PREVENTION OF HYPOXIC HEART INJURY BY AN ANTIOXIDANT OF THE HYDROXYPYRIDINE CLASS

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UDC 616.12-008.922.1-008.64-092.9-06-085.243.4

KEY WORDS: antioxidant; hypoxic heart injury.

Hypoxic damage to the heart plays an important role in the development of heart failure and is a subject of extensive research. If the heart is working under hypoxic conditions, a momentary fall of systolic pressure is followed by gradual development of contracture of the heart, due to a disturbance of the working of the calcium pump [8-10], and enzymes from the damaged myocardial cells are liberated into the perfusion fluid [11]. It was shown previously that activation of lipid peroxidation (LPO) develops during anoxia in the heart muscle [1, 2], and antioxidant inhibitors of this process limit the size of areas of necrosis developing in the heart muscle under the influence of ischemic anoxia [2, 3].

In this connection it seems likely that the development of hypoxic contracture and liberation of enzymes from the heart under the influence of hypoxia are also determined by activation of LPO, and damage by LPO products to membranes of the sarcolemma and sarcoplasmic reticulum responsible for calcium transport.

To test this hypothesis, in the investigation described below the effect of preliminary administration of an LPO inhibitor — a water-soluble antioxidant of the hydroxypyridine class (OP-6) — on the development of hypoxic contracture and on loss of creatine phosphokinase (CPK) by isolated rat heart was studied. The experiments were carried out on isolated heart of control animals and on the heart of rats exposed before sacrifice to emotional, pain-induced stress [6], for exposure to stress is known to significantly reduce the resistance of the heart to hypoxia [4].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. There were four series of experiments, in each of which 8-10 animals were used. The animals of series I served as the control; animals of series II were given OP-6 by intraperitoneal injection in a dose of 100 mg/kg body weight daily for the 3 days before their heart was removed. The animals of series III were exposed to severe emotional, pain-induced stress 2 h before removal of their heart, in the form of an anxiety neurosis induced by Desiderato's method [6]. The animals of series IV were given OP-6 for the 3 days before their heart was removed in the same doses as the animals of series II, but were exposed to emotional, pain-induced stress. The hearts from animals of all series were removed under urethane anesthesia and placed in cold Krebs—Henseleit solution, after which they were perfused with the solution at 37°C. The solution was oxygenated by a gas mixture containing 95% O_2 and 5% CO_2. Under these conditions the contractile function of the heart was investigated under isovolumic conditions by the method described in [7]. The right atrium was removed and a latex balloon with constant volume was introduced into the left ventricle; compression of the balloon caused isovolumic contraction of the ventricle. Contractions of a certain frequency were applied by means of an ÉSL-1 electrostimulator, and the pressure inside the latex balloon was recorded by an electromanometer of the Mingograph-34 apparatus (Elema-Schönander). From the curves thus recorded the systolic, diastolic, and developed pressures and the rate of contraction and relaxation were determined. In the course of the experiment a stable frequency of contraction of 120/min was imposed and the coronary blood flow was measured. After the heart had worked

Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 281-283, September, 1981. Original article submitted April 10, 1981.

0007-4888/81/9209-1167$07.50

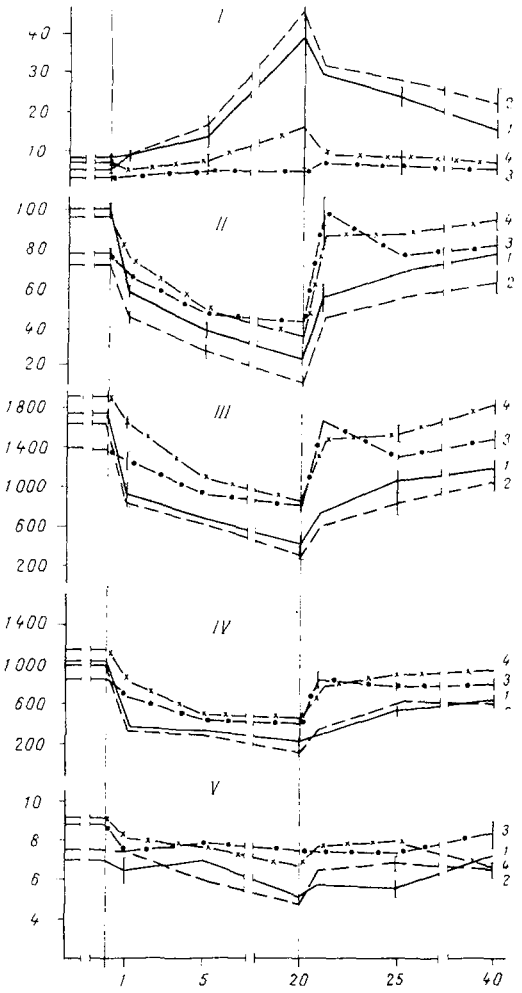

Fig. 1. Effect of antioxidant OP-6 on main parameters (M ± m) of contractile function of isolated heart during hypoxia (a) and reoxygenation (b). Abscissa, time (in min); ordinate: I) diastolic pressure (in mm Hg), II) developed pressure (in mm Hg), III) velocity of contraction of heart, IV) velocity of relaxation, V) coronary flow (in ml/min). 1) Control; 2) emotional, pain-induced stress; 3) OP-6; 4) OP-6 + emotional, pain-induced stress.

for 90 min under conditions of normal oxygenation (with minimal escape of CPK into the perfusion fluid) the oxygenated Krebs–Henseleit solution containing glucose was replaced by an unoxygenated solution not containing glucose. These hypoxic conditions continued for 20 min, after which reoxygenation was carried out. Under conditions of isovolumic contraction of the heart the dynamics of the pressure reflected completely the dynamics of systolic and diastolic contraction of the heart muscle. Correspondingly in these experiments the curve of rising diastolic pressure during the hypoxic test was a direct quantitative criterion of the developing hypoxic contracture of the heart. CPK activity was determined at the 90th minute of normal oxygenation, at the 20th minute of hypoxia, and at the second minute of subsequent reoxygenation by Bergmeyer's method [5].

EXPERIMENTAL RESULTS

The curves illustrated in Fig. 1 show that exposure to stress reduced by 28-30% the force of contraction, the velocity of contraction, and also the velocity of relaxation during

TABLE 1. Effect of OP-6, Hypoxia, and Reoxygenation on CPK Activity in Perfusion Fluid of Isolated Rat Heart (M ± m)

Series of expts.	Experimental conditions		CPK activity, i.u./liter		
			perfusion for 90 min	20 min of hypoxia	2 min of reoxygenation
I	Control	5	9,4±2,2	27,8±4,2	45,5±10
II	OP-6	7	10,1±1,6	12,4±1,6	23,8±3,9
	P_{I-II}		0,05	0,01	0,05
III	Stress	14	11,4±1,3	51,9±5,2	60,3±4,1
	P_{I-III}		>0,05	<0,01	>0,05
IV	OP-6 + stress	4	10,4±3,9	6,8±2,4	8±1,4
	P_{I-IV}		>0,05	<0,01	<0,01
	P_{III-IV}		>0,05	<0,001	<0,001

the period of normal oxygenation ($P_{1-2} < 0.05$). Preliminary injection of OP-6 into intact animals itself reduced these parameters, but on administration to animals which were subsequently exposed to stress, it completely prevented disturbance of the contractile function of the heart usually induced by stress. All parameters of contractile function were significantly higher ($P_{2-4} < 0.01$) than during the isolated action of the antioxidant or of emotional, pain-induced stress, and they did not differ significantly from the control level.

It will also be clear from Fig. 1 that the main difference between the series is that in animals not receiving the antioxidant a contracture developed in response to hypoxia, whereas this did not happen receiving the antioxidant. Analysis of the curves in fact shows that the diastolic pressure in the control animals increased gradually, and at the 20th minute of action of hypoxia it reached 40 mm Hg; the velocity of relaxation under these circumstances was reduced by three-quarters. The diastolic pressure in the heart of animals exposed to stress reached 47 mm Hg and the velocity of relaxation was reduced by 7-8. These changes are unambiguous evidence of the development of hypoxic contracture and, coupled with the fall in the systolic pressure, they led to a decrease in the developed pressure in the control animals by three-quarters, and in animals exposed to stress by four-fifths. Since the developed pressure largely determines the stroke volume of the heart, such a decrease under conditions of the whole animal would amount to the development of cardiac failure. The diastolic pressure in the heart of the control animals protected with OP-6 was 5-6 mm Hg at the 20th minute of hypoxia and, consequently, no contracture developed. In the series receiving OP-6 and exposed to stress, the diastolic pressure was 16 mm Hg and, consequently, the degree of contracture was very small. Administration of the antioxidant thus completely prevented the development of a hypoxic contracture in the control animals and reduced its degree in the animals exposed to stress.

During reoxygenation recovery from hypoxic contracture and restoration of the contractile function of the heart took place very slowly in animals not protected by the antioxidant, and was not complete even after 20 min (Fig. 1). In animals protected by the antioxidant, however, no contracture was present and recovery of the contractile function was complete after 20 min. Antioxidant protection thus ensured absolute recovery of contractile function during reoxygenation.

The data on liberation of CPK from the myocardial cells into the perfusion fluid, given in Table 1, are of great importance for the analysis of these facts. After a long period of normal oxygenation, liberation of CPK into the perfusion fluid was minimal and was the same for the animals of all series. In response to hypoxia liberation of CPK from the heart of animals not protected by the antioxidant increased: threefold for hearts of the control animals, by 4.5 times for the hearts of animals exposed to stress. The hearts of animals of both series receiving OP-6 did not respond to hypoxia by increased liberation of the enzyme. OP-6, an LPO inhibitor, thus prevented both hypoxic contracture and release of the CPK from the hearts of animals exposed to the action of hypoxia; consequently, activation of LPO is in fact an essential stage in the pathogenesis of hypoxic contracture.

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MECHANISMS OF ACTIVATION OF LIPID FREE-RADICAL PEROXIDATION DURING REGIONAL ISCHEMIA FOLLOWED BY REPERFUSION OF THE HEART

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UDC 616.12-005.4-092.9-07: 616.127-008.939.15-074

KEY WORDS: myocardium; postischemic reperfusion; lipid peroxidation.

Previous investigations showed that during reperfusion of a previously ischemized zone of the heart, indices of its contractile function are restored to close to the initial values only after brief (10-15 min) myocardial ischemia (MI). After a longer period of MI (more than 20-120 min) a progressive decline of contractility is observed despite resumption of the coronary blood flow [4, 7, 8]. It has also been shown that transient coronary insufficiency is characterized by a biphasic increase in the adrenalin concentration in the myocardium: in the initial stage of MI and during subsequent reperfusion [6]. At the same time, exogenous adrenalin in large doses is known to activate free-radical lipid peroxidation (FRLPO) of the myocardium [2, 3]. Products of FRLPO have a considerable harmful effect on the lipid components of cells, above all their membranes, and also on the enzyme systems of cardiomyocytes [1, 9].

With the above data in mind it was decided to study the pattern of dynamics of FRLPO and the possible mechanisms of its activation during MI of varied duration and during postischemic reperfusion of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on 115 noninbred male albino rats weighing 200 ± 10 g. Transient coronary insufficiency was produced by the method described previously [5, 7] under urethane anesthesia (1200 mg/kg) with artificial ventilation of the lungs with atmospheric air. The duration of the period of MI was 10, 20, 40, and 120 min, and reperfusion lasted 40-60 min. Lipids were extracted from the damaged area of the heart by the method in [13]. The intensity of FRLPO was determined from the spontaneous chemiluminescence (CL) of lipids on a constant-temperature (38°C) photon counting instrument for recording weak photic fluxes [1]. The partial pressure of oxygen in the myocardial tissue was recorded on the LP-7E polarograph (Czechoslovakia) by means of a "floating" platinum needle electrode, and the total calcium ion concentration was measured by flame photometry on a Hitachi spectrophotometer (Japan). The animals were divided into two groups: experimental (the dynamics of FRLPO during brief coronary insufficiency) and control (dynamics of FRLPO after a mock operation).

EXPERIMENTAL RESULTS

Biphasic activation of FRLPO during brief coronary insufficiency was revealed by the experiments: an "ischemic" wave during the MI period and a "reperfusion" wave during subse-

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 0007-4888/81/9209-1170$07.50