CHANGES IN ACETYLCHOLINESTERASE AND ATPase ACTIVITY AND SOME STRUCTURAL CHANGES IN THE ERYTHROCYTE MEMBRANE IN EXPERIMENTAL MYOCARDIAL ISCHEMIA

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KEY WORDS: erythrocytes; myocardial ischemia; erythrocyte membrane; enzyme activity.

One of the factors disturbing the microcirculation during the development of a myocardial infarct is a decrease in the ability of the erythrocyte membrane to undergo deformation, with the formation of pathological erythrocyte aggregates in the blood stream [6]. Changes in the mechanical properties of the erythrocytes, evidently connected with functioning of the actin–myosin-like spectrin system [11], have been well studied under normal and some pathological conditions [14]. Some workers consider that the shape and the maximal degree of deformation of the erythrocytes can be controlled by metabolic means also, for example, through the phosphorylation of spectrin or a change in the intracellular ATP concentration [7, 10].

In this investigation activity of Na,K-ATPase, Ca,Mg-ATPase, and acetylcholinesterase (AChE) of the erythrocytes of rats, in which the microcirculation was disturbed by experimental induction of a myocardial infarct, was studied. The structure of the membrane of erythrocyte ghosts was studied by means of the phthalocyanine dye Direct turquoise (DT) and the fluorescent probe 1-anilino-naphthalene-8-sulfonate (ANS).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. A myocardial infarct was induced by ligation of the descending branch of the left coronary artery. During the period of formation of the infarct, blood samples were taken from the rats on the 3rd, 7th, and 30th days. Heparin was used as anticoagulant. Erythrocytes were washed with isotonic NaCl solution (pH 7.4) and erythrocyte ghosts were isolated by the method in [4]. The protein concentration was measured by Lowry's method [9]. ATPase activity in the erythrocyte ghosts was determined by measuring the accumulation of inorganic phosphate [13] under the conditions described previously [3]. AChE activity in the erythrocytes was recorded by Elman's method [8]. Light absorption by DT was measured on the SF-4A spectrophotometer at 618 nm and the dissociation constant (K_{dis}) was calculated [1]. The intensity of fluorescence of ANS was recorded at 480 nm on an MPF-4 spectrofluorometer (Hitachi, Japan). Aggregation of erythrocytes was determined microscopically and the index of erythrocyte aggregation was calculated [5].

EXPERIMENTAL RESULTS

On the 7th day of development of the myocardial infarct the index of erythrocyte aggregation in whole blood of the rats was 1.76 (Fig. 1). The presence of rouleaux and of aggregates of 5 or 6 or more erythrocytes was observed. These observations correlate with the maximal level of spontaneous erythrocyte aggregation observed on the 8th day in patients with myocardial infarction [2]. The index of erythrocyte aggregation in the blood of intact rats was 1, and rouleaux formation was not observed. After the erythrocytes had been washed three times to remove plasma the index of erythrocyte aggregation of the experimental animals fell to 1.31 and the aggregates consisted of not more than three or four cells.

Consequently, the removal of inducers of aggregation such as fibrinogen and globulins [6], together with the plasma, did not lead to complete destruction of the erythrocyte aggre-

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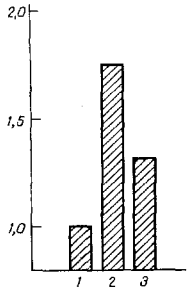

Fig. 1. Index of erythrocyte aggregation (in relative units) in blood of intact animals (1), or rats with 7-day-old myocardial infarct (2), and of erythrocytes from rats with infarct, washed to remove plasma (3).

TABLE 1. Parameters of Interaction between DT (K_{dis}) and ANS (I_{fl} — intensity of fluorescence), Bound with Erythrocyte Ghosts (M±m)

Experimental conditions	$K_{dis} \cdot 10^{-5}$ M	I_{fl}, relative units
Intact animals (control)	2,70±0,23	100
Experimental animals:		
with 3-day-old infarct	4,86±0,65	98±6
with 7-day-old infarct	7,09±0,12	102±4
with 30-day-old infarct	6,16±0,43	102±4

gates. This can evidently be explained on the grounds that, besides an increase in the plasma fibrinogen concentration in myocardial infarction [2], aggregation also is accompanied by certain changes in the cell membrane.

It was accordingly decided to study the activity of certain membrane enzymes and structural features of the erythrocyte membrane during the development of experimental myocardial infarction.

On the 7th day after the formation of the infarct AChE activity was reduced by 22% ($P < 0.05$), and by the 30th day it had regained the level of its activity in erythrocytes of intact rats (Fig. 2). Activity of NA,K-ATPase in the erythrocyte ghosts was increased by 45% ($P < 0.02$) on the 7th day and it fell to its original level by the 30th day. Similar changes were found in Ca,Mg-ATPase activity (Fig. 2).

The dissociation constant of DT adsorbed by the erythrocyte ghosts was considerably higher for rats with myocardial infarction (Table 1). The maximal (2.5 times) increase in K_{dis} was observed in the acute period of development of the infarct (7th day). Meanwhile, the intensity of fluorescence of ANS bound with erythrocyte ghosts remained unchanged. It

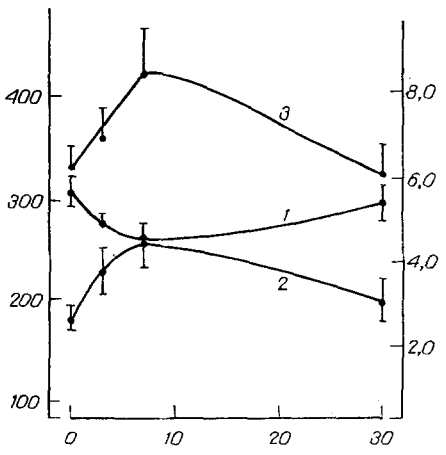

Fig. 2. Changes in acetylcholinesterase activity (1) of erythrocytes and Na,K-ATPase (2) and Ca,Mg-ATPase (3) activity of erythrocyte ghosts obtained from rats with experimental myocardial infarction at different stages of development. Abscissa, time (in days); ordinate: on left — ATPase activity (in nanomoles P_{inorg}/mg protein/h), on right — AChE activity (in micromoles thiocholine/min/ml of erythrocytes).

should be noted that ANS is localized in hydrophobic regions of the membrane [12], whereas DT is bound only by membrane proteins and is localized on the outer surface of the plasma membrane [1]. It can accordingly be postulated that only the surface regions of the membrane were changed in the erythrocytes of rats with myocardial infarction.

The 7th day is thus the critical period of development of experimental myocardial infarction in rats. This period is characterized by the most marked mechanical and functional changes in the erythrocytes. The increase in erythrocyte aggregation observed at this stage of development of the infarct and the maximal changes in activity of the membrane enzymes and in the structure of the erythrocyte membrane suggest that the changes taking place in functional and mechanical processes in this disease are interconnected. The development of a myocardial infarct, especially in the acute period, not only leads to a disturbance of the rheologic properties of the blood, but also, probably, significantly changes the metabolic state of the erythrocytes.

LITERATURE CITED

1. S. V. Levin, Structural Changes in Cell Membranes [in Russian], Leningrad (1976).
2. V. A. Lyusov, A. S. Parfenov, and Yu. B. Belousov, Kardiologiya, No. 6, 42 (1978).
3. A. S. Shevchenko and S. N. Orlov, Biokhimiya, 42, 906 (1977).
4. T. A. Bramley, R. Coleman, and J. B. Finean, Biochim. Biophys. Acta., 241, 752 (1971).
5. S. Chien, S. Usami, K. G. Dellenback, et al., Science, 157, 829 (1967).
6. L. Dintenfass, Blood Microrheology, Viscosity Factors in Blood Flow, Ischemia and Thrombosis, New York (1971), p. 183.
7. A. M. Ehrly and W. Scheinpflung, Bibl. Anat. (Basel), 13, 117 (1975).
8. G. L. Elman, Arch. Biochem., 82, 70 (1959).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. K. Nakashima and E. Beutler, Proc. Natl. Acad. Sci. USA, 75, 3823 (1978).
11. F. J. Lionetti, et al., Blood, 44, 583 (1974).
12. G. K. Radda and J. Vanderkooi, Biochim. Biophys. Acta, 265, 509 (1972).
13. W. B. Rathbun and M. B. Bethlach, Anal. Biochem., 28, 436 (1969).
14. R. I. Weed, Am. J. Med., 49, 147 (1970).

INVESTIGATION OF CARDIAC EXCITABILITY THRESHOLDS DURING ELECTRICAL STIMULATION

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KEY WORDS: excitability of the myocardium; pulse charge; pulse duration; pulse voltage; threshold characteristics.

The threshold characteristics of the excitability of the myocardium during its electrical stimulation are of great practical interest, for they are widely used when implanted artificial pacemakers are designed, and in particular, when the parameters of the stimulating pulse are chosen.

In this paper we examine defects of known methods of obtaining threshold characteristics of excitability of the heart and we suggest a simple method of determining them, based on direct measurements of the pulse charge.

During stimulation of the heart by square pulses [1-3] (as is used in the overwhelming majority of implanted pacemakers), to give a complete quantitative description of excitabil-

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