Histochemical Study of Myocardial Metabolism in Experimental Massive Pulmonary Embolism

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The period immediately following massive pulmonary embolism largely determines its further course, that is, whether compensation will occur or whether heart failure will ensue. Prognostically favorable or unfavorable histochemical characteristics of myocardial metabolism during this period are revealed in this study.

Key Words: *experimental massive pulmonary embolism; heart; histoenzymology*

Our previous study looked at the histoenzymological changes taking place in the ventricular myocardium during two variants of acute massive embolism of the pulmonary arteries (PAME): uncomplicated or complicated by the development of cardiac insufficiency [14]. In the former case the material for investigation was collected 6 h after the induction of PAME, in the latter after the animal's death, which ensued suddenly and, as a rule, early. Hence, further studies were carried out with due consideration for the time differences in order to compare the time course of metabolic changes in the ventricular myocardium during different variants of experimental PAME.

MATERIALS AND METHODS

Forty mongrel dogs weighing 15 to 20 kg were used in the study, with closed chest and spontaneous respiration. Premedication consisted of intramuscular promedole in a dose of 10 mg/kg, and narcosis during the experiment was provided by fractionated intravenous infusion of sodium thiopental in a dose of 20 mg/kg. Catheterization of the heart and blood vessels, recording of hemodynamic parameters, and simulation of acute PAME were carried out as described previously [3]. The protocol of the experimental and control studies is presented in Fig. 1. The experimental animals were divided into 3 groups, one group consisting of animals (Fig. 1, *I*) which developed heart failure eventuating in death during the first 30 min after PAME was induced (decompensated PAME) and the other two groups (Fig. 1, *II, III*) of animals without signs of circulatory insufficiency (compensated PAME).

Specimens for morphological study were taken from the right and left ventricles, fixed in 10% neutral formalin buffered after Lillie, and embedded in paraffin. Slices 5-7 μ thick were stained with hematoxylin-eosin and Schiff's reagent with amylase control. The glycogen content in the myocardium was assessed using a five-point scale. Histoenzymological study was carried out on 10-μ cryostat slices. The activities of succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), glyceraldehyde phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH), gluceraldehyde phosphate dehydrogenase NADPH diaphorases were detected routinely [2,11] and assessed on a five-point scale. The activities of SDH, LDH, NADH and NADPH diaphorases were measured using a Microvideomat television device (Opton) and a Wang-720 computer [4,5]. The data were processed by mathematical statistics using Student's *t* test.

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RESULTS

Since ICDH and GAPDH participate in the most important reactions of the citric acid and glycolysis cycles [7,10] the ratio of their activities reflects to a certain extent the balance of the mitochondrial and cytoplasmic energy-producing processes. By the end of the first hour of the control investigation, the ICDH/GAPDH ratio was 1.3/1.0 in the right and 1.5/1.0 in the left ventricle, this pointing to the important role of cell respiration in myocardial metabolism. The initial period of compensated PAME (Fig. 2, *a*) is characterized by an increase of GAPDH and a decrease of ICDH activity in both ventricles. The ICDH/GAPDH ratio is 1.0/1.2 in the right and 1.0/1.6 in the left ventricle, this reflecting an appreciable shift of metabolism towards glycolysis. The content of glycogen in the ventricles is somewhat decreased, but is still higher in the right ventricle than in the left (1.5/1.0). The ratio of NADH/NADPH diaphorases, reflecting the balance of catabolic and anabolic reactions [1,5,7,10], does not differ from the control ratios for the same period and is 1.6/1.0 for both ventricles. Besides the above-mentioned histoenzymological changes that are similar for both ventricles, differences are observed: the activities of SDH, GDH, and NADPH diaphorase are increased only in the right ventricle.

After 6 h of compensated PAME (Fig. 2, *b*) the activity of GAPDH drops in both ventricles. The ICDH/GAPDH ratio is 3.5/1.0 in the right and 4.6/1.0 in the left ventricle, which is higher than the control values for the same period (2.1/1.0 and 2.0/1.0, respectively). This means a pronounced shift of energy-producing processes towards cellular respiration. The content of glycogen falls in the myocardium of both ventricles, but remains higher in the right ventricle (1.7/1.0). The activity of NADPH diaphorase appreciably decreases in both ventricles. The NADH/NADPH diaphorase ratio is 3.3/1.0 in the right and 4.6/1.0 in the left ventricle, this being higher than in the control (2.1/1.0 and 3.2/1.0, respectively). At this time the differences between the ventricles augment as regards the activities of enzymes participating in catabolic reactions. In the right ventricle the activities of SDH, MDH, LDH, and GDH increase. Conversely, in the left ventricle the activities of these enzymes decrease or remain at the initial level. The abatement of the catabolic processes in the left-ventricular myocardium corresponds to the reduction of NADH diaphorase in it.

Hence, during a compensated course of PAME the pattern of histoenzymologic shifts in the ventricular myocardium largely depends on the time of examination: directly after the induction of PAME

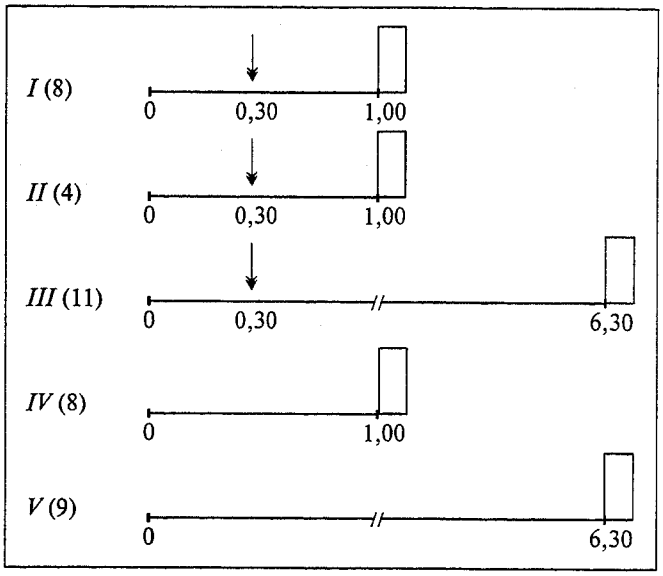

Fig. 1. Scheme of experimental (*I, II, III*) and control (*IV, V*) studies. Abscissa: time (h, min) elapsed after catheterization of the heart and vessels. The arrows show PAME induction. Bars show death (*I*) or sacrifice (*II–V*) of animals with a lethal dose of sodium thiopental and collection of material for histochemical study. The number of animals per group is shown in parentheses.

or 6 h later. In the first case glycolysis activation is observed, which is usually regarded as a reaction characteristic of the "emergency stage of compensatory cardiac hyperfunction" [9]. However, we observed such a reaction in both ventricles, even though their loading changes in different directions in PAME [3]. This suggests that the observed activation of glycolysis is largely caused by the effects of systemic factors, primarily neurohumoral [3,16,17]. During the same period the level of NADPH diaphorase increases in the right ventricle, reportedly [9] due to its hyperfunction. In the second case, that is, 6 h after PAME induction, glycolysis is inhibited and the balance of energy-producing reactions shifts towards mitochondrial respiration in both ventricles. The activity of NADPH diaphorase falls off in the same period, attesting to abatement of the anabolic processes in both ventricles, although the right ventricle is still operating under conditions of sharply increased postloading [3]. We believe that the suppression of biosynthesis in the right-ventricular cardiomyocytes is one compensatory adaptive mechanism which helps redistribute the energy reserve of cells towards the performance of external work and thus maintains the pumping function of the heart during the first hours after PAME reproduction. It is clear that the regenerative processes can only be allowed to taper off in the absence of pronounced intracellular lesions, in other words, if the plastic reserve of cells remains intact [8]. Previous studies [6,15] showed that signs of damage are seen after 6 h of compensated

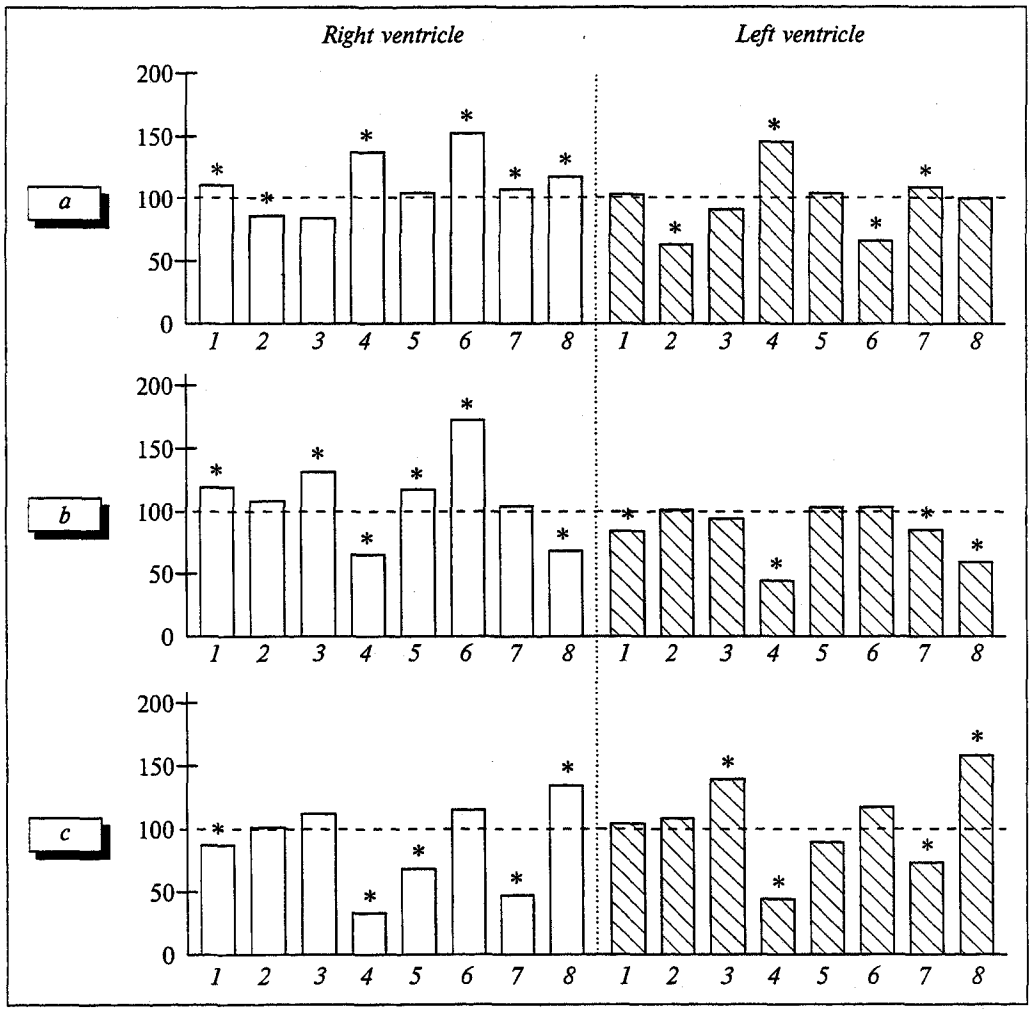

Fig. 2. Histoenzymological changes in the ventricular myocardium during different variants of experimental PAME. Ordinate: activities of enzymes in % of control values for the same period (taken as 100% and shown with a broken line). *a*) 30 min after PAME reproduction (compensated course); *b*) 6 h after PAME induction (compensated course); *c*) 30 min after PAME induction (development of cardiac failure with a lethal outcome). *1*) SDH; *2*) ICDH; *3*) MDH; *4*) GAPDH; *5*) LDH; *6*) GDH; *7*) NADH diaphorase; *8*) NADPH diaphorase. *$p<0.05$ *vs.* the control.

PAME in the cardiomyocytes of both ventricles, but these lesions are reversible and not associated with a reduction in the number of cell organelles.

On the whole, myocardial metabolism in compensated PAME is characterized by the following features. First, catabolic prevail over anabolic processes in both ventricles, and this predominance is the more expressed the longer the period that has elapsed since PAME induction. Second, as the glycogen reserve in the myocardium progressively shrinks, its content remains higher in the right ventricle. Third, differences are observed in the metabolic reactions in the right and left ventricle, which correspond to variously directed changes of their function in PAME.

When the period directly following the induction of PAME is complicated by the development of cardiac insufficiency (Fig. 2, *c*), the activity of glycolytic enzymes drops in the myocardium of both ventricles. The ICDH/GAPDH ratio is 4.2/1.0 for the right and 3.7/1.0 for the left ventricle and reflects the minor specific significance of glycolysis in the processes of energy production. The glycogen reserves are depleted in both ventricles, but more so in the right one, so that the ratio of glycogen content in the ventricles levels to 1.1/1.0. The activity of NADH diaphorase decreases, and that of NADPH diaphorase increases in both ventricles. The NADH/NADPH diaphorase ratio becomes inverse: 1.0/1.7 in the right and 1.0/1.5 in the left ventricle, this indicating a pronounced shift of metabolism towards anabolic reactions. As mentioned above, an increase of NADPH diaphorase activity in the right ventricle may to a certain degree be caused by its hyperfunction. However, a similar reaction is observed in the left ventricle, whose work slackens in PAME [3], so that we cannot attribute the activation of biosynthesis in the ventricular myocardium solely to changes in the work of the ventricles. We think that the intensification of regeneratory processes in this variant of PAME is largely a reaction to deep-seated damage in cardiomyocyte ultrastructure, which is in line with other data [12]. Such a hypothesis is based on the results of our previous studies [15], which demonstrated pronounced irreversible damage in cardiomyocytes of both ventricles in PAME cases associated with cardiac insufficiency. It is noteworthy that,

in contrast to the compensated variant of PAME, in the decompensated condition no heterogeneous histoenzymological changes are detected in the ventricular myocardium. Except for the biosynthetic ones, the examined metabolic processes are characterized by enzymopathy, which evidently underlies the manifest dystrophic changes in the cardiomyocytes of both ventricles [13].

The results lead to the following conclusion. The period directly following the induction of PAME is transitory and in many respects determines its further course: compensated or associated with cardiac failure, eventuating in death. The study revealed prognostically favorable or unfavorable histochemical characteristics of myocardial metabolism during this period. The favorable ones are: glycolysis activation; preservation of the glycogen reserve and its higher content in the right ventricle in comparison with the left; a predominance of energy-producing over biosynthetic processes; heterogeneity of metabolic reactions in the two ventricles due to variously directed changes in their work in PAME. The unfavorable characteristics are: a drastic reduction of the activities of glycolytic enzymes; depletion of the glycogen reserve, particularly in the right ventricle; a shift of the balance of catabolic and anabolic reactions towards the latter with an appreciable increase of the NADPH diaphorase level in the myocardium of both ventricles.

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