PHYSIOLOGY

Typical Changes in Electrophoretic Mobility of Erythrocytes under Stress Conditions

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Various stress procedures (swimming, hypobaric hypoxia, and exotoxin administration) induced similar changes in electrophoretic mobility of rat erythrocytes. The degree and directionality of phasic changes in electrophoretic mobility depended on the concentration of stress hormones.

Key Words: *erythrocytes; electrophoretic mobility; stress*

The study of characteristic changes in cells during pathological processes of different genesis is an important problem. New data in this field will help to understand the general biological mechanism of alterative changes in the organism and to diagnose them at early stages. Electrophoretic mobility of erythrocytes (EPME) is an important parameter of human homeostasis. Recording of blood cell migration in the electric field characterizes their electrokinetic potential (*i.e.*, morphofunctional state of the membrane) and state of homeostasis in the whole organism. Reduced negative charge and decreased EPME attest to changes in rheological characteristics of the blood determining enhanced aggregation capacity of erythrocytes and facilitating thrombus formation [9]. Previous studies showed that EPME decreases in various pathologies, including cholestatic hepatitis [7], infections, tumor growth, chronic renal failure [2], and respiratory diseases [1]. The decrease in EPME under extreme and pathological conditions probably reflects a nonspecific reaction of the organism to negative stimuli. Since stress of different severity underlies progression of various pathological processes [3] it can be hypothesized that changes in EPME can serve as an early and universal (for different types of stress) marker of stress response. Here we studied typical changes in EPME in animals subjected to different stress exposures.

MATERIALS AND METHODS

Experiments were performed on male rats weighing 180-200 g. Physical exercise, hypoxia, and administration of exotoxin (bee venom, a typical stressogenic factor) served as the models of stress [6]. Physical exercise consisted in single 15-min swimming session (26-28°C water temperature) with a load equal to 10% body weight. Hypoxia was modeled in an altitude chamber at a height of 11,000 m for 5 min. Exotoxin test was modeled by intraperitoneal injection of bee venom in a dose of 0.5 mg/kg. The control groups included intact rats (experiments with physical exercise and hypoxia) and animals receiving physiological saline (experiments with exotoxin).

The blood was taken from the sublingual vein 5 min before and 30, 60, 90, 120, 150, and 180 min after stress. Erythrocytes were washed 3-fold with 0.85% NaCl (1000 rpm, 15 min). EPME was estimated by the method of electrophoresis [5]. In *in vitro* experiments washed erythrocytes were incubated with epinephrine and cortisol. The intensity of lipid peroxidation (LPO) was measured photometrically by accumulation of ma-

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lonic dialdehyde (MDA) [4]. The results were analyzed by Student's *t* test.

RESULTS

EPME in rats of all groups underwent similar biphasic changes (Fig. 1). EPME initially decreased (phase I), but then increased and surpassed the baseline level (phase II). We revealed only quantitative and temporal differences, which depended on the type of stress.

Thirty minutes after stress EPME decreased similarly in rats of different groups. One hour after swimming and administration of bee venom EPME returned to normal, while after hypoxia EPME progressively decreased to 78% of the control level (1.32±0.09 and 1.69±0.10 μ×cm/V/sec, respectively, $p<0.05$).

Two hours after injection of bee venom EPME increased from 1.04±0.05 to 1.37±0.04 μ×cm/V/sec ($p<0.05$). These changes were less significant after hypoxia and swimming; 180 min after stress EPME practically did not differ from the baseline level.

Our results suggest that the stress response of the organism is accompanied by typical changes in electrokinetic characteristics of erythrocytes. It is well known that the sympathoadrenal and hypothalamic-pituitary-adrenal systems play a key role in the humoral and hormonal mechanisms of stress. The decrease in EPME (phase I) is probably associated with modification of the erythrocyte membrane with endogenous catecholamines. These compounds affect integral membrane sialoglycoproteins that determine a negative charge on the cell surface. Catecholamines are released during the stress response, stimulate secretion of ACTH, and contribute to an increase in blood concentration of adrenocortical hormones. It can be hypothesized that excretion of corticosteroids suppressing phase I of the stress reaction probably determines increased EPME (phase II) due to their interaction with glucocorticoid receptors.

To verify this hypothesis, erythrocyte were *in vitro* incubated with epinephrine or cortisol. Epinephrine was used in a concentration corresponding to blood hormone level during stress (1 μg/liter). Epinephrine decreased EPME by 15% (from 1.33±0.09 to 1.13±0.06 μ×cm/V/sec, $p<0.05$). Incubation of erythrocytes with cortisol in a concentration of 100-500 μg/liter was followed by a significant dose-dependent increase in EPME (by 9-16%). Typical changes in EPME under stress conditions are probably related to variations in the concentration of stress hormones in the blood.

Our assumption can explain the existence of quantitative differences in phasic changes in EPME under stress conditions. The anxiety stage is most pronounced during hypobaric hypoxia. This state is accompanied by activation of the sympathoadrenal system and increase in blood epinephrine concentration. Administration of bee venom is followed by long-term activation of the adrenal cortex [6]. Forced swimming produces a weaker stress and less pronounced changes in EPME.

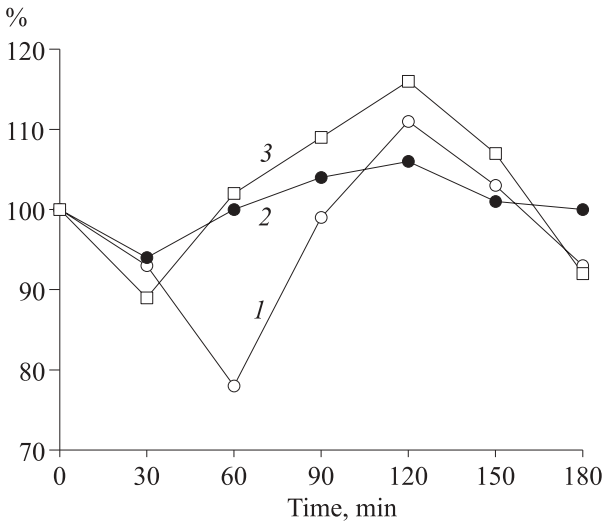

Fig. 1. Electrophoretic mobility of erythrocytes after hypoxia (*1*), forced swimming with a load (*2*), and administration of bee venom (*3*). Control: 100%.

Measurement of LPO in erythrocytes confirmed our assumption that the degree and directionality of changes in EPME depend on the type of stress. The intensity of LPO is proportional to the severity of alteration changes in the organism [8]. MDA concentration was maximum 30 min after hypobaric hypoxia (337% of the baseline level). The concentration of MDA was lower after swimming stress (153% of the baseline level). Administration of bee venom was followed by a significant and long-term release of corticosteroids. MDA concentration decreased by 14-16% 30 min after administration of bee venom. *In vitro* experiments showed that MDA concentration increases over 3-h incubation with epinephrine (by 22%, from 3.68±0.21 to 4.50±0.35 nmol/ml), but decreases 2 h after treatment with cortisol (by 12%).

Our findings suggest that biphasic changes in EPME are a characteristic response to various types of stress. The degree of these changes corresponds to the severity of stress phases. EPME can be used as an early and valid criterion of pathological changes in the organism and activity of the stress-realizing systems.

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