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EFFECT OF ETHANOL AND THE CATALASE INHIBITOR AMINOTRIAZOLE ON LIPID PEROXIDATION IN THE RAT MYOCARDIUM

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The peroxisomal enzyme catalase, which takes part in the detoxication of hydrogen peroxide, is one component of the antioxidative protective system of the cell [3, 5]. It was shown previously that during long-term combined administration of 3-amino-1,2,4-triazole (aminotriazole) and ethanol ultrastructural changes characteristic of the morphological picture of alcoholic cardiomyopathy in man are observed in the cardiomyocytes of rats [9]. In the case of separate administration of the two substances the pathological process does not develop. The necessity for inactivation of catalase when the experimental model of alcoholic cardiomyopathy is created may indicate a probable role of peroxide processes in the pathogenesis of this disease.

The aim of this investigation was to study the effect of chronic administration of ethanol and aminotriazole on the level of lipid peroxidation (LPO) in the rat myocardium. The action of natural (vitamin E, reduced glutathione) and artificial (dibunol*) antioxidants on alcohol-induced lipid peroxidation also was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 160-180 g were used. The animals were kept on a semisolid diet, balanced with respect to the principal components (proteins, lipids, carbohydrates), which included vitamins and mineral salts [1]. Parallel experiments were carried out on rats of four groups: 1) control, 2) ethanol, 3) aminotriazole, 4) ethanol + aminotriazole. The animals of groups 2 and 4 received ethanol (34-36% of the total calorific value of the diet, 10-12 g/kg body weight/day) as part of their diet. Aminotriazole, in the form of a 10% aqueous solution, was injected intraperitoneally in a dose of 1 g/kg 3 times a week. The total duration of the experiments was 12 weeks. The rats were deprived of food and the last injections of aminotriazole were given 18-20 h before sacrifice. Immediately after decapitation blood was collected from the animals, and the ethanol concentration in it was determined by gas chromatography. The ethanol concentration in the blood at the time of sacrifice of the animals did not exceed 0.5-0.7 mM. After thoracotomy the heart was perfused with cold isolation medium (0.15 M KCl, 20 mM Tris-HCl buffer, pH 7.4). The organ was removed and quickly frozen in dry ice, after which pieces of tissue were kept at −80°C for not more than 2-3 weeks. The heart tissue was homogenized in isolation medium in a Dounce homogenizer

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

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TABLES 1. Changes in Biochemical Parameters in Rats' Heart during Long-Term Administration of Ethanol and Aminotriazole (M ± m)

Parameter	Group of animals			
	1 (control)	2 (ethanol)	3 (aminotriazole)	4 (ethanol + aminotriazole)
Catalase, relative units	0,97±0,09	1,70±0,16**	0,33±0,06***	0,39±0,04***
Glutathione, μmoles /g tissue	0,78±0,03	0,92±0,07	0,88±0,06	0,85±0,08
TBA, nmoles/min/g tissue:				
"nuclear-free" homogenate	3,0±0,5	8,7±3,4	19,0±4,6**	19,5±5,8*
fraction of particles	6,4±1,1	11,4±2,1*	20,0±2,2***	21,0±2,4***
Protein, mg/g tissue	68±3	70±3	68±1	76±4
Relative weight of heart, %	0,281±0,005	0,306±0,009*	0,280±0,008	0,262±0,006*

Legend. Protein concentration was determined in whole heart tissue homogenate. Number of animals in each group 8-10. *p < 0.05, **p < 0.01, ***p < 0.001 compared with control.

(glass/Teflon). The homogenate was filtered through nylon gauze and centrifuged at 3000 rpm for 10 min. To isolate the total fraction of particles the resultant supernatant was recentrifuged at 15,000 rpm for 40 min (RS-6 centrifuge). Catalase activity was determined at 25°C [5] and expressed in relative units/g tissue. The reduced glutathione concentration was measured with the aid of Ellman's reagent [12]. In the study of LPO the level of chemiluminescence (ChL) and the rate of accumulation of products reacting with 2-thiobarbituric acid, TBA, were determined [2]. To initiate the LPO process 0.1 mM $FeCl_3$ and 1.5 mM ADP were used in both cases, and ascorbic acid, in a concentration of 0.1 mM, was used as the reducing agent for the iron [7]. To determine the level of ChL 1 ml of a sample of "nuclear free" homogenate or of the total fraction of particles was introduced into 22-ml glass flasks for radioactivity measurement, containing 4 ml of isolation medium. After incubation for 25-30 min in a darkened incubator at 25°C the spontaneous weak luminescence (zero time) was measured in the samples. Next, LPO initiators (Fe/ADP and ascorbate) were added to the flasks, and the increase in the level of ChL was recorded at definite time intervals, using a scintillation counter (Roche-Bioelectronique, France), programmed for two-channel pulse counting. As the control, flasks containing 5 ml of isolation medium were used. Each flask was kept on measurement mode for not less than 30 sec. The ChL level was expressed in cpm/sample. The rate of TBA accumulation was determined in samples containing 0.4-0.5 mg protein in a final volume of 1 ml. The duration of incubation of the samples (at 25°C) in the presence of LPO initiators was 30 min. The reaction velocity was expressed in nanomoles malonic dialdehydes (ε_{532} = 156 $mM^{-1} \cdot cm^{-1}$) per min per g tissue. Protein was determined by Lowry's method [10].

EXPERIMENTAL RESULTS

Chronic administration of ethanol as a component of an isocaloric diet was accompanied by an increase of 12% in the relative weight of the rats' heart (Table 1). However, no increase was observed in the absolute weight of the heart. The differences can be explained on the grounds that under the influence of ethanol the rate of increase of the absolute body weight of the rats fell significantly (during 12 weeks of the experiment the animals of the control group gained in weight by 48%, compared with only 27% in the group of rats receiving ethanol). After combined administration of ethanol and aminotriazole a decrease in both the absolute and the relative weight of the rats' heart was observed (Table 1). This may indicate the development of degenerative processes in the myocardium of the animals of this group. This conclusion is confirmed by morphological observations [9]. In the rats of groups 3 and 4, under the influence of aminotriazole there was a marked decrease in catalase activity, whereas in the animals of group 2 activity of this enzyme increased. Meanwhile ethanol and aminotriazole had no effect on the concentration of reduced glutathione or of protein in whole myocardial homogenate (Table 1).

Two independent experimental approaches were used to record LPO: determination of the ChL level and measurement of the rate of accumulation of TBA. The level of peroxidation in nuclear-free homogenate of rat heart was found to be raised in all the experimental groups; the highest values, moreover, were found in animals of group 4 (Fig. 1). Similar results were observed during measurement of the rate of TBA accumulation (Table 1). In the total fraction of particles, consisting mainly of mitochondria and fragments of the sarcoplasmic reticulum, activation of peroxidation also took place under the influence of ethanol and aminotriazole

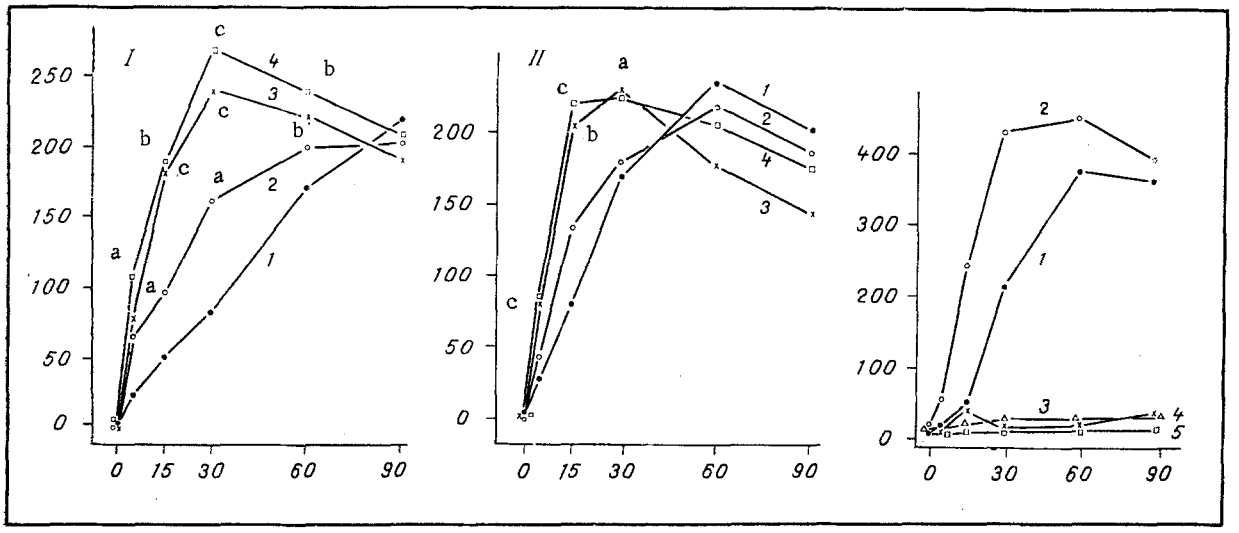

Fig. 1 Fig. 2

Fig. 1. Level of ChL in rat myocardium during long-term administration of ethanol and aminotriazole. Abscissa, time after addition of peroxidation initiators (in min); ordinate, level of ChL (in cpm•10^{-3} per sample). I) "Nuclear free" heart homogenate (final protein concentration in sample 0.9-1.1 mg/ml; II) total fraction of particles (0.5-0.6 mg protein/ml). 1) Control; 2) ethanol; 3) aminotriazole; 4) ethanol + aminotriazole. For each point, standard deviations from mean value did not exceed 10-15%. In each group the number of animals was five. Significance of differences from control: a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.

Fig. 2. Effect of antioxidants on ChL level in vitro. Dependence of ChL level on duration of incubation determined with the aid of total fraction of particles (final protein concentration in sample 0.9-1.0 mg/ml), obtained from heart tissue homogenate of rats of groups 1 (control, curve 1) and 2 (ethanol, curves 2, 3, 4, 5). Reduced glutathione (final concentration 0.1 mM, curve 3) and also lipid antioxidants in the form of alcoholic solutions (10 µl) in final concentration: vitamin E 5 µM (curve 4), dibunol 10 µM (curve 5) were added immediately before incubation. Remainder of legend as to Fig. 1.

(Fig. 1, II; Table 1). The phenomenon observed was reproduced in three independent series of experiments. To prove the role of LPO in the mechanism of appearance of very weak luminescence, natural (glutathione, vitamin E) and artificial (dibunol) antioxidants were used (Fig. 2). On the addition of these substances in vitro the ChL level in nuclear-free heart homogenate virtually did not increase throughout the period of incubation (90 min, 25°C). We know that vitamin E and dibunol are lipid antioxidants, interacting directly with radicals of polyunsaturated fatty acids, and thereby interrupting the process of peroxidation [2, 13]. The protective action of reduced glutathione, when used in low concentrations (0.1 mM), may evidently be connected with activation of the membranotropic protein factor whose antioxidant action is potentiated by vitamin E [8].

The results are evidence of a possible role of activation of peroxidation in the pathogenesis of the dystrophic changes observed in the rat myocardium during combined administration of ethanol and aminotriazole. There is reason to suppose that the mechanism of this phenomenon includes a disturbance of hydrogen peroxide metabolism in cardiomyocyte microperoxisomes. We know that during chronic administration of ethanol the number and size of the cardiac microperoxisomes increase, as also does activity of catalase, the marker enzyme of these organelles (Table 1) [6, 11]. As the preliminary results show, besides activation of catalase, an increase in activity of acyl-CoA-oxidase, an enzyme generating peroxide, and also located in the microperoxisomes, takes place in the rat cardiomyocytes. It may thus be expected that catalase activation in this case is a compensatory reaction to the increased peroxide-producing capacity of the cardiomyocyte microperoxisomes. Inhibition of catalase by aminotriazole is accompanied by disturbance of the balance between hydrogen peroxide production and detoxication in favor of hyperproduction of this highly toxic metabolite which, in turn, activates intracellular peroxidation processes.

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EFFECT OF CHOLERA ENTEROTOXIN ON CARBOHYDRATE METABOLISM IN THE LIVER AND SMALL INTESTINAL MUCOSA OF RABBITS

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Carbohydrate metabolism in the liver is connected with changes in the cAMP level in the cell. An increase in the cAMP concentration is accompanied by release of glucose from glycogen, stimulation of gluconeogenesis, and inhibition of glycolysis. Cholera enterotoxin is a factor known to increase adenylate cyclase activity, and thereby to increase the cAMP concentration in various tissues, including the liver [9]. There is evidence in the literature that cholera toxin, in primary hepatocyte culture, activates glycogenolysis and inhibits lactic acid formation, probable evidence of the inhibition of glycolysis [9]. This effect of the toxin on carbohydrate metabolism is in agreement on the whole with the action of other factors such as glucagon and adrenalin, which raise the cAMP level in the liver.

Cholera enterotoxin irreversibly activates adenylate cyclase in the mucosa of the small intestine [6]. It has been shown in biopsy specimens from the mucosa of the jejunum in vitro that cholera toxin inhibits pyruvate kinase, which catalyzes an irreversible glycolysis reaction, and stimulates fructose-1,6-diphosphatase [12, 13].

One of the least studied enzymes of carbohydrate metabolism both in the liver and in the small intestinal mucosa, is glucose-6-phosphatase, which unites two metabolic pathways in the liver: gluconeogenesis and glycogenolysis. Glucose-6-phosphatase catalyzes the irreversible reaction of glucose-6-phosphate hydrolysis to glucose and inorganic phosphate and it is possibly one of the regulatory enzymes of gluconeogenesis. The view is held that this enzyme also discharges dephosphorylated glucose into the channels of the endoplasmic reticulum, and consequently into the intercellular space.

In the mucosa of the small intestine glucose-6-phosphatase evidently plays an additional role in glucose transport, for glucose is found inside the cell in a phosphorylated form [3].

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