Medical Sciences)

0007−4888/93/0002−0126$12.50

TABLE 1. Na^+ and K^+ Flux Rates Ratio, Total Phosphorus Content, and Phospholipid Composition of Erythrocyte Membrane in Patients with Essential Hypertension.

Patients	Total phosphorus, μg P/10^{12} erythrocytes	LPC	PS	SPM	PC	PE	PA	Na^+/K^+ flux rates ratio
Control n=8	47.2±1.96	7.45±0.61	6.12±0.54	7.63±0.54	8.72±0.51	7.55±0.24	10.4±0.68	3.63±0.47
Essential hypertension n=10	32.86±1.32 $p<0.01$	4.83±0.48 $p<0.05$	2.76±0.22 $p<0.01$	3.46±0.40 $p<0.01$	5.47±0.57 $p<0.01$	8.76±1.53	4.56±1.42 $p<0.01$	1.84±0.20 $p<0.01$

of the membrane (about 60%) and play an important role in the formation and function of the erythrocytes.

The aim of the present investigation was a comparison study of the phospholipid composition of erythrocyte membranes and their permeability for Na^+ and K^+ in patients with EH and in healthy subjects.

MATERIALS AND METHODS

Ten patients with mild EH aged 20 to 43, with arterial pressure of 155-170/80-95 mm Hg without concomitant diseases were examined. The control group included 8 healthy male subjects aged 27 to 41, with arterial pressure of 110-125/60-70 mm Hg and no family history of hypertension (only first-degree relatives were taken into consideration). The erythrocyte membrane permeability for monovalent cations was assayed after Garay and Meyer [7]. Na^+ and K^+ content was determined with a flame photometer (Germany). Total phosphorus and major PL were determined by thin-layer chromatography [2]. PL separation was performed in a thin layer of silica gel and developed with chloroform:methanol:25% ammonium (14:6:1). Silica gel for TLC-L 5/40 microns Chamapal (Czechoslovakia) was used. The following PL fractions from the erythrocyte membrane were determined: lysophosphatidylcholines (LPC), phosphatidylserines (PS), sphingomyelins (SPM), phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidic acids (PA). To evaluate the PL concentration the number of erythrocytes was determined. The total phosphorus content was expressed as μg P/ 10^{12} erythrocytes.

RESULTS

The experiments showed that the permeability for Na^+ and K^+ is altered in the erythrocyte membranes in the patients with EH. In particular, Na^+ retention in the erythrocytes was more pronounced in EH as compared with that in healthy subjects ($p<0.05$), whereas the rate of K^+ flux revealed opposite changes.

The difference between the EH patients and the control group became more significant when the Na^+/K^+ ratio was used. We thus used this index in the further discussion (see Table 1).

We also found a substantially lower content of total phosphorus in the erythrocytes of EH patients versus the control, which seems to be connected with its decrease in all PL fractions except PE (Table 1).

Taken together, these data suggest that the structural changes in the membrane, expressed in a decrease of major PL content, correspond to a lowering of the membrane permeability for Na^+ and K^+. The exact molecular mechanisms underlying the alteration of plasma membrane structure in EH are not known.

When analyzing the causes of the functional changes of cell membranes in EH, it is possible to single out the main factors contributing to membrane structure formation: the PL composition, the relative cholesterol content, and the state of the proteins of the membrane cytoskeleton.

Recent data suggest that a primary defect responsible for disorders of membrane function is located in a protein component of the cell membrane [1,10].

Several studies concerning the lipid composition of membranes are known where the authors do not find any difference in the major PL content between EH patients and healthy subjects [8], nor has any difference been discovered in the cholesterol content in these groups [9].

From our investigations we conclude that the disturbances in erythrocyte membrane permeability in EH are caused both by protein changes and by changes in PL composition.

These results supplement our previous electronmicroscopic study of the shape and surface of erythrocytes in patients with EH, which exhibit a 15% increase of abnormal erythrocyte types [3]. Undoubtedly, the quantitative changes observed in membrane PL composition must affect the transformation of the phospholipids.

REFERENCES

1. P. V. Gulak, S. N. Orlov, V. A. Shnyrov, *et al.*, *Kardiologiya*, №12, 59 (1983).
2. E. V. Dyatlovitskaya, T. I. Torkhovskaya, L. D. Bergel'son, *et al.*, *Biokhimiya*, **34**, №1, 177 (1969).

3. V. G. Kavtaradze, N. A. Aleksidze, N. I. Nadiradze, *et al., Byull. Eksp. Biol.*, №5, 554 (1989).
4. G. M. Kravtzov, S. N. Orlov, I. N. Pokudin, *et al., Kardiologiya,* №11, 88 (1982).
5. Yu. V. Postnov, S. N. Orlov, A. S. Shevchenko, *et al., Kardiologiya,* 10, 65 (1976).
6. E. Ambrosioni, F. Tartagni, L. Montebugnoli, *et.al., Clin. Sci.* 57, 3255 (1979).
7. R. P. Garay and P. Meyer, *The Lancet* 1, №109, 349 (1979).
8. R.Preiss, R.Prumke, *et al., Intern.J. Clin. Pharmacol. Ther.Toxicol.* 20, №3, 105 (1982).
9. K. Tsudo, T. Tamaki, J. Kusuiama, *et al.*, 10th International Society Hypertension Meeting, Abstracts, Interlaken (1984) p.873
10. J. Virit, D. Daveloose, G. Tostilier, *et al., Trav. Sci. Cherch.Serv.Santirmees*, №2, 384 (1981).

Low-Calcium Water Diet and Hypertensive Plasma Activity of WKY Rats

S. K. Churina, N. Z. Klueva, G. T. Eschanova, and D. B. Ryzhov

UDC 616.12.008.331.1−02:547.41]−07

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 115, № 2, pp. 137−138, February, 1993. Original article submitted July 10, 1992.

Key Words: *normotensive rats; drinking water; hypertensive plasma activity; parathyroid hypertensive factor*

Disturbances in calcium metabolism [4], as well as changes in the transport of Ca^{2+} and other cations across the membranes of a variety of cells, in particular erythrocyte membranes [3], have been found to play an important role in the pathogenesis of essential hypertension. These changes are most expressed under conditions of a Ca^{2+}-deficient diet [1]. An inversely proportional dependence between the Ca^{2+} content in the diet, notably in the drinking water, and both the frequency and degree of arterial pressure (AP) increase has been shown in epidemiological studies [4,5].

A search of the humoral factors responsible for the development of arterial hypertension (AH) under conditions of a Ca^{2+}-deficient diet has resulted in the discovery of a plasmatic factor in spontaneously hypertensive rats [6-8], in addition to the well-known erythrocytic factor [9,10].

The present study is based upon data concerning the broad prevalence of cardiovascular diseases under conditions of a diet deficient in Ca^{2+} and Mg^{2+}, especially in the regions with soft drinking water (Saint-Petersburg, Finland, Sweden, Norway, which rank first in incidence of AH), as well as upon data on the universal role of Ca^{2+} ions in the regulation of all cell functions. Additionally, it should be noted that there is a lack of physiological studies concerning the mechanism of the exogenous influence of Ca^{2+} on the formation of vascular tone as well as cardiac output.

The purpose of the present work was to study the influence of low and normal Ca^{2+} concentrations in drinking water on both the AP level and plasma hypertensive activity in WKY rats.

MATERIALS AND METHODS

Rats of the Wistar-Kyoto line (males) weighting 250-280 g at the age 12-16 weeks were used in the experiments. From the moment of the transition to the definitive nourishment the animals were kept on a standard ration with 0.6% Ca^{2+} content. The rats were given water ad libitum. The first (control) group starting from 4-week age was given drinking water with a

Laboratory of Experimental and Clinical Cardiology, Pavlov Institute of Physiology, Russian Academy of Sciences, St.Petersburg
(Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

0007−4888/93/0002−0128$12.50