depending on which part of the heart was subjected to ischemic damage, and for how long the coronary blood flow was disturbed. Prolonged ischemia, causing the development of irreversible damage to parts of the heart, evidently also damages the intracardial nervous system, which becomes unable to control the afferent flows. It is important to note that during the first minutes of ischemia the heart also "slips" from descending influences. It is stated in the literature that stimulation of the vagus nerve during myocardial ischemia does not lead to the development of bradycardia [6], but no analysis of the phenomenon observed is given. Comparison of the results of the first three series of experiments and those of series IV suggest a single biological explanation for these responses. Limitation of afferent flows from the damaged heart to the CNS may in fact facilitate defense of the body and of the heart itself against the development of cardiac pain and the appearance of pathological foci of excitation in CC and deep brain structures. In turn, the "slipping" of the heart from the control of central descending influences may prevent involvement of the myocardium in responses which, if hemodynamically sustained, may lead to aggravation of the pathology. All this suggests that the patterns of regulation of the heart described in this paper under conditions of ischemic damage to the ventricular myocardium are a manifestation of a defensive–adaptive response, which is one of a combination of responses of the body aimed at restoring function predominantly of the pathologically changed organ.

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INCREASE IN CARDIOPROTECTIVE ANTIOXIDANT ENZYME ACTIVITY DURING ADAPTATION OF RATS TO SHORT-TERM STRESS

T. G. Sazontova, Yu. V. Arkhipenko, and F. Z. Meerson

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KEY WORDS: protective antioxidant enzymes; stress; adaptation; rat heart.

Emotional–painful stress, which can itself activate lipid peroxidation (LPO) in the heart [3], lowers the resistance of the isolated atria to the action of LPO inducers [4]. Conversely, adaptation of animals to short-term stress significantly increases the resistance of the isolated atria to the arrhythmogenic action of H_2O_2 [2]. It has accordingly been suggested that the efficiency of antioxidant protection is enhanced during adaptation of the animal to stress situations and to other environmental factors [6].

The aim of this investigation was to study the effect of stress and of adaptation to short-term stress on the activity of enzymes of antioxidant protection: catalase (CT), superoxide dismutase (SOD), and glutathione peroxidase (GPO), and also on the α-tocopherol (TP) concentration in the myocardium.

Laboratory of Pathophysiology of the Heart, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Activity of Protective Antioxidant Enzymes and TP Concentration during Stress, Adaptation to Short-Term Stress, and Stress Combined with Adaptation (M ± m)

Parameter studied	Control	Stress	Adaptation	Adaptation + stress
CT, mmoles H_2O_2/g tissue/min	1,82±0,11 (100)	1,47±0,18* (80,7)	2,52±0,27* (135,3)	1,96±0,21 (107,8)
SOD, conventional units/g tissue/min	58,7±2,1 (100)	56,1±2,1 (95,3)	67,9±6,5 (115,7)	64,0±2,5 (109,3)
GPO, μmoles NADPH/g tissue/min	19,2±0,6 (100)	18,7±0,4 (95,8)	19,0±0,7 (98,9)	19,4±0,2 (101,2)
TP, μg/g tissue	66,7±2,1 (100)	63,4±4,3 (95,1)	64,5±2,9 (96,6)	67,8±2,6 (101,6)

Legend. Value of parameter in percent shown between parentheses. *$p < 0.05$ compared with control.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The animals were divided into four groups: 1) control, 2) stress: induction of an anxiety neurosis by the method in [8] for 6 h; 3) adaptation to short-term stress (eight exposures to stress, each lasting 1 h, on alternate days); 4) adaptation to short-term stress followed by exposure to stress for 6 h, 1 day after the end of adaptation. The rats were killed by decapitation 2 h after long-term stress or 24 h after the end of adaptation. The hearts were removed, washed with cold physiological saline, and frozen in liquid nitrogen until required for use.

Two hearts were minced in a Polytron homogenizer (type 2 × 7 sec) at a speed of 12,000 rpm in 10 volumes of medium containing 20 mM imidazole and 100 mM NaCl (pH 7.4) at 4°C. The homogenate was used for determination of: CT, spectrophotometrically at 240 nm, based on the decrease in the quantity of added H_2O_2 [11]; SOD, spectrophotometrically at 560 nm, by recording the difference between the velocities of generation of the superoxide radical in a xanthine–xanthine oxidase–butyl system, before and after addition of the homogenate [9], and GPO, based on the decrease in NADPH recorded at 340 nm in a coupled system of reduced glutathione–glutathione reductase, using tert-butyl hydroperoxide as the substrate [1]. The TP concentration in the cardiac homogenate was determined spectrofluorometrically after preliminary washing and extraction of the specimens with hexane [14]. LPO in the homogenate was induced by a system of Fe^{++} (0.1 mM) + ascorbate (0.2 mM) in a medium of 20 mM Tris-HCl (pH 7.0) at 25°C. The reaction was stopped by the addition of ionol in a dose of 10 nmoles/mg protein and cooling. The degree of oxidation was determined by measuring accumulation of products interacting with 2-thiobarbituric acid [3]. The results were subjected to statistical analysis with determination of the standard deviation and the significance of differences by Student's test.

EXPERIMENTAL RESULTS

Of all the protective antioxidant enzymes the most labile was CT, whose activity was depressed by long-term stress by 20% compared with the control (Table 1). Activity of SOD and GPO changed by not more than one-third: SOD activity had a tendency to increase (by 15%), whereas GPO activity did not change significantly. When these facts are interpreted it must be recalled that under these experimental conditions it was always activity of the same protective antioxidant enzyme which showed the greatest change, namely CT, which was depressed during stress and raised during adaptation to stress. This result is in agreement with the view that CT plays the most important role in limiting the H_2O_2 concentration in pathological states associated with an increase in the concentration of this LPO inducer [13]. It has correspondingly been shown that CT activity is depressed by a greater degree than activity of the other antioxidant enzymes during ischemia and reperfusion [10] and infarction [7], and is raised by the greatest degree in, for example, hyperbaric oxygenation under a small excess pressure [12]. In this connection it is a very interesting fact that during a gradual increase in the oxygen concentration in the incubation medium, in which epithelial cells of the hamster testis were cultured [15], a phenomenon perfectly analogous to that observed during

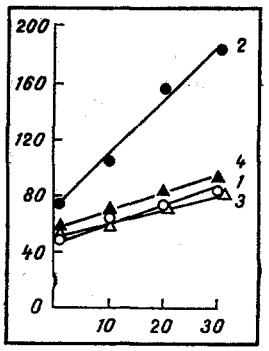

Fig. 1. LPO in vitro in heart homogenate during stress, adaptation to short-term stress and to long-term stress associated with adaptation. Abscissa, duration of oxidation (in min); ordinate, malonic dialdehyde accumulation (in nmoles/g tissue). 1) Control, 2) stress, 3) adaptation, 4) adaptation + stress.

adaptation to short-term stress developed, viz., CT activity increased considerably whereas SOD and GPO activity increased by a lesser degree. The agreement between these results can evidently be attributed to the fact that stress and hyperbaric oxygenation activate LPO, and in response to this, activity of induced enzymes increases.

It must be pointed out that in the present experiments neigher long-term stress nor adaptation to short-term stress caused any significant changes in the TP concentration in the myocardium (Table 1). This may be attributed either to compensating activation of other antioxidant systems, as was shown in our case for CT and SOD, or to redistribution of vitamin E in the body, for we know that the liver is a special depot for this antioxidant.

The end result of adaptation is thus increased activity of protective antioxidant enzymes and a decrease in CT activity. Conversely, activity of CT and SOD has a tendency to be maintained after stress at levels which may be higher than in the control. It can be tentatively suggested that the reason for this is the readiness of the protein-synthesizing apparatus of the cells of adapted animals to undertake the rapid formation of increased quantities of protective antioxidant enzymes necessary to restrict LPO during stress.

The results obtained in vivo for the effect of stress and adaptation on the state of the antioxidant systems of heart muscle were confirmed by subsequent experiments in vitro. During induction of LPO in heart homogenates after stress (Fig. 1) oxidation took place more than 3 times faster than in the control, whereas after adaptation to short-term stress it took place only two-thirds as fast. In the series of experiments with adaptation followed by exposure to stress, the velocity of oxidation was comparable with that in the control.

Thus as a result of long-term stress CT activity was reduced, whereas the vitamin E concentration remained constant and activity of the other protective antioxidant enzymes was unchanged. Adaptation to short-term stress led to a sharp increase in efficiency of the antioxidant system of the heart, and could thus limit the damage sustained by it in connection with activation of LPO. In complete agreement with this it was shown that adaptation of this kind to short-term stress increases the resistance of the heart not only to stress-induced, but also to ischemic [5] damage. It must accordingly be expected that procedures accompanied by activation of antioxidant systems may act as a factor protecting the myocardium against stress-induced damage.

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EFFECT OF DIBUNOL* AND VERAPAMIL ON SERUM CREATINE KINASE AND MYOGLOBIN LEVELS IN DOGS DURING POSTISCHEMIC CORONARY REPERFUSION

A. P. Golikov, O. A. Avilova, V. Yu. Polumiskov, A. A. Berestov, E. A. Konorev, S. P. Smotrov, and I. N. Sharipova

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Restoration of the coronary blood flow after ischemia accelerates the release of intracellular high-molecular-weight proteins from the heart [4, 14]. The mechanisms of these changes have not been fully elucidated. It has recently been shown that postischemic reperfusion aggravates structural damage to the cardiomyocyte membrane [9], and the most important role in the formation of this damage is played by intensification of lipid peroxidation (LPO) [1, 2] and a disturbance of calcium transport [3].

The aim of this investigation was to study the effect of the antioxidant dibunol and the calcium antagonist verapamil on postreperfusion release of myoglobin (MG) and creatine kinase (CK) from the heart in experimental myocardial infarction due to coronary occlusion.

EXPERIMENTAL METHOD

Experiments were carried out on 30 dogs weighing 7-20 kg. Myocardial infarction was produced by application of an atraumatic vascular clip to the anterior descending branch of the left coronary artery under pentobarbital sodium anesthesia (40 mg/kg, intraperitoneally) with artificial ventilation of the lungs by the RO-6-03 apparatus. The myocardium was revascularized by removal of the clip 3 h after its application. Dibunol was injected intraperitoneally in a dose of 30 mg/kg 2 and 3 h after coronary occlusion, in the form of an oily emulsion in Tween-80; verapamil was injected intravenously (a bolus dose of 0.1 mg/kg followed by 0.2 mg/(kg·h) by the drip method, starting with 2 h after application of the clip to the artery). The serum MG level of the dogs was determined by solid-phase enzyme immunoassay, using the sandwich technique. This method is based on detection of the MG–antibody complex with the aid of peroxidase-labeled γ-globulin against MG. Antiserum to MG was obtained by immunizing rabbits with a purified preparation of MG, isolated from dog skeletal muscles by the method in [12]. Antibodies to MG were isolated from the immune serum by affinity chromatography on CNBr-sepharose 4B (Sigma, USA). Peroxidase-labeled γ-globulin from rabbit antiserum was synthesized by the periodate method [7]. MG was determined by consecutive treatment of 9-well polystyrene plates (Labsystems, Finland) with sensitized antibodies, with the dog serum to be

*4-Methyl-2,6-di-tert-butylphenol.

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