Dynamics of Correlation between Erythrocyte Electrophoretic Mobility and Volume in Stress

V. B. Matyushichev, V. G. Shamratova, and D. R. Gutsaeva

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At rest, rat erythrocyte electrophoretic mobility varied independently on cell volume. Emotional and physical stress gave rise to a short-lived moderate negative correlation between erythrocyte volume and the coefficient of asymmetry of electrophoretic mobility distribution, probably originating from accompanying metabolic and systemic influences.

Key Words: *erythrocyte electrophoretic mobility; erythrocyte volume; rats; stress; correlation*

Electrophoretic mobility (EPM) of erythrocytes as a function of their surface charge is a vitally important parameter determining cell aggregation resistance, blood fluidity, and microcirculation in capillaries. It is of interest, therefore, to identify the factors participating in EPM regulation. Cell volume may be one of such factors [7]. Surface charge is proportional to cell size [11]. Young erythrocytes have higher mobility compared to old cells [10]. Young mature erythrocytes are larger than aging cells [2], and therefore are characterized by higher EPM. To clarify this question we studied the correlation between EPM and erythrocyte volume using the model of emotional and physical stress in rats.

MATERIALS AND METHODS

Experiments were carried out on 30 outbred male albino rats weighing 160-180 g. The animals were forced to swim in ice-cold water until exhaustion and decapitated under ether narcosis 10 min (n=15) and 1 h (n=15) after swimming session. EPM parameters, erythrocyte and reticulocyte count, hematocrit, and mean erythrocyte volume were determined. The control group (n=10) comprised intact rats of the same weight.

Erythrocyte EPM was measured automatically with a "Parmoquant-2" apparatus at 25°C in a 0.02-ml blood sample diluted (1:200) with 0.1 M phosphate buffer (pH=7.4). Electrophoretic scanograms were input to a computer. The mean EPM for each sample was calculated by averaging data of 250 individual measurements. Coefficients of asymmetry (As) and excess (Ex) for EPM distribution were determined for each sample and for the whole groups. The significance of differences was evaluated by Student's t test. Correlation analysis was performed using standard software.

RESULTS

Ten minutes after swimming, the hematocrit and the mean volume of erythrocytes increased, while the reticulocyte count and total erythrocyte concentration remained unaffected (Table 1). The mean EPM did not differ from the control, but its distribution (Ex) became considerably flattened. One hour after swimming erythrocyte count decreased, against the background of further activation of erythropoiesis (increased reticulocyte count) and further increase in erythrocyte mean volume. In other words, rejuvenation of the erythrocyte population was accompanied by accelerated elimination of red blood cells from circulaion; hematocrit returned to the control level. The mean EPM signifi-

Institute of Physiology, St. Petersburg State University; Bashkirian State University, Ufa; Institute of Evolutional Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg

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cantly decreased and EPM distribution was characterized by negative asymmetry and distinct negative excess.

Correlation analysis was applied to investigate the relationships between EPM and other blood indices. In control rats, high correlations were revealed between the reticulocyte count and EPM distribution parameters, in particular, variance (r=0.78) and As coefficient (r=-0.66), as well as between hematocrit and Ex coefficient (r=-0.71). The increase in reticulocyte count is accompanied by high EPM variability and accumulation of cells with high EPM. These data suggest that the right part of the erythrogram, which determines negative asymmetry of the distribution curves, is partially presented by rejuvenated erythrocyte population.

In rats exposed to emotional and physical stress the correlations were different. Ten minutes after swimming the character of the EPM distribution was determined by erythrocyte concentration and mean volume. Accumulation of cells with high EPM (decrease of As) correlated with the increase in erythrocyte mean volume (r=-0.68) and the decrease in their concentration (r=0.73). Judging by the Ex coefficient, the erythrocyte concentration noticeably modulated erythrocyte population heterogeneity (r=-0.72). Furthermore, in this group the level of significance was reached by the correlations between erythrocyte and reticulocyte concentrations (r=-0.81) and between reticulocyte count and erythrocyte mean volume (r=0.72).

One hour after swimming, the correlations between the standard red blood parameters weakened, while their correlations with EPM became stronger. The total content of erythrocytes and reticulocytes and hematocrit negatively correlated with EPM variance (r= -0.81, -0.63, and -0.90, respectively) and the Ex coefficient (r=-0.65, -0.88, and -0.65, respectively) and positively correlated with the As coefficient (r=0.75, 0.62, and 0.75, respectively). Therefore, variability of erythrocyte EPM decreased with increasing the number of erythrocytes and reticulocytes and total volume of erythrocytes, while population heterogeneity and the proportion of cells with low EPM increased.

It should be noted that the structure of correlations between various erythrocyte parameters also underwent significant changes during adaptation to extreme conditions. In the control group, a strong correlation (r=-0.94) was found only between erythrocyte count and their mean volume. This correlation persisted after swimming (r=-0.79), and additional correlations between these parameters and reticulocyte content reached the level of significance. All these correlations disappeared 1 h after swimming.

Thus, changes in erythrocyte EPM correlated with variations in their concentration and mean volume. Negative correlation between the concentration and mean volume of erythrocytes in rats has been reported previously [6]. We showed that extreme conditions gradually deteriorate the mechanism maintaining the total respiratory area of erythrocytes. Cell volume varies independently of mean EPM, but modulates the character of EPM distribution in cell population: the larger erythrocytes the higher proportion of cells with high EPM.

The absence of a correlation between the As coefficient of EPM distribution and erythrocyte volume is not confusing. More important is that the revealed correlations are unstable, depend on the situation and the subject of observation. This indicates that these correlations are not primary but subordinate to some other variables, not considered in this study.

For instance, when studying the correlation between EPM and volume, we did not consider the intensity of metabolism and, in particular, activity of ion transport systems involved in the autoregulation of erythrocyte volume [7] and EPM control [5]. It can be assumed that young and functionally mature cells have

TABLE 1. Rat Blood Indices under Normal Conditions and after Emotional and Physical Stress ($M\pm m$)

Index	Control	Time after stress	
		10 min	1 h
Hematocrit, %	44.2±0.8	47.4±1.1*	44.6±1.5
Erythrocytes, 10^{12}/liter	7.12±0.24	6.96±0.32	6.21±0.19*
Reticulocytes, per 1000 erythrocytes	25.4±0.9	28.1±1.2	32.1±1.3*
Erythrocyte mean volume, fl	62.2±1.7	68.1±2.1*	71.9±2.4*
Erythrocyte EPM, 10^{-8} m^2/V×sec	1.04±0.01	1.03±0.01	0.99±0.01*
As	-0.08±0.05	0.02±0.05	-0.35±0.05*
Ex	0.21±0.10	-0.23±0.10*	0.43±0.10*

Note. *p<0.05 in comparison with the control.

high EPM because of high metabolic activity and energy supply rather than large size. There seem to be no direct relationships between erythrocyte EPM and volume and statistical correlation between these parameters arise from their common dependence on metabolism. This correlation does not manifest itself under normal conditions, which can be explained by low contribution of metabolic processes to maintenance of cell volume when it remains constant [8]. It is likely that the volume of circulating erythrocytes changes during stress, thus activating the autoregulatory mechanism. Correspondingly, immediately after stress, mobilizing energy resources at the expense of elevation of blood adrenaline sharply activating erythrocyte metabolism [9], the linear correlation between erythrocyte volume and the As coefficient of EPM distribution becomes significant. It disappears again after exhaustion of metabolic energy resources under the massive attack of metabolites [12], which makes the ion transport systems unable to provide the optimum level of EPM (it decreases) or the effective correction of erythrocyte volume. The observed dynamics of correlations can also be explained by stress-induced mobilization of defective and "old" erythrocytes with low activity of transport ATPases and altered membranes [4] and by some other mechanisms.

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