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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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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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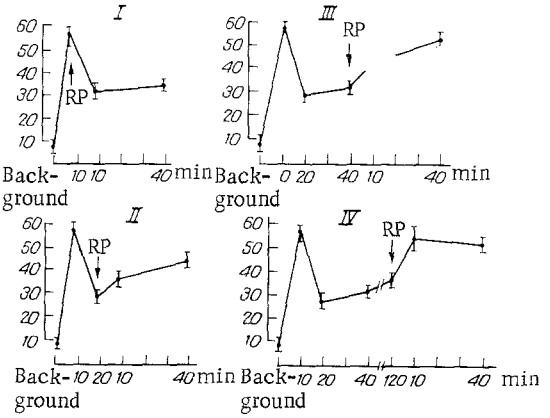

Fig. 1. Dynamics of FRLPO during ischemia and subsequent reperfusion of the myocardium (M ± m). Abscissa, time (in min); ordinate, intensity of FRLPO in myocardium reflected in CL criterion (pulses/sec). RP) End of period of myocardial ischemia and beginning of reperfusion period. Number of tests at each point was 35. I) Animals with myocardial ischemia for 10 min, II) 20 min; III) 40 min, IV) 120 min.

quent reperfusion (Fig. 1). The dynamics of FRLPO differed significantly during the two periods. In MI a considerable (by 6-7 times compared with the background) increase in CL lipids was observed initially, most marked at the 10th minute of the period. The intensity of CL then fell, but was 3.7 and 4.5 times higher than the background level at the 40th and 12th minutes of MI respectively. It can be tentatively suggested that the increase in CL in the initial stage of MI was due to an increase in the concentration of FRLPO substrates and, in particular, of nonesterified free fatty acids, and of active pro-oxidants, namely catecholamines, ADP, AMP, and orthophosphates, and also to a decrease in the concentration and (or) activity of antioxidants. The chain of events leading ultimately to stimulation of FRLPO can be represented as follows. MI is accompanied by a marked increase in the catecholamine concentration in the myocardium [6, 8], which leads to the development of a hyperkinetic response of the heart [6] and intensification of lipolysis [15] and FRLPO [2, 3]. The latter effect leads to an increase in the concentration of products of this reaction, namely polyunsaturated fatty acids (substrates for FRLPO), in the myocardium which, in the presence of MI, accumulates mainly in the cytosol [14]. At the same time the hyperdynamia of the heart during the first 10-15 min of ischemia was due to a considerable increase in the concentration of FRLPO initiators (hydrolysis products of HEP) in the myocardial cells [1]. The coincidence of these two processes in time, leading to accumulation of substrates and activators of FRLPO, and also, evidently, the decrease in activity of antioxidant systems, lie at the basis of intensification of CL in the initial stage of MI. The subsequent (at the 20th-40th minute) small decrease in the concentration of FRLPO substrates and initiators was accompanied by a tendency for its intensity to fall, although it still remained considerably higher than the background level.

The resumption of coronary perfusion in the previous ischemized area of the heart was accompanied by a second — "reperfusion" — wave of intensification of FRLPO. However, by contrast with the ischemic period, reperfusion was accompanied by a progressive rise in CL. The intensity of FRLPO also was found to depend on the duration of the period of ischemia that preceded reperfusion of the myocardium. If the blood flow in the coronary arteries was reviewed after a long period (20, 40, and 120 min) of MI a continuous increase in CL of lipids was observed. For instance, 40 min after the beginning of reperfusion the intensity of CL after a previous 20-min period of MI was 5.3 times higher than the background level, and after MI for 40 and 120 min it was 6.4 and 6.7 times higher respectively. Reperfusion after a short period (10 min) of MI was accompanied by an initial (for the first 10 min of the period) decrease in FRLPO, followed by an increase, and at the 40th minute of the period CL was almost 4 times higher than the background level.

An important role in the development of the "reperfusion" wave of FRLPO may be played by the same factors as during MI. Meanwhile the increase in the intensity of CL after re-

sumption of the coronary bloodflow is evidence of the mobilization of additional factors of FRLPO activation. One of the most important of these is the 10-15-fold increase in the partial pressure of oxygen in the myocardium compared with the MI period, and this is a powerful initiator of FRLPO. Another factor leading to activation of CL during reperfusion, especially after a long period of MI, is injury (or even destruction) of the membranes, more especially of the mitochondria, as a result of intensive uptake of calcium ions by the membranes from the hyaloplasm [11, 12] and activation of phosphorylases A by these ions [10]. The total Ca^{++} ion concentration in the myocardium in the period of postischemic perfusion increased considerably, proportionally to the duration of the MI period. For instance, by the 40th minute of reperfusion the Ca^{++} concentration had risen by 10, 586, and 617% compared with the period of MI after MI lasting 10, 40, and 120 min respectively. Destruction of the mitochondria, and also of the other structures of the myocardial cells, is accompanied by decomposition of the membranes, liberation of their lipid components (substrates for FRLPO), and intensification of their free-radical oxidation. The observations described above suggest that the chief substrate for FRLPO during reperfusion of the heart after MI lasting 20-120 min (just as also in the later stages of MI) are cell membrane phospholipids, whereas in the initial stage of MI the main substrates are the free unsaturated fatty acids of the cytosol.

These results as a whole are evidence that during transient coronary insufficiency activation of lipid CL is observed both during MI and during subsequent reperfusion. The increase in FRLPO when the coronary blood flow is restored is more marked after prolonged ischemia. The inducing factors of this process are evidently an increase in the concentration of powerful FRLPO activators (adrenalin, oxygen, and also calcium ions) in the reperfused myocardium. Intensification of free-radical lipid oxidation in the myocardium during its postischemic reperfusion may be one of the main factors responsible for the development of arrhythmias and depression of the contractile function of the heart, which are the essence of the cardiac reperfusion syndrome [6, 7]. Considering that transient coronary insufficiency, reproduced in animals, is an experimental model of angina and the states following revascularization of the myocardium in the acute (ischemic) period of infarction [6, 7], this suggests that the above mechanisms may participate in the development of these forms of coronary heart disease in man.

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