EFFECT OF CHEMICAL DESYMPATHIZATION ON APase AND LIPID PEROXIDATION IN ERYTHROCYTE MEMBRANES OF RATS WITH MYOCARDIAL INFARCTION

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The development of a myocardial infarct is accompanied by changes in various structural and functional parameters of erythrocytes. Investigations have shown that in the acute period of myocardial infarction the erythrocyte count and their hemoglobin content are increased [7]. Changes arise in the levels of Na^+, K^+, and Ca^{++} ions in the erythrocytes [5, 6] and, as a result, in Na,K-ATPase and Ca, Mg-ATPase activity also [9]. Deformability of the erythrocyte membranes is reduced and the ability of the cells to aggregate is increased [11]. Both these factors are concerned in the disturbance of the microcirculation arising during infarction and complicating its course. The changes observed are considered to be due to hypoxia and disturbance of neurohumoral regulation [7, 8].

One method of studying the role of the sympathetic nervous system in the onset and development of myocardial infarction is chemical desympathization, which results in complete destruction of peripheral sympathetic neurons [2].

In the investigation described below the functional characteristics of erythrocyte membranes were studied after desympathization in animals with myocardial infarction. For this purpose activity of Mg-ATPase and the intensity of lipid peroxidation (LPO) in the erythrocyte membranes were determined.

EXPERIMENTAL METHOD

Experiments were carried out on 77 Wistar rats aged 2 months. Desympathization was produced by subcutaneous injection of isobarin* (from Pliva), in a dose of 20 mg/kg, into newborn animals, starting from the 2nd day of life and thereafter daily for 4 weeks. Intact rats from the same litter, receiving similar doses of physiological saline, served as the controls. Infarction was induced by ligation of the descending branch of the left coronary artery in the desympathized and control rats. Blood was taken under superficial ether anesthesia from the bifurcation of the abdominal aorta. Erythrocytes were separated from plasma and white blood cells by centrifugation, and then they were washed in isotonic NaCl solution (pH 7.4). Erythrocyte ghosts [12] were isolated and protein [13], Mg-ATPase activity [10], and LPO [3] were determined in them. Inorganic phosphorus was measured spectrophotometrically [15]. The results were subjected to statistical analysis by Fisher's and Student's tests.

EXPERIMENTAL RESULTS

Desympathization of the rats had no significant effect on Mg-ATPase activity in the erythrocyte membranes. Activity of this enzyme rose by 54% in erythrocytes of the nondesymphathized rats 2 h after ligation of the descending branch of the left coronary artery. A high level of enzyme activity persisted 1 day after the operation. The normal levels were restored 30 days after production of the infarct. In desympathized rats Mg-ATPase activity also was increased 2 h after ligation of the artery, but by the 30th day the normal situation had not been restored (Table 1). This was evidently connected with the more severe disturbances of the structure of the erythrocyte membranes. This conclusion is based on data in

*Guanethidine.

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TABLE 1. Effect of Desympathization on Mg-ATPase Activity (in moles inorganic phosphorus/mg protein/h) of Erythrocyte Membranes in Rats with Myocardial Infarction (M ± m)

Experimental conditions	Mg-ATPase activity of erythrocyte membranes
normal	3,12±0,13 (7)
After creation of infarct	
2 h	4,82±0,15* (5)
24 h	4,61±0,15* (7)
30 days	3,35±0,08 (7)
Desympathization	3,46±0.22 (8)
Desympathization + infarct	
2 h	4,64±0,12* (6)
24 h	4,86±0,28* (8)
30 days	4,38±0,06* (10)

*P < 0.05 compared with corresponding control.
Legend. Number of animals in parentheses.

TABLE 2. MDA Concentration (in μ moles/mg protein) in Erythrocyte Membranes of Desympathized Rats with Infarction (M ± m)

Time after addition of Fe^{++}, min	Control (C)	Desympathization (D)	Time after creation of infarct			
			24 h		30 days	
			C	D	C	D
0	4,32±0,07 (7)	3,59±0,04 (7) $P<0,5$	2,82±0,09 (6) $P_1<0,01$	4,10±0,06 (7)	4,50±0,02 (10)	5,33±0,04 (7)
10	5,56±0,10 (7) $P_2<0,05$	5,89±0,07 (7) $P_2<0,05$	4,66±0,08 (6) $P_2<0,01$	7,60±0,12 (7) $P_2<0,01$ $P_3<0,01$	5,70±0,04 (10)	6,20±0,06 (7)

Legend. P) Significance of differences between C and D; P_1) between animals with infarct and without infarct, during endogenous LPO; P_2) increase of MDA concentration 10 min after addition of Fe^{++}; P_3) significance of differences between data with and without infarct. Number of animals in parentheses. Conditions of determination: 1-2 mg protein in 1 ml incubation medium (10 mM Tris-HCl, pH 7.4, 52 mM KCL) 12×10^{-6} M Fe^{++}. Extinction measured at 535 nm. Quantity of MDA formed was calculated by using coefficient of molar extinction of 156×10 $M^{-1} \cdot cm^{-1}$.

the literature on the relationship between Mg-ATPase activity and the shape of erythrocytes in experiments *in vitro* [14] and the decrease in deformability of the erythrocyte membrane in myocardial infarction [11].

On the basis of data indicating that transport of materials through membranes is linked with the intensity of LPO in them [1], the concentration of malonic dialdehyde (MDA), as an end product of LPO, was determined. As Table 2 shows, desympathization of the rats reduced by 16% the original MDA concentration in the erythrocyte membranes. This agrees with data in the literature, showing a decrease in the MDA concentration in erythrocytes and in their hemoglobin content after desympathization [4], and this may perhaps be one cause of the weakening of oxidative processes in the organs and tissues. The MDA concentration 10 min after stimulation of LPO by Fe^{++} ions was increased by 64% in the desympathized rats and by 28% in the erythrocyte membranes of the control animals, i.e., the ability of erythrocyte membranes of desympathized animals to take part in lipid peroxidation reactions was greater than that of the control rats.

Endogenous LPO in erythrocytes of the control animals by 34.8% was observed 24 h after ligation of the coronary artery and the formation of an ischemic focus in the heart. Under these conditions the intensity of LPO stimulated by Fe^{++} was 85% higher in the desympathized animals (from 4.1 to 7.6 units) than in the control (by 64% — from 2.82 to 4.66 units). On the 30th day after production of an infarct the intensity of LPO in erythrocyte membranes of intact rats and of desympatheized rats did not differ from the corresponding controls.

The results of these experiments thus showed that chemical desympathization does not affect Mg-ATPase activity of erythrocyte membranes. However, when a myocardial infarct is produced after desympathization of the animals, this enzyme activity reveals certain special features which, on the basis of data in the literature, can be interpreted as an indication of a disturbance of erythrocyte membrane structure. This view is confirmed by the increase in LPO induced in erythrocyte membranes of desympathized animals, which is particularly marked in the acute period of myocardial infarction.

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INVESTIGATION OF PENETRATION OF TRITIATED CYCLIC AMP INTO VARIOUS MOUSE TISSUES

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The question of permeability of cèll membranes to tritiated cyclic AMP (cyclic-AMP-^{3}H) [4, 5, 7, 11, 12] has not yet been settled. Meanwhile cyclic AMP is known to have a role in the regulation of cell metabolism, and, in particular, during tumor growth [2, 9], and disturbances of extracullular cyclic AMP metabolism have been found in cancer patients [8]. Accordingly, in the investigation described below, penetration of cyclic AMP-^{3}H was studied into certain organs and tissues of intact mice and of mice with transplanted tumors (Ehrlich's carcinoma and sarcoma 180).

EXPERIMENTAL METHOD

Experiments were carried out on 60 male SHR mice weighing 22-24 g. Penetration of cyclic AMP-^{3}H into the tissue was studied simultaneously in intact animals and in mice with a trans-

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