ACTIVATION OF PEROXISOMAL ACYL-CoA-OXIDASE AND OF LIPID PEROXIDATION IN THE RAT MYOCARDIUM DURING LONG-TERM ETHANOL INTAKE

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During chronic ethanol intake by rats increased catalase activity is observed in the myocardium, and the number and size of the microperoxisomes also are increased [7]. Besides catalase, peroxisomes of different tissues also contain several peroxide-generating oxidases, including acyl-CoA-oxidase, which oxidizes long-chain fatty acids [4]. In rats receiving ethanol and the highly specific catalyase inhibitor 3-amino-1,2,4-triazole (aminotriazole), ultrastructural changes similar to the morphological picture of alcohol cardiomyopathy in man develop in the myocardium [13]. The information given above points to a possible role of catalase and of other peroxisomal enzymes in the pathogenesis of alcohol cardiomyopathy.

The aim of this investigation was to study the effect of chronic alcoholic intoxication on activity of peroxisomal acyl-CoA-oxidase, and also of the enzymes of antioxidative protection of the cell. Parameters characterizing the level of peroxidation processes in the rat myocardium also were 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 its principal components (proteins, lipids, carbohydrates), to which vitamins and minerals also were added [1]. Rats of the experimental groups received 10% ethanol with their food (34-36% of the total calorific value of the food, 10-12 g/kg body weight daily), whereas animals of the control group received sucrose with the same calorific value. The duration of the experiments, including adaptation of the animals to the semisolid diet, was 8-10 weeks. The rats were deprived of food 18-20 h before killing them. After thoracotomy, the heart was perfused with cold isolation medium (0.15 M KCl, 20 mM Tris-acetate, pH 7.4), after which the heart was removed, weighed, cut into pieces with scissors, and then carefully homogenized in isolation medium in a Dounce homogenizer (glass/Teflon, 30 tractions). In some cases blood cells, fragments of connective tissue, and nuclei were removed by centrifugation of the 10% homogenate at 3000 rpm for 10 min (TsLR centrifuge, small rotor). The nuclear-free homogenate thus obtained was used to measure the level of chemiluminescence (CL) and also to determine catalase and acyl-CoA-oxidase activity. To isolate the total fraction of particles, the nuclear-free homogenate was recentrifuged at 15,000 rpm for 40 min [12]. Enzyme activity was determined on a Gilford model 250 spectrophotometer at 250°C: catalase by the method in [3] and superoxide dismutase (SOD) by the method in [10]. Acyl-CoA-oxidase activity was measured by the use of palmitoyl-CoA as the substrate [6]. The substrates for selenium-dependent glutathione peroxidase [5] and for glutathione-S-transferase [9] were H_2O_2 and 1-chloro-2,4-dinitrobenzene, respectively. Glutathione reductase and glucose-6-phosphate dehydrogenase activity was determined [5] by measuring the rate of reduction of $NADP^+$. Catalyase [3] and SOD [10] activity was expressed in relative units, and activity of the remaining enzymes in nanomoles/min/mg protein of the whole heart homogenate. The parameters used to characterize peroxidation processes were the CL level and the rate of accumulation of products reacting with 2-thiobarbituric acid (TBA) [2]. To initiate lipid peroxidation (LPO) 0.1 mM $FeCl_3$ and 1.5 mM ADP were used in both cases, with 0.1 mM absorbic acid as the reducing agent for the iron [8]. To determine the CL level 2 ml of 10% nuclear-free heart homogenate was transferred into glass flasks containing 3 ml of iso-

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TABLE 1. Effect of Chronic Ethanol Intake on Enzyme Activity in Rat Myocardium (M ± m)

Enzyme	Control	Experiment
Catalase	5,4±0,4	10,8±1,1**
Acyl-CoA-oxidase	0,47±0,05	1,16±0,17*
SOD	5,4±0,5	5,0±0,6
Glutathione peroxidase	261±25	282±30
Glutathione reductase	7,3±0,6	7,1±0,6
Glutathione-S-transferase	56,0±5,4	67,0±11,6
Glucose-6-phosphate dehydrogenase	6,9±0,8	7,8±0,5
Glutathione, μmoles/g tissue	0,66±0,06	0,65±0,06
Protein, mg/g tissue	78,6±3,5	71,1±4,7

Note. Catalase activity expressed in relative units ($\times 10^3$). Number of animals in each group was 8-10. *P < 0.01, **P < 0.001 compared with control.

lation medium (final protein concentration 1.3-1.5 mg/ml). After incubation for 25-30 min in a darkened thermostat at 30°C spontaneous CL was measured in the sample (zero time). Next, the LPO initiators ($FeCl_3$/ADP and ascorbate) were added to the flasks and, after certain time intervals, the increase in CL was recorded. Flasks containing 5 ml of isolation medium were used as the control. The measuring procedure with each flask took at least 30 sec. The CL level was expressed in pulses per minute per sample. The measurements were made on a Roche Bioelectronique liquid scintillation counter (France), with two-channel pulse counting. The rate of accumulation of products reacting with TBA was determined in the total fraction of particles by the method in [4]. Each sample contained 0.4-0.5 ml protein of particles in a final volume of 1 ml; duration of incubation (37°C) in the presence of LPO inhibitors was 30 min. The rate of accumulation of TBA-products was expressed in nanamoles/min/mg protein. The quantity of reduced glutathione in the heart tissues was investigated with the aid of Elman's reagent [11]. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Chronic alcoholic intoxication was accompanied by a moderate increase in the relative weight of the rat heart (control 0.287 ± 0.012%, experiment 0.321 ± 0.019%; P < 0.001, n = 9), although the absolute weight of the organ was unchanged (0.78 ± 0.02 and 0.76 ± 0.03 g respectively). The difference can be explained by the unequal increase in body weight, which was smaller in animals of the experimental group (239 ± 13 g) than in the control (271 ± 7 g, P < 0.05). Under the influence of ethanol the specific catalse activity in the rat heart homogenate was increased (Table 1), confirming activation of this enzyme during long-term alcohol intake reported in the literature [7]. It will be clear from Table 1 that, parallel with the increase in catalase activity in the nuclear-free myocardial homogenate, activity of the key enzyme of the peroxisomal chain for β-oxidation of fatty acids, namely peroxide-generating acyl-CoA-oxidase, also was increased. According to preliminary results, activity of this enzyme in the liver, like activity of other components of the peroxisomal β-oxidation chain, is unchanged by ethanol. No differences likewise could be found in activity of other peroxisomal peroxide-generating oxidases in the liver and heart: urate oxidase, D-amino-acid oxidase, and L-α-hydroxy-acid oxidase. Under these circumstances activity of the last two enzymes in heart tissue was lower than 0.05 nmole/min/mg protein of the homogenate, whereas urate oxidase activity could not be detected either in the control or in the experimental samples. Unlike catalase, other components of the antioxidative enzyme system of the cardiomyocytes did not change their activity during long-term ethanol intake (Table 1). No changes likewise were found in the content of the reduced form of glutathione in the freshly-isolated heart homogenate (Table 1). Incidentally, activity of the majority of enzymes of antioxidative cell protection in heart tissue was found to be substantially lower than in the liver (data not given).

To study the effect of ethanol on the level of peroxidation processes in the rat myocardium two independent experimental approaches were used: determination of the concentration of LPO products interacting with TBA, and also direct measurement of the velocity of the LPO

chain process on the basis of intensity of CL, mainly determined by recombination of the free radicals [2]. To activate LPO, substances most widely used in experimental practice ($FeCl_3$ and ADP) were used, with ascorbic acid as the iron-reducing agent. The mechanism of activation of LPO by the agents mentioned above has been studied in fair detail [5]. In both cases the results unequivocally demonstrated activation of peroxidation processes in the cardiomyocytes during long-term alcohol intake by the rats. The CL level in the nuclear-free heart homogenate of rats of the experimental group imcreased on average by 3-4 times compared with the control, whereas the rate of accumulation of TBA-products in the fraction of particles was doubled.

On the whole the results indicate a possible role for activation of peroxidation processes in the pathogenesis of experimental alcohol cardiomyopathy, developing in rats in response to combined administration of ethanol and aminotriazole. In particular, the need for the combined action of the catalase inhibitor and of alcohol on the heart tissue can be understood and in this case, on the one hand there is an increase in the peroxide-producing capacity of the peroxisomes on account of activation of acyl-CoA-oxidase, whereas on the other hand hand there is inhibition of the detoxicating function of catalyase by aminotriazole. It will be evident that it is only under those conditions that maximal exhaustion of the compensatory powers of the cardiomyocytes and the rapid development of the pathological process, due to induction of LPO, can arise.

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