Effect of chronic ethanol treatment on peroxisomal acyl-CoA oxidase activity and lipid peroxidation in rat liver and heart

L. F. Panchenko, S. V. Pirozhkov, S. V. Popova and V. D. Antonenkov*

All-Union Research Center for Medico-biological Problems in the Prevention of Alcohol Abuse and Alcoholism, Kropotkinsky per. 23, Moscow 119034 (USSR), 30 June 1986

Summary. Chronic ethanol administration was shown to increase catalase and acyl-CoA oxidase activities in rat myocardium but did not alter the activity of liver peroxisomal enzymes. As a result of alcohol consumption a 2–3-fold increase in the level of lipid peroxidation was observed in the heart tissue while in the liver the induction was much less pronounced.
Key words. Ethanol; peroxisomes; lipid peroxidation; alcoholic cardiomyopathy; rat liver; rat heart.

It is well established that rat liver and heart peroxisomes contain catalase and enzymes for β-oxidation of long-chain fatty acids, including H_2O_2-generating acyl-CoA oxidase[1,2]. Chronic ethanol administration has been shown to induce a marked proliferation of heart peroxisomes[3]. The increase in peroxisomes is accompanied by a significant elevation of catalase activity[3,4]. At the same time, ethanol has no effect on the activity of hepatic and erythrocyte catalase[4].

In the present paper we report on the influence of chronic ethanol administration on the activity of acyl-CoA oxidase and some other peroxisomal H_2O_2-producing oxidases in rat liver and heart. We also describe the elevation of the Fe-ascorbate-induced lipid peroxidation in homogenates and subcellular fractions of these tissues after chronic ethanol feeding.

Materials and methods. 2-Thiobarbituric, ascorbic and p-hydroxybenzoic acids were from the Sigma Chemical Co. 4-Aminoantipyrine was obtained from Aldrich Chemical Co. The sources of all other reagents have been given previously[5,6].

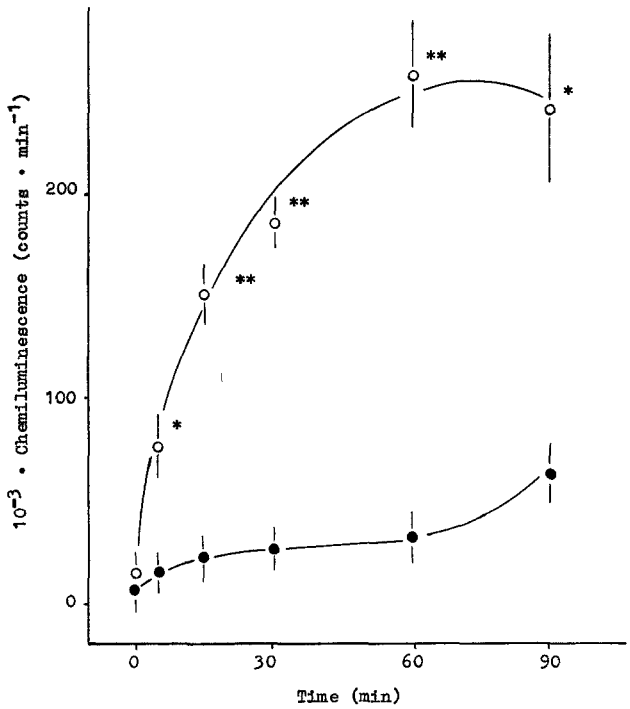
Wistar male rats with an initial body weight of 150–170 g were used. Animals were fed with a liquid ethanol (about 36% of the total calories) or control diet (modified from Lieber at al.[7]). All rats were maintained on the control diet for 1 week before the experimental group was placed on the alcohol containing diet. Ethanol dose was gradually increased for the period of 1 week. After 6 to 8 weeks of feeding, the rats were fasted overnight and then killed by decapitation. Heart and liver were perfused with saline to wash out erythrocytes, weighed and homogenized in 9 vols. of ice-cold isolation medium (0.15 M KCl, 20 mM Tris-HCl buffer, pH 7.4). The homogenates were filtered through nylon cloth, and in some cases heart homogenates were centrifuged at 1000 g for 10 min to remove unbroken cells, nuclei and cell debris ('postnuclear' homogenate). Rat liver mitochondria and microsomes were obtained as described earlier[6]. The heart cells total membrane fraction was obtained by centrifugation of the 'postnuclear' homogenate for 40 min at 12000 g. Acyl-CoA oxidase activity was measured with palmitoyl-CoA as substrate[8]. Catalase[9], urate oxidase[10], D-amino acid oxidase and L-α-hydroxy acid oxidase[11] were assayed according to the published procedures. Heart catalase and acyl-CoA oxidase activities were measured in the 'postnuclear' homogenates. For lipid peroxidation experiments, subcellular fractions (0.5 mg protein/ml) were incubated in 1 ml of isolation medium, containing 0.1 mM $FeCl_3$, 1.5 mM ADF and 0.1 mM ascorbate for 30 min at 37 °C. The amount of malondialdehyde was determined by the thiobarbituric acid method[12] using an extinction coefficient of 156 mM^{-1} cm^{-1}. For measurement of a low-level chemiluminescence the 'postnuclear' homogenates (total vol. 5 ml, about 1.3 mg/ml) were incubated in the isolation medium containing $FeCl_3$, ADP and ascorbate at 25 °C in the dark-adapted 22 ml glass scintillation vials. The amount of chemiluminescence was determined in a Roche-Bioelectronique liquid scintillation counter operated in the out-of-coincidence mode[13]. Each vial was counted for at least 30 s. The chemiluminescence measurements are expressed as counts/min (c.p.m.) per vial. Protein concentration was estimated[14] using bovine serum albumin as standard.

Results and discussion. Both groups of rats gained weight during the 8-week period: those on the control diet from 190 ± 9 g to 273 ± 12 g, and those in the ethanol-treated group from 191 ± 8 g to 246 ± 14 g (means ± SD). The absolute heart weight was similar in both groups: control 0.746 ± 0.081 g, ethanol

Table 1. Effect of chronic ethanol administration on the activities of peroxisomal enzymes in rat liver and heart homogenates

Enzyme		Activity (nmol/min per mg protein) Control	Ethanol
Liver			
Catalase (units × 10^3)	(8)	114 ± 27	135 ± 17
Acyl-CoA oxidase	(5)	3.9 ± 0.5	3.5 ± 0.3
Urate oxidase	(5)	15.9 ± 0.8	14.3 ± 1.5
D-Amino acid oxidase	(5)	1.2 ± 0.2	1.4 ± 0.2
L-α-Hydroxy acid oxidase	(8)	2.6 ± 0.2	2.5 ± 0.1
Protein (mg/g tissue)	(15)	211.5 ± 5.8	193.4 ± 4.9*
Heart			
Catalase (units × 10^3)	(10)	5.4 ± 0.4	10.8 ± 1.1***
Acyl-CoA oxidase	(10)	0.27 ± 0.03	0.67 ± 0.10**
Protein (mg/g tissue)	(10)	78.6 ± 3.5	71.4 ± 4.7

Catalase activity was expressed as described[5]. Mean values ± SD are shown for specific activities and the number of animals in each group is given in parentheses; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



Effect of chronic ethanol consumption on the rate of lipid peroxidation measured as low-level chemiluminescence.
Heart 'postnuclear' homogenates from control (●) and ethanol-treated (○) rats were incubated at 26 °C. Values are expressed as the means (± SD) of 5 separate experiments; * $p < 0.01$; ** $p < 0.001$.

Table 2. Susceptibility of the liver and heart subcellular fractions to lipid peroxidation after chronic ethanol consumption

Fraction		Rate of lipid peroxide formation (nmol malondialdehyde/min per mg protein) Control	Ethanol
Liver			
Mitochondria	(8)	1.08 ± 0.08	1.39 ± 0.12*
Microsomes	(7)	1.03 ± 0.12	1.46 ± 0.16*
Heart			
Total membrane fraction	(5)	0.64 ± 0.10	1.35 ± 0.12**

Subcellular fractions were incubated for 30 min at 37 °C. Each value represents means ± SD, the number of animals used is shown in parentheses; * $p < 0.05$; ** $p < 0.01$.

0.752 ± 0.060 g, but the liver weight was slightly increased after alcohol treatment: control 10.9 ± 0.6 g, ethanol 11.6 ± 0.9 g ($p < 0.05$). Chronic ethanol administration leads to an elevation of the relative liver weight (organ weight/body weight) by 17% ($p < 0.01$; $n = 15$) and heart weight by 12% ($p < 0.001$; $n = 9$). In the heart homogenate of the alcohol-fed group there was an increase in the catalase specific activity (table 1) in keeping with the previous reports about activation of this enzyme after prolonged ethanol ingestion [3,4]. At the same time ethanol failed to induce catalase activity in the liver. Similarly ethanol had no effect on the activity of a number of peroxisomal oxidases in the liver such as acyl-CoA oxidase, D-amino acid oxidase, urate oxidase and L-α-hydroxy acid oxidase. In contrast with this, in the 'postnuclear' homogenate of rat myocardium a 2-fold increase in acyl-CoA oxidase activity was demonstrated (table 1). The activity of all the oxidases studied was markedly lower in the heart muscle than in the liver tissue. The myocardial D-amino acid oxidase and L-α-hydroxy acid oxidase activities were lower than 0.05 nmol/min per mg of homogenate protein and urate oxidase activity was not detected either in the control or in the ethanol-treated group.

Two methods were employed in the study of the ethanol effects on lipid peroxidation. The first one dealt with the assay of the thiobarbituric acid-reactive products (mainly malonic dialdehyde), and the second included the registration of the low-level chemiluminescence in the 'postnucelar' myocardial homogenate of the ethanol-fed rats which was increased 3–4-fold as compared to the control levels. In the liver homogenate from the treated rats this parameter surpassed the control levels by 15–20% (data not shown). Similar results were obtained when the rate of accumulation of the thiobarbituric acid-reactive products was measured in the peroxidizing subcellular fractions from the liver and heart homogenates (table 2). In this case the intensity of lipid peroxidation in liver microsomes and mitochondria was augmented by 30–40% under the influence of ethanol while in the total particulate fraction from the heart homogenate the increase was more than 2-fold.

The data obtained suggest that, in contrast to the situation in liver tissue, in the heart chronic ethanol administration leads to an activation of the two main peroxisomal enzymes, namely catalase and acyl-CoA oxidase. This process is possibly followed by a significant elevation of the content of acetaldehyde in the heart; acetaldehyde is generated from ethanol via the catalase pathway[15]. It is known that acetaldehyde exerts toxic effects on the cardiomyocytes[16,17]. Activation of the peroxisomal enzymes is accompanied by a significant increase in the Fe-ascorbate-induced lipid peroxidation in the heart tissue. Although the experimental design used in this study does not enable us to estimate the in vivo level of lipid peroxidation[18], the results obtained indicate the appearance of the conducive conditions for 'oxidative stress'[19] in the cardiomyocytes under the action of ethanol. According to the data by Kino[20], in rats treated simultaneously with ethanol and the highly specific catalase inhibitor 3-amino-1,2,4-triazole, ultrastructural changes developing in the myocardium are similar to those occurring in human alcoholic cardiomyopathy. On the basis of our results we suppose that the onset of the alcoholic cardiomyopathy under a partial catalase inhibition is associated with the activation of lipid peroxidation in cardiomyocytes as a result of an elevation of the hydrogen peroxide-producing capacity of heart peroxisomes. This process may be stimulated by an induction of the acyl-CoA oxidase and potentiated by an inhibition of catalase under 3-amino-1,2,4-triazole administration to rats. Further investigations are needed to confirm this suggestion.

* To whom reprint requests should be addressed.

1 Lazarow, P.B., and de Duve, Ch., Proc. natn. Acad. Sci. USA *73* (1976) 2043.
2 Norseth, J., and Thomassen, M.S., Biochim. biophys. Acta *751* (1983) 312.
3 Fahimi, H.D., Kino, M., Hicks, L., Thorp, K.A., and Abelman, W.H., Am. J. Path. *96* (1979) 373.
4 de Master, E.G., Kaplan, E., Chasler, E., Alcoholism: clin. exp. Res. *5* (1981) 45.
5 Panchenko, L.F., Antonenkov, V.D., and Gerasimov, A.M., Int. J. Biochem. *7* (1976) 409.
6 Panchenko, L.F., Pirozhkov, S.V., and Antonenkov, V.D., Biochem. Pharmac. *34* (1985) 471.
7 Lieber, C.S., Jones, D.F., and de Carli, L.M., J. clin. Invest. *44* (1965) 1009.
8 Horie, S., Ishii, H., and Suga, T., Life Sci. *29* (1981) 1649.
9 Beers, R.F., and Sizer, J.W., J. biol. Chem. *195* (1952) 133.
10 Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, St., and de Duve, Ch., J. Cell Biol. *37* (1968) 482.
11 Hayashi, H., Suga T., and Niinobe, S., Biochim. biophys. Acta *252* (1971) 58.
12 Ernster, L., and Nordenbrand, K., in: Methods in Enzymology, vol. 10, p. 574. Academic Press, New York 1967.
13 Trush, M.A., Wilson, M.E., and Van Dyke, K., in: Methods in Enzymology, vol. 57, p. 462. Academic Press, New York 1978.
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
15 Chance, B., Sies, H., and Boveris, A., Physiol. Rev. *59* (1979) 527.
16 Schreiber, S.S., Oratz, M., Rothschild, M.A., Reff, F., and Evans, C., J. molec. cell. Cardiol. *6* (1974) 207.
17 Kupary, M., Lindros, K., Hillbom, M., Heikkila, J., and Ylikahri, R., Alcoholism: clin. exp. Res. *7* (1983) 283.
18 Dianzani, M.U., Alcohol Alcoholism *20* (1985) 161.
19 Cadenas, E., and Sies, H., Adv. Enzyme Regul. *23* (1985) 217.
20 Kino, M., J. molec. cell. Cardiol. *13* (1981) 5.

0014-4754/87/050580-02$1.50 + 0.20/0