FUNCTION AND MORPHOLOGY OF THE MYOCARDIUM IN EXPERIMENTAL MASSIVE PULMONARY ARTERIAL EMBOLISM

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Massive thromboembolism of the pulmonary artery is one of the most threatening complications in surgery and is attended by a high mortality [8, 9]. Accordingly, the experimental study of various aspects of this pathology is extremely urgent. Until recently virtually no comprehensive experimental study of the pathology and causes of death of acute massive pulmonary embolism (MPE) have been undertaken. The aim of the present investigation was to compare structural and metabolic changes in the myocardium of the right and left ventricles in acute MPE.

EXPERIMENTAL METHOD

Experiments were carried out on 19 mongrel male and female dogs weighing 15-20 kg, under closed chest conditions and with natural breathing. Premedication consisted of intramuscular injection of trimeperidine (10 mg/kg) and the animals were anesthetized by fractional intravenous injection of thiopental-sodium (20 mg/kg). The chambers of the heart and the aorta were catheterized without thoracotomy through the peripheral vessels using Edman's catheters. Longitudinally divided fragments of the dog's sartorius muscle (5 × 50 mm) were used as emboli. The ECG, the pressure in the aorta, right ventricle (RV), right atrium, and left ventricle (LV), and the rate of contraction (+dp/dt) and relaxation (—dp/dt) of the ventricles were recorded.

All parameters were recorded on a Mingograf-82 (Siemens-Elema, Sweden). Samples were taken from the aorta and right ventricle to study the gas composition and acid—base balance of the blood, using a microanalyzer (Corning-178, USA). In 13 dogs which constituted the experimental group, MPE was produced by injection of 5-11 muscle emboli into the external iliac vein. In the control group (6 dogs) the chambers of the heart were catheterized only. Material for morphological and electron-microscopic investigation was taken immediately after the animals of both groups were killed by intravenous injection of a lethal dose of thiopental-sodium, 6 h after the experiment began. Material from RV, LV, and its anterior papillary muscle (APM) for morphological investigation was fixed in 10% neutral formalin, buffered by Lillie's method, embedded in paraffin wax, after which sections 5-7 μ thick were cut and stained with hematoxylin and eosin and by Van Gieson's method. The enzyme histochemical investigation was carried out on frozen sections 10 μ thick. Activity of enzymes of the citric acid cycle (SDH, ICDH, MDH), of glycolysis (GAPDH, LDH), and of the pentose phosphate shunt, (G6PDH), of enzymes of enzyme-transport systems (NADH- and NADPH-diaphorases) and of protein catabolism (GDH) was determined. Activity of the oxidoreductases was demonstrated with the aid of nitro-BT by the usual methods [2, 4, 7] and was determined by visual semiquantitative assessment. To verify the accuracy of this method, quantitative determination of SDH and LDH activity was carried out in the same preparations using a "Microvideomat" television system (Opton, West Germany), with data processing by Wang-720C computer (USA), and by regression analysis of correlation between the two methods. Material for electron-microscopic investigation was fixed for 1.5 h in 2.5% glutaraldehyde, made up in phosphate buffer (pH 7.4). Besides by the standard method, preparations were made by incubation in 1% tannic acid solution after treatment in 1% OsO_4 solution, followed by dehydration

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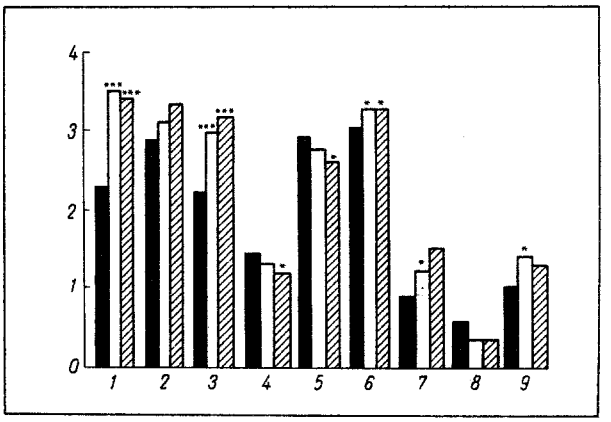

Fig. 1. Enzyme-histochemical profile of cardiomyocytes in control group. Ordinate, enzyme activity (conventional units); abscissa, enzymes: 1) SDH; 2) ICDH; 3) MDH; 4) GAPDH; 5) LDH; 6) NADH-diaphorase; 7) NADPH-diaphorase; 8) G6PDH; 9) GDH. Black columns indicate RV, unshaded columns LV, obliquely shaded columns APM. Asterisks indicate significant differences compared with RV: *$p < 0.05$, ***$p < 0.001$.

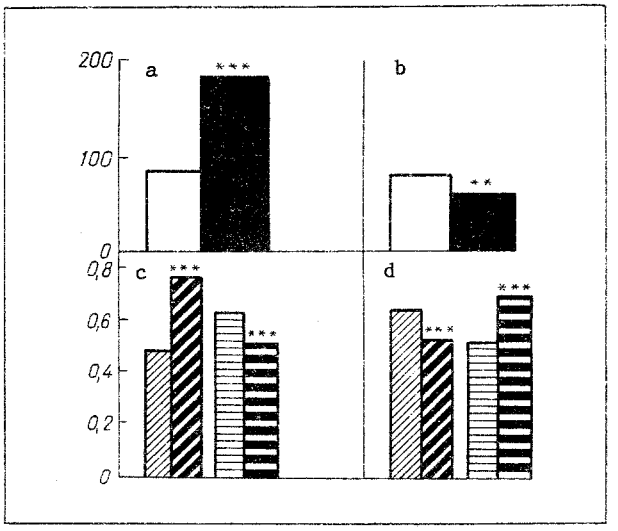

Fig. 2. Changes in pressure and metabolism in RV (a, c) and LV (b, d) after 6 h of MPE. Unshaded columns — control, black columns — MPE, obliquely shaded columns — SDH, horizontally shaded — LDH; a, b) systolic pressure (ordinate, in per cent of initial value); c, d) enzyme activity (ordinate, conventional optical density units). Asterisks indicate significant changes compared with control values: **$p < 0.01$, ***$p < 0.001$.

of the tissue and embedding in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the HU-12A transmission electron microscope. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The enzyme-histochemical profile of the cardiomyocytes in the control group was characterized by a distinctive ratio between levels of activity of the enzymes studied in the right and left sides of the heart (Fig. 1), emphasizing the metabolic heterogeneity of the myocardium [5]. On comparing the control values, activity of enzymes of cell respiration (SDH, ICDH,

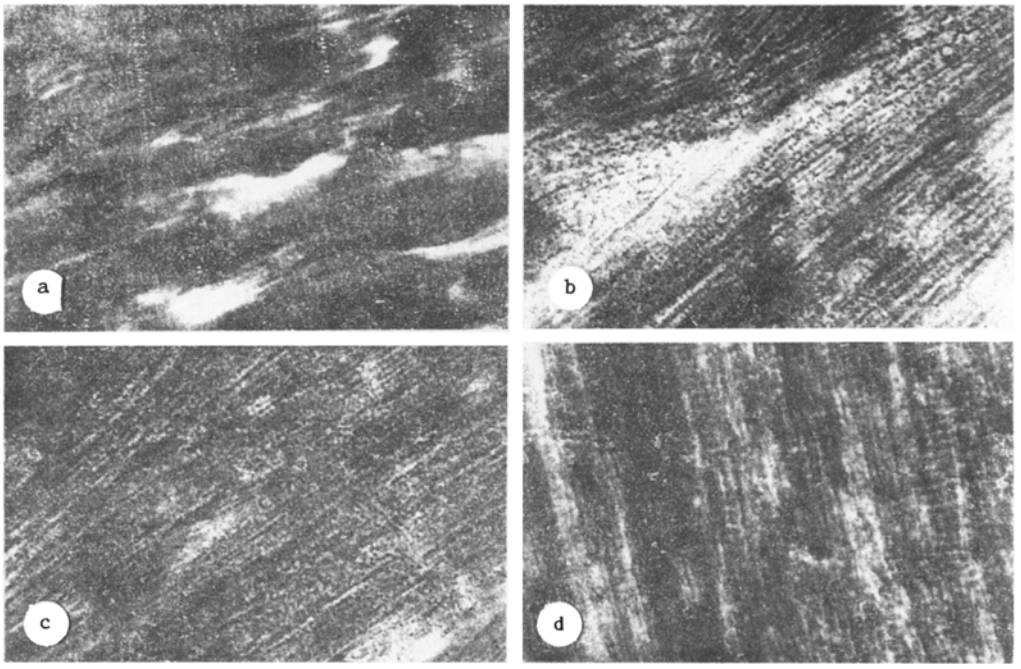

Fig. 3. Changes in enzyme activity in cardiomyocytes of RV and LV after 6 h of MPE. a) High SDH activity in RV. 240×; b) low SDH activity in LV. 240×; c) low LDH activity in RV. 240×; d) high LDH activity in LV. 240×.

MDH-2) was higher in LV and APM than in RV. This reflects the ratio of the energy demands of the myocardium of the ventricles, and in turn it correlates with their relative functional loads.

The fact that the control GDH levels corresponded to hemodynamic loads indicates more active processes of amino acid deamination in LV.

The control group was characterized by a somewhat higher LDH level in the myocardium of RV than in LV and APM (Fig. 1, Fig. 2c, d).

The electron-microscopic investigation showed that the numerical density of mitochondrial profiles was $7.0 \cdot 10^7 \pm 0.7 \cdot 10^7$ cm^0/cm^2 in the subendocardial parts of LV and $4.7 \cdot 10^3 \pm 0.4 \cdot 10^7$ cm^0/cm^2 in corresponding parts of RV ($p < 0.05$), whereas the relative surface area of the mitochondrial profiles was 30.7 ± 3.4 and 18.8 ± 2.1% respectively ($p < 0.05$). Thus the results of the ultrastructural investigation confirm the relative intensities of aerobic metabolism of the myocardium in the parts of the heart studied in the control group.

Acute MPE was accompanied by marked changes in the hemodynamics and metabolism of both the right and the left parts of the heart.

The systolic pressure in RV 6 h after creation of MPE was 185.0 ± 14.0%, the rate of its contraction was 171.0 ± 13.0%, and the rate of its relaxation 201.0 ± 24.0% of the initial levels, significantly higher than the control values ($p < 0.001$). The end-diastolic pressure in RV did not differ from the initial and control values, confirming the state of hemodynamic compensation. The systolic pressure in LV was reduced to 69.0 ± 4.0% of the initial value, significantly below the control level ($p < 0.01$). The velocity of contraction of LV was 71.0 ± 8.0% and the velocity of its relaxation 85.0 ± 11.0% of the initial level, not very different from the control values ($p > 0.05$). The intraaortic pressure was reduced to 72.0 ± 5.0% of the initial value, significantly lower than in the control ($p < 0.05$). The heart rate was increased to 136.0 ± 18.0%, higher than the control values ($p < 0.05$). Against the background of an increased frequency of respiratory excursions (to 212.0 ± 38.0%; $p < 0.01$) a marked degree of hypoxia (pO_2 59.8 ± 3.2 mm Hg, $p < 0.01$) and a moderate degree of hypocapnia (pCO_2 27.7 ± 1.9 mm Hg, $p < 0.01$) were observed. No significant changes could be found in the acid–base balance of the blood.

The significant increase in the after-load on RV, increasing the energy demands on the myocardium of this portion of the heart, was accompanied by intensification of aerobic metabolism (Fig. 2a, c; Fig. 3a). Against the background of relaxation of the work of LV, opposite changes in metabolism were discovered (Fig. 2b, d; Fig. 3b).

The distribution of energy processes between the mitochondria and cytoplasm can be judged by the dynamics of changes in SDH and LDH activity. An increase in the relative role of enzymes of the Krebs' cycle in the structure of energy metabolism, accompanied by swelling of the mitochondria, for which SDH is regarded as the marker [3, 6], and by an increase in their number, is proof of an increase in the functional load on the cell [1]. Similar changes were discovered in the subendocardial zones of RV during MPE, mainly a tendency toward an increase in the relative surface area (26.3 ± 4.3%, $p > 0.05$) and in the numerical density of profiles ($6.0 \cdot 10^7 \pm 0.9 \cdot 10^7$ cm^0/cm^2, $p > 0.05$) of the mitochondria. The changes discovered by ultrastructural methods correlate with the dynamics of SDH in RV during MPE. The increase in the relative area and numerical density of the mitochondria found in RV is an indirect indication of weakening of the role of the hyaloplasm in the provision of energy for the cardiomyocytes. A decrease in the relative surface area of the mitochondria (17.1 ± 2.0%, $p < 0.01$) and a tendency for their density to decrease ($6.4 \cdot 10^7 \pm 0.6 \cdot 10^7$ cm^0/cm^2, $p > 0.05$) were observed in the subendocardial zones of LV, corresponding to the trend of changes in SDH activity in this part of the heart during MPE.

An increase of 15% ($p < 0.05$) in MDH activity in RV may indicate intensification of metabolism of reducing equivalence between the cytoplasm and mitochondria, whereas its reduction by 19% ($p < 0.01$) in LV may be evidence of weakening of this type of metabolism in the malate shuttle mechanism [10].

These opposite changes in the hemodynamic loads on different parts of the heart were accompanied by intensification of protein catabolism in RV (GDH activity was increased by 53%, $p < 0.01$) and by some degree of weakening of this process in LV (GDH activity was reduced by 10%, $p > 0.05$). By the 6th hour of MPE a small fall of the LDH level in RV and a rise of its level in LV could be observed (Fig. 2c, d; Fig. 3c, d).

Thus the structural and metabolic features of the ventricular myocardium discovered in the control group correspond on the whole to the functional loads on these parts of the heart. The opposite changes in hemodynamic loads on RV and LV during acute compensated MPAE correlate with the opposite changes in their cellular respiration, accompanied by adequate responses of intracellular structures. Elevation of the LDH level in the myocardium of LV can evidently be explained by the action of the hypoxic factor. The small reduction of LDH in RV toward the 6th hour of MPAE may be the result both of complex coordination of respiration and anerobic glycolysis in the myocardium of this part of the heart in response to a sudden increase in its load [10] and also an increase in permeability of cell membranes, accompanied by release of LDH into the blood stream.

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