STRUCTURAL AND METABOLIC CHANGES IN THE VENTRICULAR MYOCARDIUM IN EXPERIMENTAL MASSIVE PULMONARY EMBOLISM

M. S. Tverskaya, V. V. Karpova, A. O. Virganskii, V. V. Banin, L. A. Tsareva, I. Zh. Satylganov, R. V. Rogulenko, E. I. Tikhvinskaya, M. A. Chumakova, and I. G. Saganelidze

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A leading place in the mechanisms of death from acute massive embolism of the pulmonary artery (MEPA) is occupied by right-ventricular failure. The comparative study of the morphology and function of the myocardium of the right (RV) and left (LV) ventricles is of great importance to the understanding of the causes leading to its development. The writers previously [5] studied the structural and metabolic changes in the myocardium of RV and LV in acute compensated MEPA. The aim of the present study was to undertake similar investigations in acute MEPA accompanied by the development of irreversible decompensation.

EXPERIMENTAL METHOD

Experiments were carried out on 29 male and female mongrel dogs weighing 15-20 kg, under closed chest conditions and with natural respiration. Premedication consisted of intramuscular injection of trimeperidine (10 mg/kg). Anesthesia was maintained during the experiment by fractional intravenous injection of thiopental sodium (20 mg/kg). Longitudinally divided fragments of the dog's sartorius muscle were used to produce embolism. The chambers of the heart and the aorta were catheterized through peripheral vessels without thoracotomy. The ECG, heart rate (HR), respiration (RR), and pressure in the aorta and RV and LV were recorded. A detailed account of the method of producing a model of MEPA, and the schedule of catheterization and methods of recording was given previously [2]. Animals in which a model of MEPA was produced were divided into two groups. The first experimental group consisted of 13 dogs in which no signs of circulatory failure were observed during the 6 h after production of MEPA. The second experimental group consisted of 10 dogs in which acute MEPA was accompanied by the development of irreversible decompensation. The control group consisted of six dogs. Animals of the control and first experimental groups were sacrificed by intravenous injection of a lethal dose of thiopental sodium 6 h after the experiment began.

Material for morphologic investigation was taken from RV and LV, fixed in Lillie's 10% buffered neutral formalin, and embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin-eosin and by Van Gieson's method. The histoenzymologic investigation was conducted on frozen sections 10 μ thick. Activity of enzymes of the citric acid cycle (SDH, ICDH, MDH), of protein catabolism (GDH), of the phosphogluconate pathway (G6PDH), and of NADH- and NADPH-diaphorases was determined. Oxidoreductase activity was determined with the aid of nitro-BT by the traditional methods [1, 6, 9] and estimated visually and semiquantitatively. Activity of SDH and of NADH- and NADPH-diaphorases was determined on the same sections quantitatively by means of a "Microvideomat" television system ("Opton," Germany) and a Wang 720C computer (USA).

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TABLE 1. Parameters (M ± m) of Systemic Hemodynamics and Respiration in Compensated MEPA or MEPA Accompanied by Development of Irreversible Decompensation

Group of animals studied	Parameters in % of initial level (100%)					
	systolic pressure in RV, mm Hg	systolic pressure in LV, mm Hg	systolic pressure in aorta, mm Hg	diastolic pressure in aorta, mm Hg	heart rate, beats/min	respiration rate, cycles/min
Control group	82,0±6,0	90,0±4,0	85,0±7,0	92,0±6,0	113,0±21,0	90,0±11,0
1st experimental group (MEPA without circulatory failure)	185,0±14,0***	69,0±4,0**	73,0±4,0	72,0±5,0*	136,0±18,0	212,0±38,0*
2-nd experimental group (MEPA accompanied by development of decompensation)	255,0±33,0***,+	75,0±9,0	62,0±6,0*	58,0±6,0**	99,0±5,0	260,0±30,0***

Legend. Significant differences compared with control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) and between experimental groups (+$p < 0.05$) are shown

Material from the subendocardial regions of RV and LV for electron-microscopic investigation was fixed for 1.5 h in 2.5% glutaraldehyde, made up in phosphate buffer (pH 7.4). The specimens were prepared by standard procedures and also by incubation in 1% tannic acid solution after treatment in 1% OsO_4 solution, followed by dehydration 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

Our previous investigations [2, 4] showed changes in the hemodynamic loads on RV and LV in different directions in acute compensated MEPA. A considerable increase in the after-load on RP was accompanied by intensification of catabolism, increased activity of the enzymes of cell respiration, and an increase in the numerical density of mitochondrial profiles in the myocardium of this part of the heart. Weakening of the work of LV was accompanied by opposite structural-metabolic changes.

Table 1 gives parameters of the systemic hemodynamics and respiration recorded in animals of the control and first experimental groups 5-10 min before sacrifice, and also in animals of the second experimental group 5-10 min before the development of irreversible decompensation. The absence of any marked differences between the parameters of MEPA, when compensated or accompanied by the subsequent development of decompensation, will be noted. The end-diastolic pressure in RV did not differ from its initial value and the control levels in animals of the first experimental group and was significantly higher than these levels in animals of the second experimental group (0.0, 0.3 ± 0.7, and 4.2 ± 1.7 respectively, $p < 0.05$).

Comparison of the metabolic changes in the ventricular myocardium in MEPA accompanied by the development of decompensation (Fig. 2), and with changes in compensated MEPA (Fig. 1) reveals two fundamental differences. First, despite the considerable level of the after-load on RV, reduced NADH-diaphorase activity and activity of HAD-dependent catabolic enzymes was observed in the myocardium of this part of the heart (Fig. 2), including enzymes of cellular respiration (ICDH, MDH). Second, in MEPA accompanied by subsequent development of irreversible decompensation, in both ventricles there was a marked increase in the NADPH-diaphorase level. The differences specified are clearly illustrated in Fig. 3, which gives a direct comparison of the results of the histochemical studies in the two experimental groups.

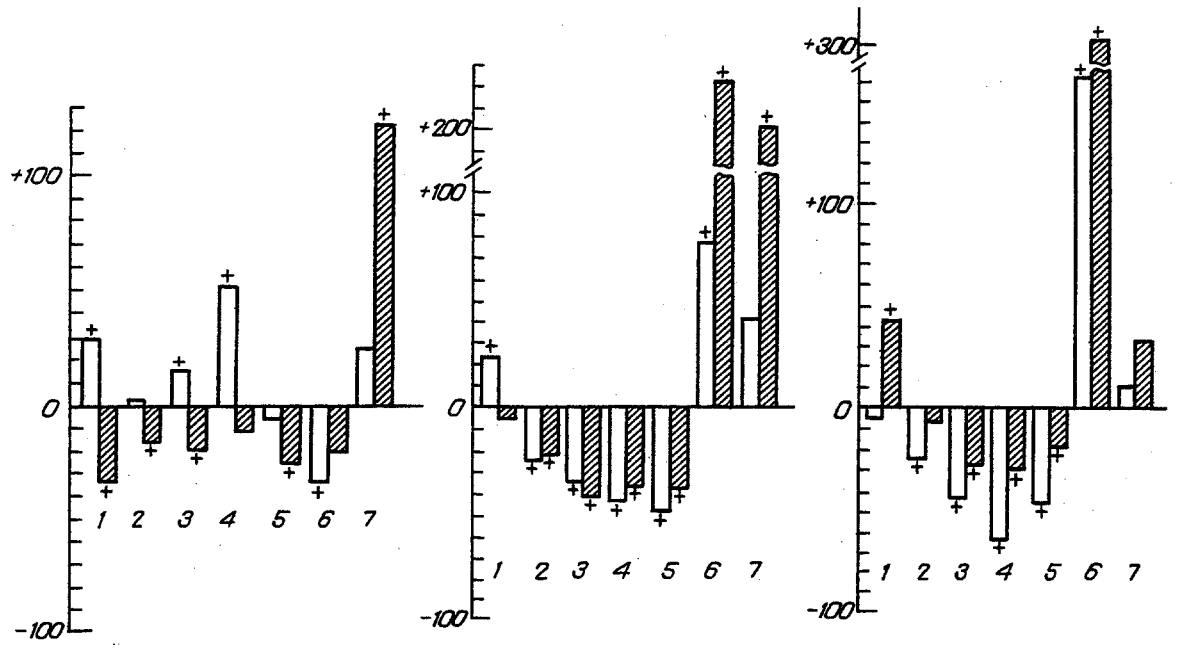

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Histochemical changes in ventricular myocardium in acute compensated MEPA. Ordinate, changes (M ± m) in enzyme activity in % of their control levels; abscissa, enzymes: 1) SDH, 2) ICDH, 3) MDH, 4) GDH, 5) NADH-diaphorase, 6) NADPH-diaphorase, 7) G6PDH. Unshaded columns – RV, shaded columns – LV. +) Significant changes compared with control values at $p < 0.05$.

Fig. 2. Histochemical changes in ventricular myocardium in acute MEPA, accompanied by development of irreversible decompensation. Legend as to Fig. 1.

Fig. 3. Comparison of results of histochemical study in two experimental groups. Ordinate, changes (M ± m) in enzyme activity in % of their levels in compensated MEPA. + indicates significant changes compared with values obtained with compensated MEPA at $p < 0.05$ level.

In the myocardium of LV changes in the activity of enzymes involved in catabolism in the two experimental groups were similar (Figs. 1 and 2) and correlated directly with reduction of the hemodynamic load on this part of the heart (Table 1). In MEPA accompanied by the development of irreversible decompensation, the electron-microscopic study revealed a decrease in numerical density of mitochondrial profiles per unit area of section (1 cm^2). Compared with compensated MEPA it was reduced in the myocardium of RV ($6.0 \cdot 10^7 \pm 0.9 \cdot 10^7$ and $4.3 \cdot 10^7 \pm 0.4 \cdot 10^7$ cm^0/cm^2, $p < 0.05$) and had a tendency to fall in the myocardium of LV ($6.4 \cdot 10^7 \pm 0.6 \cdot 10^7$ and $5.5 \cdot 10^7 \pm 0.3 \cdot 10^7$ cm^0/cm^2 $p > 0.05$). The mitochondrial fraction in which injuries were observed (disorganization of the cristae, destruction of the outer membrane) was increased in the myocardium of both ventricles. Whereas in compensated MEPA there was only a tendency for the fraction of injured mitochondria to be increased compared with the control (from 23.3 ± 5.4% to 33.3 ± 5.5% in RV, $p > 0.05$; from 20.5 ± 4.8% to 30.2 ± 3.9% in LV, $p > 0.05$), in MEPA accompanied by the development of decompensation there was a significant increase in this fraction (to 39.4 ± 4.5% in RV, $p < 0.05$; to 40.9 ± 2.7% in LV, $p < 0.01$).

The ratio NADH/NADPH is known to reflect the balance between metabolism and biosynthesis in the cell [1, 6, 8]. The ratio NADH-/NADPH-diaphorases for RV and LV was 2.0/1.0 and 3.5/1.0 respectively in the control group and 2.8/1.0 and 3.3/1.0 in the group with compensated MEPA. These results are in agreement with those of our previous investigation [4], confirming the initially more marked shift of metabolism toward energy processes in the myocardium of LV, and also an increase in this shift in the myocardium of RV in compensated MEPA. In cases of MEPA accompanied by the later development of irreversible decompensation, the NADH-/NADPH-diaphorase ratio becomes inverted, amounting to 1.0/1.7

in RV and 1.0/1.5 in LV, indicating a significant shift of metabolism toward biosynthesis in the myocardium of both ventricles. This conclusion is confirmed by elevation of levels of NADPH-diaphorase and G6PDH (Fig. 2) in both ventricles, the latter being regarded as a marker of the phosphogluconate pathway [3], activation of which is accompanied by accumulation of NADPH.

Thus, whereas in compensated MEPA the structural-metabolic reactions in the ventricular myocardium are on the whole adequate to meet their hemodynamic loads [4], in MEPA accompanied by the development of decompensation changes are found in the myocardium of RV which do not correlate with the increase in the after-load on this part of the heart, namely: a decrease in activity of enzymes involved in catabolism (including enzymes of cellular respiration), a decrease in the number of mitochondria, an increase in the fraction of injured mitochondria. In acute MEPA accompanied by the development of irreversible decompensation a marked shift of metabolism is observed toward biosynthesis, with a significant rise of the NADPH-diaphorase and G6PDH levels in the myocardium of both ventricles.

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EFFECT ON NUMBER AND ORIENTATION OF MICROGRAFTS ON WOUND EPITHELIZATION IN RATS

V. N. Shvets, Z. E. Vnukova, and A. T. Loshakov

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Deep wounds can be epithelized by the aid of skin microautografts (MAG) [1-4]. Can the MAG method be used in the case of extensive burns, when the donor's reserves of skin are depleted? An indicator of the efficacy of the skin grafting method is the transplantation coefficient (the ratio of the area of the graft to the area of the wound). We know that the transplantation coefficient attainable by the use of UAG is up to 1:30 in rabbits [3, 4] and up to 1:40 in rats [2]. These values, however, may perhaps not be limiting. The MAG method also differs from traditional grafting of a skin flap in that the pieces of skin are transplanted arbitrarily. The question naturally arises of the ability of MAG to epithelize the wound if they are strictly oriented relative to the wound surface (to the dermis, the epidermis, or laterally). The aim of this investigation was to study these problems.

Research and Production Center for Medical Bioengineering, Ministry of Health of the USSR, Moscow.

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