Changes of the Sympatheticoadrenal System during Experimental Massive Pulmonary Embolism

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UDC 616.127−091+616.131−088.331−07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.115, № 4, pp.347−350, April, 1993
Original article submitted November 18, 1992

Key Words: *experimental massive pulmonary embolism; sympatheticoadrenal system*

We have already shown in previous investigations [9,10] that acute massive pulmonary embolism (MPE) is attended by pronounced structural and metabolic alterations of the ventricular myocardium. The state and functional activity of the cardiomyocytes depend on their metabolism, which is closely connected with the neurohumoral regulation, including the sympatheticoadrenal system (SAS) . Numerous investigations attest to an important role of cathecholamines in the heart compensatory processes and in the development of myocardial dystrophy and heart failure [4-6,12].

The aim of the present research was to study the state of the SAS during acute experimental MPE either complicated with heart failure or not.

MATERIALS AND METHODS

Experiments were carried out on 37 mongrel dogs weighing 15-20 kg. The chest was maintained intact and the breathing remained natural. The animals received promedol injected i.m. (10 mg/kg) as a preliminary medication, and sodium thiopental was injected fractionally i.v. (20 mg/kg) to produce general anaesthesia. The methods of the cardiac and vessel catheterization, registration of the hemodynamic indexes and the modeling of acute MPE were described previously [2]. The experiments conformed to the scheme presented in Fig. 1. It should be pointed out that the results of neurohistological study obtained in the first control group (Fig. 1, I) were accepted as the initial ones for all other experimental series. A separate group comprised four dogs with MPE followed by the rapid development of heart failure with a lethal outcome. The specimens for neurohistological study were dissected from the middle third of the right ventricle (RV) and left ventricle (LV) walls, the interventricular septum (IVS), and also from the left adrenal medulla. The specimens were frozen with dry ice, after which the cryostat sections (25 μ) were performed at -15° to -20°C. For the histochemical demonstration of norepinephrine (NE) the sections were incubated in 2% glyoxylic acid solution [13,15]. The slides were examined under a LYUMAM-I3 luminescent microscope. The relative area of the adrenergic nerve plexus was computed according to a described method [7], using a planimetric ocular grid with 960 nodes. The intensity of NE fluorescence in the adrenal medulla was measured with the use of a photometric attachment on the microscope. Blood samples for biochemical study were taken simultaneously from the RV and LV. The serum concentrations of epinephrine (E), NE, and dopamine (DA) were assayed with the radioimmune technique (Khe-

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(Presented by V.S. Savel'ev, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0004-0360$12.50

mapol kits, Czechoslovakia). The data were processed statistically using the Student t test.

RESULTS

The control animals showed a progressive time-dependent decrease of the relative area of the adrenergic nerve plexus in all specimens studied (Fig. 2, *a-c*). The RV/LV ratio of the relative area of the adrenergic plexus was 2.3 for the initial level and 1.9 and 1.4 one and six hours after treatment, respectively. These results are in good agreement with published data on a higher density of the adrenergic innervation in the RV myocardium [7]. The content of NE in the adrenal chromaffin cells was slightly increased toward the first hour and fell to the basal level by the sixth hour (Fig. 2, *d*). The serum NE concentration was not affected, the DA concentration exhibited a tendency to increase, while the E concentration significantly increased toward the third and decreased toward the sixth hour (Table 1). The E/NE ratio increased toward the third hour and then decreased. There was a negative correlation between the E and NE variations (r=-0.63, p<0.05) during the first three hours in the control experiment. A comparison of catecholamine contents in the RV and LV samples showed a lower content of NE in the blood flowing out of the lungs.

Thus, in the control there is a short-term moderate activation of the hormonal component of the SAS, which consists in an increase of cathecholamine synthesis and release from the adrenal medulla. Chromaffin cell activation in the control is probably related to the immobilization stress and cardiac catheterization, which can also be considered as a stress factor [5,6]. The decreased NE level in the myocardial nerve plexus may be related to its reduced synthesis and uptake or increased transmitter release [3,5,6]. Since the control animals exhibit no increase of serum NE, it may be assumed that the lower NE level in the nerve endings is due rather to a reduction of its synthesis. The lower NE concentration in the blood flowing away from the lungs in comparison with the mixed venous blood points to its metabolic breakdown in the lung tissue [8,14]. Besides, this fact is confirmed by the absence of activation of the SAS nervous component in the control group. The latter is also proved by the negative correlation between the E and NE variations in the blood of the control animals. These findings are consistent with the idea that the sympathetic nervous system and adrenal medulla reaction is not always parallel [11].

There was a decrease of NE content in the RV nerve plexus toward the first and, especially, toward the sixth hour of experimental MPE (Fig. 2, *a*). The

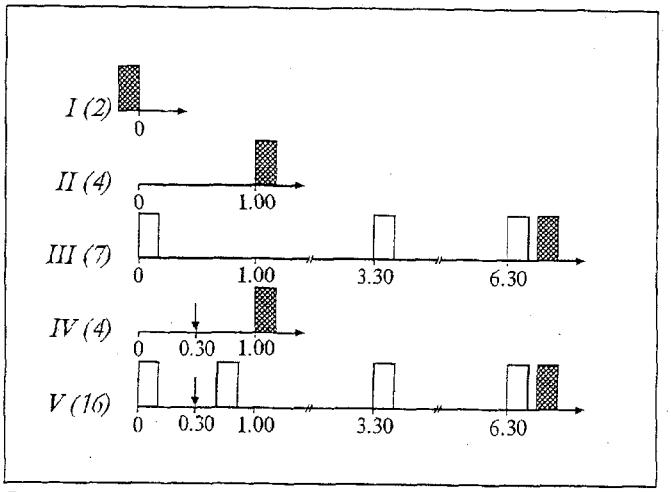

Fig. 1. Scheme of control (I, II, III) and experimental (IV, V) studies. Abscissa: time (h, min) starting from the point of completed catheterization of the heart and vessels. Arrows: MPE reproduction; open bars: biochemical sampling; dashed bars: treatment of animals with a lethal dose of sodium thiopental and collection of material for neurohistochemical assay. The number of animals in each group is given in parentheses.

content of NE in the LV nerve endings rose in the first hour and dropped by the sixth hour (Fig. 2, *b*). The RV/LV ratio of the adrenergic nerve plexus areas was 0.95 and 2.1 at these times. The norepinephrine content in the adrenal chromaffin cells fell in the first hour and by the sixth hour became higher than in the control (Fig. 2, *d*). The serum concentrations of NE, DA, and, in particular, E were elevated immediately after MPE and remained unchanged during the whole experiment (Table 1), accompanied by an increase of the E/NE ratio. A positive correlation between the E and NE variations was revealed in the period of 3-6 hours of MPE (r=0.55, p<0.05). There was a higher content of NE in the RV blood compared to the LV content.

Thus, the MPE-induced activation of the hormonal component of SAS is more pronounced then in the control. The decreased content of adrenal catecholamines and the sharp increase of their serum level toward the first hour of MPE suggest that initially catecholamine release significantly outstrips resynthesis [5]. By the sixth hour of MPE the chromaffin cell activity remains high, consisting in an increased level of cathecholamine synthesis and release from the adrenal medulla. In contrast to the control, there is MPE-induced activation of the sympathetic nervous system, whose manifestations differ in the RV and LV myocardium. In our view, since serum NE increases, the lower NE content in the nerve plexus is due to the predominance of transmitter release over its synthesis and uptake. The processes of transmitter release and synthesis are energy-dependent and can be disrupted owing to insufficient energy production [6] under a dramatic increase of

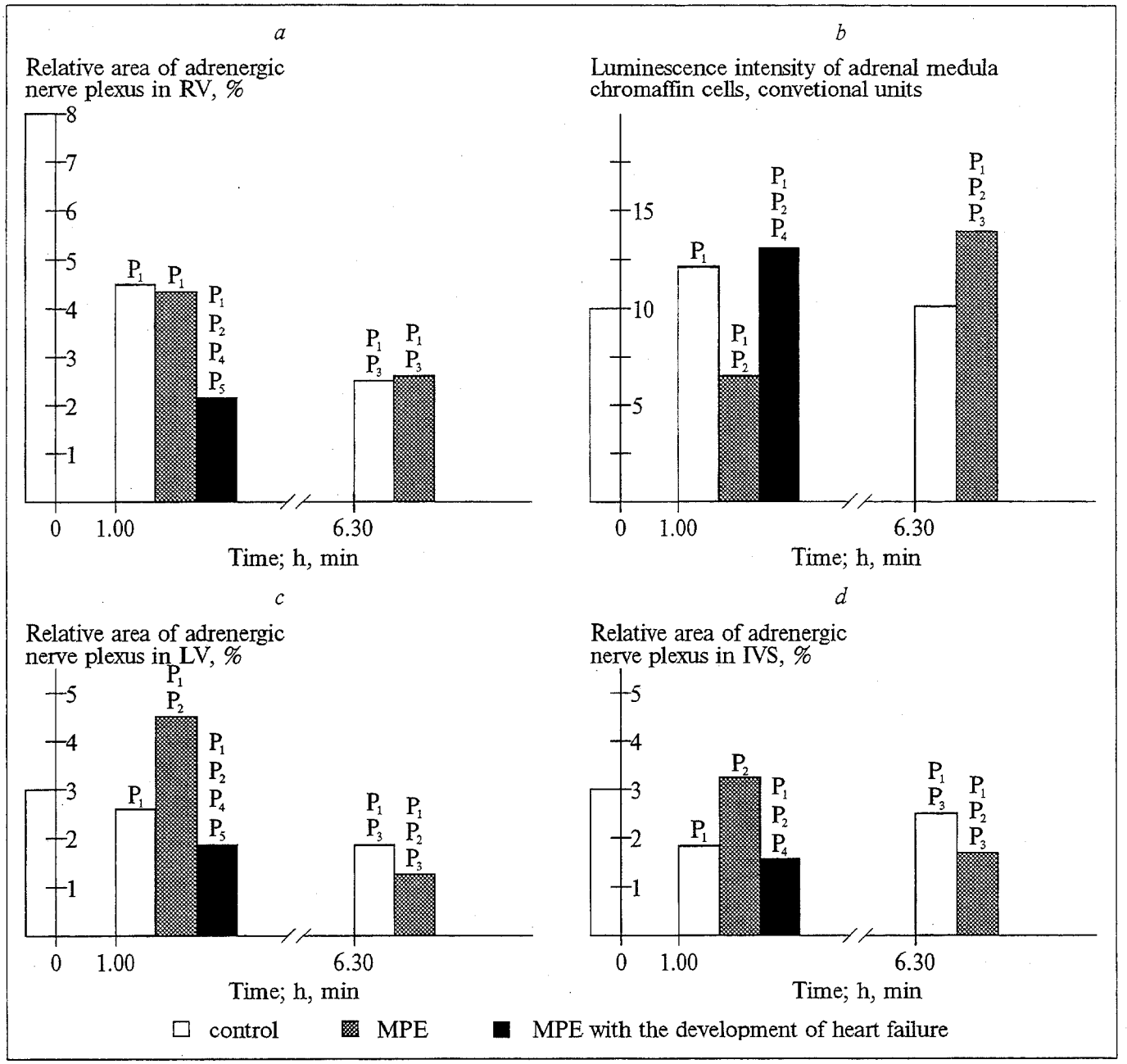

Fig. 2. Norepinephrine content in myocardium nerve plexus ($a-c$) and in adrenal chromaffin cells (d) in experimental (MPE) and control animals. There are significant differences for $p<0.05$ in comparison with the outcome (P_1), control (P_2), between the first and the sixth hours of the experiment (P_3), and between MPE with heart failure and compensated MPE in the first (P_4) and the sixth hour (P_5).

the RV work load [1]. The increased NE level in the LV nerve plexus against the background of decreasing LV contraction [1] probably reflects the dominance of transmitter synthesis and uptake over its release from the nerve endings. It has been shown [12] that moderate hypoxia in the initial stages is accompanied by an activation of transmitter synthesis and an increase of cathecholamine uptake by the myocardium. Consequently, moderate hypoxia [1] and hypercatecholaminemia may be the factors accounting for the increased NE content in the LV myocardium nerve plexus during the first 30 min of MPE. MPE-induced sympathetic nervous system activation is supported by the higher NE concentration in the blood flowing away from the lungs than in the mixed venous blood, indicating that transmitter release prevails over its breakdown in the lung tissue. A decrease of NE inactivation in the lungs may be promoted by hypoxia [16], perfusion failure [8], and capillary endothelium impairment [17], as was demonstrated in our investigations previously [1]. The positive correlation between the NE and E variations

TABLE 1. Cathecholamines Level in Arterial (a) and Venous (v) Blood of Experimental (MPE) and Control Animals ($M \pm m$).

Index	Series	Time from beginning of experiment							
		0 h 00 min		0 h 30 min		3 h 30 min		6 h 30 min	
		a	v	a	v	a	v	a	v
E, nmol/liter	Control	9.04±1.15	8.39±1.14	–	–	7.38±1.61**	18.16±1.15***	112.12±1.35	13.98±2.03
	MPE	5.97±1.30	5.08±1.22	8.31±2.85***	15.47±1.96***	20.09±3.38***	20.49±3.06***	18.72±2.90***	21.95±4.19**
NE, nmol/liter	Control	10.82±1.94	16.03±2.07	–	–	9.92±1.72	12.92±2.13	10.81±2.99	13.32±1.34
	MPE	6.27±1.18	5.78±0.76	9.43±2.18	7.83±1.91	14.14±0.92***	10.42±1.96*	13.53±1.59**	9.00±1.70
E/NE	Control	0.84±	0.52±	–	–	1.75±	1.41±	1.12±	1.05±
	MPE	0.95	0.88	1.94	1.98	1.42	1.97	1.38	2.44
DA, nmol/liter	Control	2.01±0.77	2.61±0.90	–	–	2.24±0.58	5.10±1.11	3.65±1.11	4.44±1.25
	MPE	2.00±0.54	1.68±0.50	2.76±0.64	4.45±0.90*	3.49±0.87	3.96±0.68*	4.08±1.09	3.37±0.64

Note: asterisks represent significance of differences in comparison with initial level (0 h 00 min): one – $p<0.05$, two – $p<0.01$, three – $p<0.001$.

in the blood of the experimental animals attests to a synchronous activation of the nervous and hormonal components, induced by MPE. Toward the sixth hour of MPE the NE content in the nerve endings in LV, IVS, and RV substantially decreases, indicating functional insufficiency of the energy-dependent catecholamine synthesis and uptake in all parts of the heart studied and reflecting a rapid depletion of the nervous component during SAS activation. These results are in good agreement with findings [5] which showed that the decrease of NE content in the myocardium in the sixth hour of stress arises from a predominance of transmitter release over its resynthesis and neuronal uptake, the latter suffering the most.

When MPE is followed by the development of heart failure, there is a drop of the NE content in the nerve plexus and a substantial increase of adrenal chromaffin cell NE (Fig. 2). These changes are most pronounced for a comparison of two variants of one-hour MPE development: either complicated with heart failure or not. The findings are consistent with published data on a decrease of myocardium NE content under progressive heart failure and sudden death [3,6,7], and an increase of SAS hormonal component activity as the health status of cardiac patients worsens [6]. The similar level of neurohistological indexes in acute decompensated MPE (1 hour) and relatively long-lasting compensated MPE (6 hours) should be noted. This suggests the vital importance of the rate of development of the changes in determining whether transitional processes will result in a compensated or decompensated state.

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