ACTIVITY OF ANTIOXIDANT ENZYMES OF THE ISCHEMIC MYOCARDIUM

D. V. Gutkin and Yu. A. Petrovich

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There is no longer any doubt about the important role of lipid peroxidation (LPO) processes in the pathogenesis of myocardial damage resulting from ischemia. It is confirmed, on the one hand, by data on accumulation of LPO products during myocardial ischemia [3] and, on the other hand, by investigations which have shown the favorable effect of antioxidants on the course of infarction, reduction of the area of necrosis, and the speeding up of repair processes in the infarcted zone [1, 3]. LPO activation leads to disturbance of the structure and permeability of biological membranes, which may cause swelling and disintegration of mitochondria, vacuolation of the sarcoplasmic reticulum, increased permeability of lysosomal membranes, and the liberation of acid hydrolases, followed by autolysis of the cell. It is important to note that all these processes are regularly observed in ischemic damage [6, 7].

Meanwhile the causes of LPO activation in ischemia have not yet been discovered. Theoretically there are several possible causes of LPO activation: the introduction of pro-oxidants, an increase in oxygen toxicity (for example, in hyperbaric oxygenation), weakening of the antioxidant systems of the cell — nonenzymic (tocopherols, etc.) or enzymic (superoxide dismutase, catalase, glutathione peroxidase). Since there can be no question of a primary increase in the toxicity of oxygen or of the introduction of pro-oxidants in ischemia, the most likely cause of LPO activation in this case is weakness of the protective antioxidant systems in the presence of normal saturation of the body with tocopherols, evidently on account of a reduction in activity of antioxidant enzymes.

For the reasons given above, it was decided to study the time course of changes in catalase, glutathione peroxidase, and superoxide dismutase activity in experimental myocardial ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred male albino rats weighing 180-220 g. The animals were divided into three groups: control (normal rats — 10 animals) and two experimental groups, in one of which the left branch of the descending coronary artery of the animals was divided by Kogan's method [2] (40 animals), and in the other a mock operation was performed without ligation of the coronary artery (40 animals). At different times (1 and 3 h, 1, 3, and 5 days) after the operation, which was performed under ether anesthesia, the animals were decapitated. Three areas were excised from the heart: ischemized, peri-ischemic (2 mm around the zone of ischemia), and extraischemic (myocardium of the right ventricle). A homogenate was prepared from each sample in a homogenizer with glass pestle in 0.15 M KCl solution, followed by centrifugation at 5000 g for 5 min. Superoxide dismutase activity was determined in the resulting supernatant [5] and expressed in units/mg protein (1 unit of activity was taken to be the quantity of enzyme which inhibits the velocity of the O_2^--dependent reaction by 50%). Catalase activity was determined by the method in [9] and expressed in micromoles/min/mg protein. Glutathione peroxidase activity was studied by the direct method as the change in concentration of glutathione SH groups, estimated in the reaction with dithio-bis-nitrobenzoic acid. Under these circumstances, unlike in the previous investigation [4], concentrations of glutathione (40 mM) comparable with those actually existing in myocardial cells were used, so that the activity of this enzyme could be estimated under the conditions of actual function of glutathione synthetase and glutathione reductase systems.

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TABLE 1. Activity of Myocardial Antioxidant Enzymes of Rats under Normal Conditions and at Various Times after Production of Myocardial Ischemia (M ± m)

Area of heart	Normal conditions	After operation				
		1 h	3 h	1 day	3 days	5 days
Right ventricle	28,4±0,8	22,9±1,0	16,0±1,1	30,6±1,2	27,2±0,6	33,8±2.4
Left ventricle	33,5±0,7	24,2±1,1	15,2±0,5	23,4±1,0	25,6±0,8	33,8±1.5
Ischemic zone	—	17,0±0,8	13,3±0,8	18,0±0,7	20,2±0,9	24,9±1.0
Mock operation	—	31,0±1,1	18,1±1,0	28,4±1.4	32,6±2,0	33,4±3.5
Right ventricle	57,7±2,5	22,4±1,9	54,6±2,4	63,8±4,3	62,2±3,0	54,3±6,4
Left ventricle	67,5±2,3	18,8±2,4	51,0±4,2	51,2±3,1	66,1±3,8	42.1±4.1
Ischemic zone	—	8,4±1,2	11,6±0,9	10,2±1,6	15,1±2,3	31,2±3,2
Mock operation	—	65,8±5,0	50,1±3,2	58,8±4,3	60,7±3,2	69.6±4.5
Right ventricle	15,8±1,2	19,8±0,8	15,8±1,1	18,7±0,9	23,2±0,7	18,0±1.7
Left ventricle	18,4±1,1	18,4±0,9	17,1±1,0	17,0±1,3	19,0±0,9	16,7±1,9
Ischemic zone	—	17,7±0,5	13,3±1,1	12,9±1,1	17,5±1,0	12.5±1.0
Mock operation	—	17,8±0,8	17,2±1,4	17,9±1,0	19,2±1,1	19,5±0,8

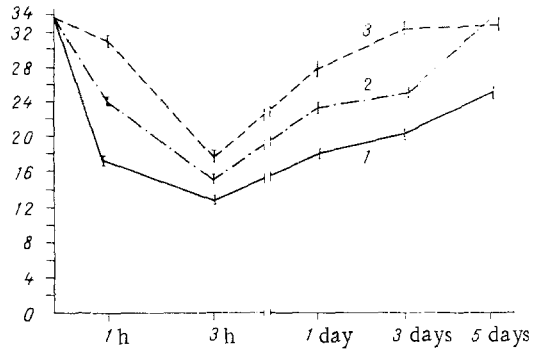

Fig. 1

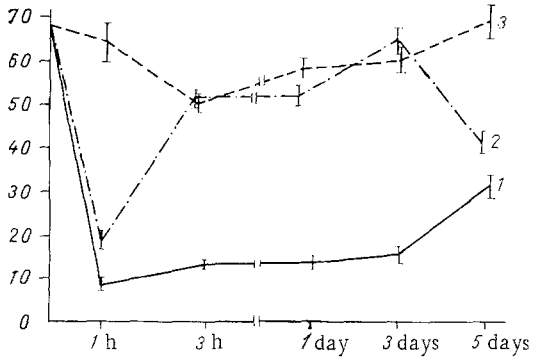

Fig. 2

Fig. 1. Superoxide dismutase activity of tissues of left ventricle of rats at different times after production of myocardial ischemia. Abscissa, time after operation; ordinate, enzyme activity (in units/mg protein). 1) Ischemic zone, 2) perischemic zone, 3) mock operation.

Fig. 2. Glutathione peroxidase activity of tissues of left ventricle of rats at different times after production of myocardial ischemia. Abscissa, time after operation; ordinate, enzyme activity (in nmoles/min/mg protein). Remainder of legend as to Fig. 1.

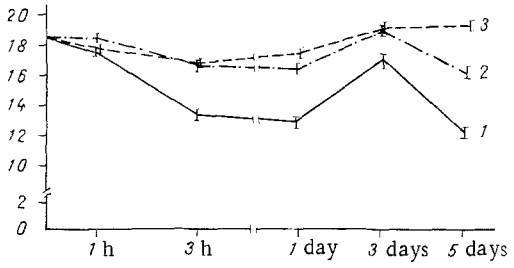

Fig. 3. Catalase activity in tissues of left ventricle of rat at different times after production of myocardial ischemia. Abscissa, time after operation; ordinate, enzyme activity (in μmoles/min/mg protein).

Protein was determined by Lowry's method [8]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The first point to note was that the activity of all these antioxidant enzymes was much higher in the left ventricle than in the right in the control group (Table 1). The reason for this may perhaps be that in the course of evolution greater protection has developed for the left ventricle because of the heavier load which it has to carry.

In the zone of ischemia (Figs. 1-3) activity of the three enzymes studied was significantly lower than in the control animals and rats undergoing the mock operation as early as after 1 h, and after 3 h it was still at a low level. The extent of the reduction of glutathione peroxidase activity (by 88% after 1 h) and superoxide dismutase activity (by 60% after 3 h) must be particularly noted. In the later stages activity of these enzymes in the ischemic zone still remained low, despite a tendency for it to rise. The difference from the control was still significant 5 days later ($P < 0.001$).

The mock operation itself caused a decrease in superoxide dismutase and glutathione peroxidase activity which was most marked 3 h after the operation. However, activity of the enzymes was changed by a lesser degree than actually in the ischemic zone, and after 3 days there was no longer any difference from the control.

In the peri-ischemic zone superoxide dismutase and glutathione peroxidase activity was significantly lower than in the rats undergoing the mock operation in the early period of observation, but later it returned to normal. Catalase activity in this zone did not change significantly at any time of observation (Fig. 3).

Determination of enzyme activity in the myocardium of the right ventricle showed virtually no change compared with that in animals undergoing the mock operation. Only glutathione peroxidase activity 1 h after the operation was significantly lower than activity in the control rats and after the mock operation.

This investigation demonstrated the character of changes in activity of enzymes of the antioxidant system of the myocardium in the zone of experimental ischemia. The decrease in glutathione peroxidase and superoxide dismutase activity was particularly marked in this zone, a fact which could reflect the importance of these enzymes in the pathogenesis of ischemic myocardial damage, whereas the less marked changes in catalase activity could reflect its less important role in antioxidant protection.

The changes described above were only slight in the peri-ischemic zone and virtually absent in the extraischemic zone. Consequently, they were presumably caused by the myocardial ischemia itself. The operation also plays a definite role in the dynamics of antioxidant enzyme activity, as shown by experiments with mock operations. The intensity of these changes did not reach a maximum at once, but 3 h after the operation, and the return to normal was observed after 1 day. The changes were evidently due to stress injury of the myocardium under the influence of operative trauma.

Analysis of the results suggests that activation of LPO observed in myocardial ischemia may be due primarily to lowering of antioxidant enzyme activity.

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