Structural and Metabolic Changes in Cardiac Conducting System during Massive Pulmonary Embolism

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We studied structural and metabolic changes in ventricular conducting cardiomyocytes during the acute phase of massive pulmonary embolism complicated or uncomplicated by cardiac insufficiency. During massive pulmonary embolism without cardiac insufficiency, glycolysis in conducting cardiomyocytes of both ventricles was activated, and its contribution to energy formation increased. Massive pulmonary embolism complicated by cardiac insufficiency was accompanied by inhibition of glycolytic enzymes and damages to conducting cardiomyocytes of the left and right ventricles. Our findings indicate that the development of cardiac insufficiency during the acute phase of massive pulmonary embolism provides structural and morphological basis for impairment of electrophysiological properties of the myocardium.

Key Words: *experimental massive pulmonary embolism; cardiac conducting system; pathomorphology; enzyme histology*

Arrhythmia and conduction disturbances often accompany and aggravate the acute phase of massive pulmonary embolism (MPE) [5]. Structural and metabolic mechanisms underlying electrophysiological dysfunction of the myocardium during MPE are poorly understood. There are no data on the state of the cardiac conducting system over the first hours of MPE. Clinical observations indicate that severe myocardial injuries play an important role in the pathogenesis of arrhythmias, which cause cardiac insufficiency and increase mortality [6]. In this respect, intramyocardial changes during MPE complicated or uncomplicated by cardiac insufficiency are of particular interest.

Here we studied structural and metabolic changes in conducting ventricular cardiomyocytes during the acute phase of uncomplicated MPE or MPE complicated by cardiac insufficiency.

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MATERIALS AND METHODS

Experiments were performed on 40 closed-chest mongrel dogs (15-20 kg) under conditions of natural ventilation. The dogs were premedicated by intramuscular injection of 10 mg/kg promedol and anesthetized by fractional intravenous administration of 20 mg/kg sodium thiopental. Cardiac and vascular catheterization, recording of hemodynamic parameters, and modeling of acute MPE were performed as described elsewhere [3].

Figure 1 shows the scheme of the experiment. Group 1 included dogs with uncomplicated MPE. Group 2 included dogs with MPE complicated by cardiac insufficiency, which developed over the first 30 min of the experiment. Group 3 dogs were euthanized after 30 min of uncomplicated MPE to compare changes accompanying complicated and uncomplicated MPE. Group 4 and 5 animals served as the control.

Specimens from the middle third of the right and left ventricles were fixed in 10% neutral formalin (Lilly's method) and embedded in paraffin. Slices (5-7 μ)

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were stained with hematoxylin and eosin, toluidine blue, Schiff reagent (amylase control), and by the methods of Goldner and Rego. The relative number of damaged cardiomyocytes was calculated on Rego-stained samples.

The samples for histochemistry were frozen in cold petroleum ether (-70°C) and stored in liquid nitrogen. The activities of succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), glyceraldehyde-3-phosphate dehydrogenase (GAPD), lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), and NADH and NADPH diaphorases were measured in 10-μ slices by routine methods and scored using a 4-point scale [1]. The ICDH/GAPD ratio reflected the balance between mitochondrial and cytoplasmic energy-forming reactions, and the NADH/NADPH diaphorase ratio reflected the state of catabolic and anabolic processes [2,11].

The results were analyzed by Student's t test.

RESULTS

Morphological picture of the ventricular conducting system in control dogs did not differ from normal [4,7]. Large conducting cardiomyocytes and contained considerable amounts of glycogen located primarily in the perinuclear zone. On preparation stained with hematoxylin and eosin, toluidine blue, or by the Goldner's method these zones looked empty. Enzyme activities in ventricular conducting myocytes were normal (Table 1) [4,7]. By the end of the 1st hour, ICDH to GAPD activity ratios in the right and left ventricles were 1.0:1.1 and 1.3:1.0, respectively, and NADH to NADPH diaphorase activity ratios were 1.7:1.0 and 1.2:1.0, respectively. By the 6th hour, ICDH to GAPD activity ratios in the right and left ventricles were 1.9:1.0 and 1.6:1.0, respectively, and NADH to NADPH diaphorase activity ratios were 2.1:1.0 and 2.4:1.0, respectively.

The number of damaged conducting cardiomyocytes (Rego staining) during MPE increased in both ventricles (Table 2). The acute phase of uncomplicated MPE was characterized by high GAPD and low MDH activities in ventricular conducting cardiomyocytes (Table 1). Activities of SDH, LDH, and NADH and NADPH diaphorases in the right ventricle increased, while SDH and ICDH activities in the left ventricle decreased. ICDH to GAPD activity ratios in the right and left ventricles were 1.0:1.2 and 1.0:1.7, respectively. The ratio between NADH and NADPH diaphorase activities in cardiomyocytes of the right ventricle was 1.2:1.0.

TABLE 1. Enzyme Activities (Points) in Ventricular Conducting Cardiomyocytes ($M \pm m$)

Enzymes	Time, h	Right ventricle			Left ventricle		
		control	MPE		control	MPE	
			uncomplicated	complicated		uncomplicated	complicated
SDH	1	1.6±0.1	1.85±0.07⁺	1.66±0.09	1.9±0.1	1.42±0.08⁺	2.03±0.11
	6.5	1.45±0.15	1.68±0.07	—	1.57±0.13	1.40±0.08	—
ICDH	1	1.93±0.09	2.03±0.08	2.72±0.15⁺	2.50±0.11°	1.42±0.10⁺	2.88±0.09⁺
	6.5	3.38±0.08*	2.54±0.06*⁺	—	3.04±0.08*°	2.70±0.07*⁺	—
MDH	1	2.00±0.09	1.60±0.08⁺	1.40±0.15⁺	2.20±0.09	1.67±0.09⁺	2.10±0.17
	6.5	2.38±0.07*	2.01±0.07*⁺	—	2.52±0.07*	2.12±0.09*⁺	—
GAPD	1	2.00±0.11	2.32±0.07⁺	1.40±0.15⁺	2.0±0.1	2.47±0.09⁺	1.7±0.1⁺
	6.5	1.75±0.10	1.64±0.06*	—	1.94±0.08	1.78±0.07*	—
LDH	1	3.30±0.08	3.65±0.08⁺	2.44±0.07⁺	3.25±0.10	3.43±0.08	3.17±0.12
	6.5	3.60±0.15	3.33±0.06*	—	2.60±0.16*°	3.45±0.06⁺	—
GDH	1	0.77±0.09	1.00±0.12	1.44±0.10⁺	1.25±0.12°	0.98±0.10	1.22±0.10
	6.5	1.77±0.11*	0.98±0.07⁺	—	1.43±0.09°	1.6±0.1*	—
NADH diaphorase	1	3.17±0.07	3.50±0.08⁺	2.67±0.09⁺	3.25±0.10	3.43±0.08	3.00±0.11
	6.5	3.05±0.17	2.57±0.06*⁺	—	3.10±0.11	2.70±0.07*⁺	—
NADPH diaphorase	1	1.93±0.09	3.00±0.16⁺	1.94±0.11	2.75±0.10°	—	2.50±0.09
	6.5	1.5±0.1*	1.28±0.09*	—	1.25±0.09*	1.32±0.10	—

Note. $p<0.05$: *compared to 1 h, ⁺compared to the control, °compared to the right ventricle.

TABLE 2. Content (%) of Rego-Positive Conducting Cardiomyocytes at Various Terms after Cardiac Catheterization

Ventricle, time, h		Control	MPE	
			uncomplicated	complicated
Right	1	12	60	88
	6.5	6	38	—
Left	1	20	60	70
	6.5	15	36	—

The number of damaged conducting cardiomyocytes (Rego staining) decreased with the progression of uncomplicated MPE (Table 2). By the 6th hour, activities of GAPD and NADH and NADPH diaphorases in cardiomyocytes of both ventricles decreased, while ICDH and MDH activities increased compared to those in the acute phase of uncomplicated MPE (Table 1, Fig. 2, *a*). ICDH/GAPD and NADH/NADPH diaphorase activity ratios in both ventricles were 1.5:1.0 and 2.0:1.0, respectively. Enzyme activities in both ventricles were below or equal to normal (except for LDH activity in conducting cardiomyocytes of the left ventricle, which considerably surpassed the control, Table 1, Fig. 2, *b*).

Most ventricular conducting cardiomyocytes appeared to be damaged during MPE complicated by cardiac insufficiency (Rego staining, Table 2, Fig. 2, *c*). Examination of the preparations incubated with amylase and stained with Schiff reagent revealed plasma infiltration of some conducting cardiomyocytes, which indicated their irreversible damages (Fig. 2, *d*) [16]. ICDH activity increased, while GAPD activity decreased in cardiomyocytes of both ventricles (Table 1). Activities of MDH, LDH, and NADH diaphorase decreased, while GDH activity increased in the right ventricle. ICDH and GAPD activity ratios in the right and left ventricles were 1.9:1.0 and 1.7:1.0, respectively; the NADH to NADPH diaphorase ratios were 1.4:1.0 and 1.2:1.0, respectively.

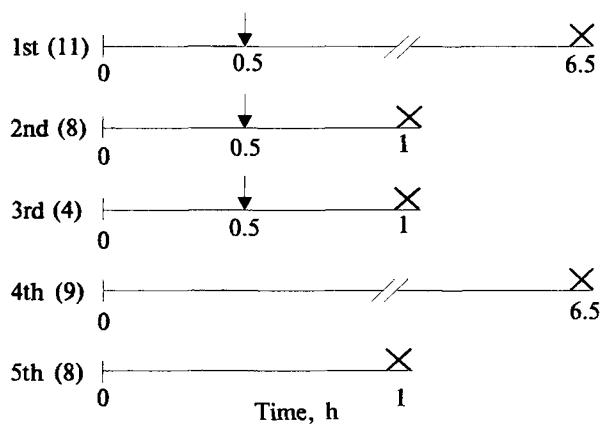

Fig. 1. Scheme of the experiment. Groups 1, 2, and 3: experiment; groups 4 and 5: control. Arrows: start of massive pulmonary embolism; cross-stitches: death (group 2) or euthanasia (groups 1, 3, 4, and 5) of animals with a lethal dose of sodium thiopental followed by sampling for morphological assay. Number of dogs is shown in parentheses.

Hence, the metabolism in ventricular conducting cardiomyocytes during the acute phase of uncomplicated MPE is characterized by activation of glycolysis and its increased contribution to energy formation. Our previous experiments demonstrated similar changes in ventricular contractile cardiomyocytes [13]. The same structural and metabolic changes in various cardiomyocytes of both ventricles, whose functions undergo opposite changes during MPE [3], indicate that activation of glycolysis is induced by systemic neurohormonal factors. Our previous studies showed that MPE is accompanied by activation of the sympathoadrenal system and arterial hypoxia [3,10,14]. Our findings are consistent with published data that hypoxia and catecholamines stimulate glycolytic processes in the myocardium [8,11,15].

Deceleration of glycolysis, intensification of cell respiration, and suppression of anabolic processes were observed in ventricular conducting cardiomyocytes with the progression of uncomplicated MPE. By the 6th hour, the metabolism in conducting cardiomyocytes did not differ from normal. The shift towards catabolic reactions persisted, and cell respiration played the major role in energy formation. Activities of most enzymes were below or equal to the control. The number of damaged cardiomyocytes decreased. This dynamics of structural and metabolic changes in conducting cardiomyocytes probably reflects normalization of their electrophysiological properties with transition from the acute to subacute phase of MPE. This assumption is confirmed by electrocardiography showing low incidence of arrhythmia and normalization of electrocardiogram by the 6th hour of MPE.

During MPE complicated by cardiac insufficiency, the metabolism in conducting cardiomyocytes was characterized by low activities of glycolytic enzymes and small contribution of glycolysis to energy formation. Similar changes were found in ventricular contractile cardiomyocytes [13]. The same structural and metabolic changes in various cardiomyocytes of both ventricles indicate that they are caused by systemic factors. Our previous studies showed that the content of norepinephrine in the nerve plexuses of the ventricular myocardium sharply decreases during the acute phase of complicated MPE [10,14]. These data attest to an interrelation between the intensity of glycolytic processes and norepinephrine concentration in the myocardium during the acute phase of MPE.

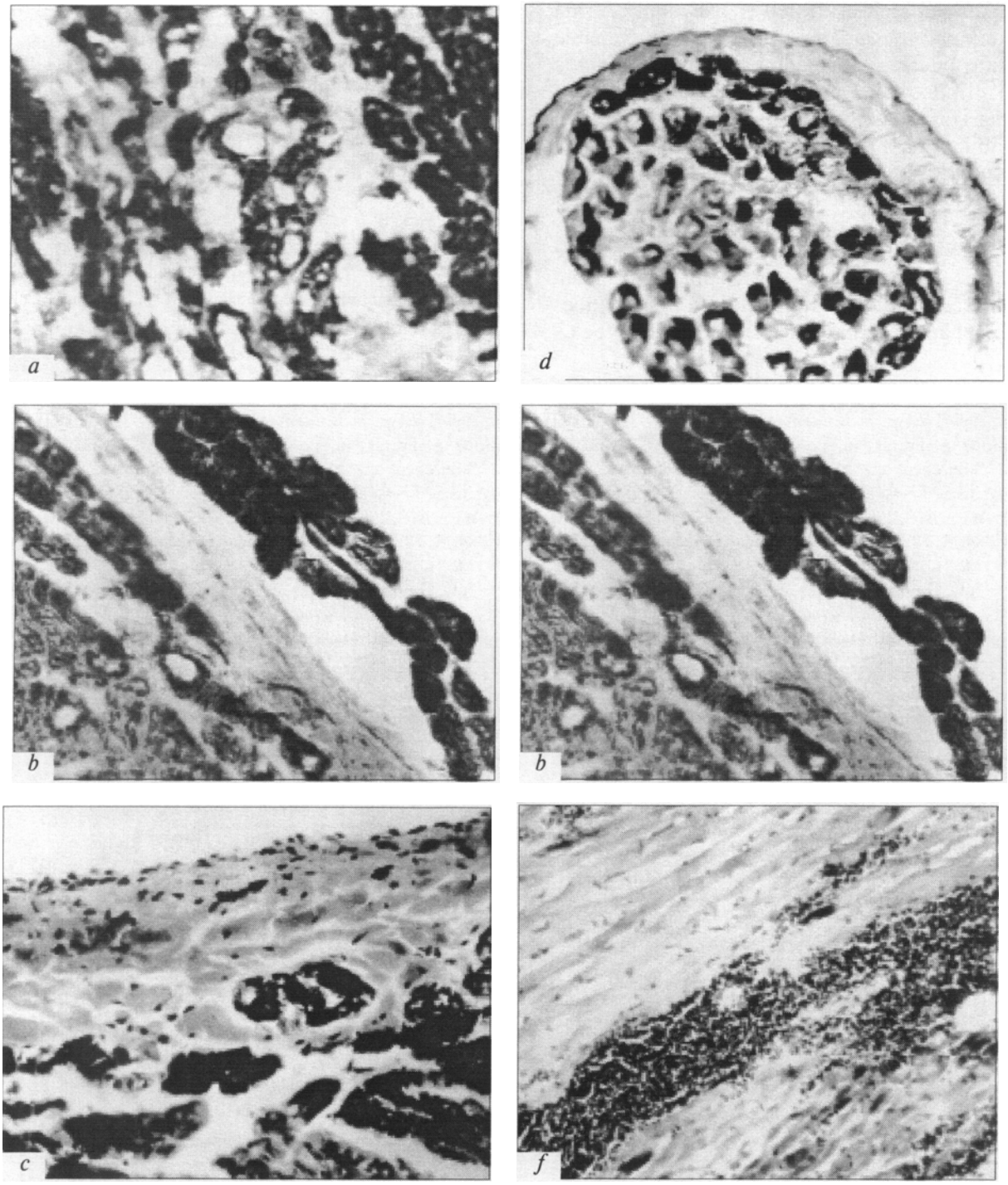

Fig. 2. Structural and metabolic changes in the cardiac conducting system during uncomplicated (*a*, *b*) and complicated (*c-f*) massive pulmonary embolism: moderate NADH diaphorase activity in conducting cardiomyocytes (CCM) of the right ventricle, reaction with NBT (×400, *a*); high LDH activity in CCM of the left ventricle, reaction with NBT (×200, *b*); damage to CCM of the left ventricle (Rego staining, ×200, *c*); plasma infiltration of CCM in the left ventricle, incubation with amylase and staining with Schiff reagent (×200, *d*); interstitial edema and hemorrhage in cardiac conducting system, subendocardial layer of the right ventricle (Goldner staining, ×400, *e*); diffuse hemorrhage in the subendocardial layer of the left ventricle (Rego staining, ×200, *f*).

It is now established that glycolysis plays an unique role in providing energy for electrophysiological processes in the myocardium [8,9,11]. Ion pumps in conducting cardiomyocytes consume energy formed during glycolysis [8]. Glycolytic disturbances promote electrical instability of the myocardium and increase the risk of arrhythmia [9]. Therefore, the inhibition of glycolysis in conducting and contractile cardiomyocytes impairs their electrophysiological properties and causes arrhythmia. This is confirmed by changes in elec-

trocardiogram and high incidence of arrhythmia during MPE complicated by cardiac insufficiency. Irreversible damages to conducting and contractile cardiomyocytes [12] and subendocardial ventricular hemorrhages impair impulse conduction in the heart and promote electrical instability of the myocardium (Fig. 2, *e*, *f*).

Our findings indicate that the development of cardiac insufficiency during the acute phase of MPE provides the structural and morphological basis for impairment of electrophysiological properties of the myocardium. These data are consistent with current notions on a close interrelation between disturbances in mechanical and electrical properties of the myocardium [6].

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