Increase of alcohol dehydrogenase and protein content of liver following chronic ethanol administration

László Buris, Gyula Csabai, Mihály Fodor and Mihály Varga

Institute of Forensic Medicine, Medical University of Debrecen, POB 25, H4012 Debrecen, Hungary

Received 24 January 1985; revised version received 18 February 1985

Increased alcohol tolerance following chronic alcohol administration has been explained by increased mitochondrial oxidation of NADH and or increased activation of MEOS. According to our experiments this increased tolerance after chronic alcohol consumption is connected with an increased activity of ADH.

Chronic ethanol consumption *Alcohol tolerance* *ADH activity* *Protein content*

1. INTRODUCTION

90% of ingested alcohol is catabolised in the liver. ADH plays the main role in the catabolism. This enzyme can be found in the cytosol fraction of liver tissue. Alcohol metabolism increases in chronic alcohol addicts [5]. One can speculate that the increased tolerance to alcohol is connected with an increase of the enzymes that participate in the catabolism of alcohol. However, many authors suggested that increased elimination of alcohol takes place without alteration of ADH activity [4,5,11]. Our aim was to determine whether, following prolonged alcohol treatment, we may expect an increase of ADH activity in liver tissue of rats.

2. MATERIALS AND METHODS

To CFY rats, weighing 100–120 g at the beginning of the experiment, an alcohol solution (20%) was given as the only drinking fluid for 5 weeks and LATI diet ad libitum. The control animals in similar circumstances were given 25% sucrose in the drinking water, with the LATI diet [8]. At the end of the experiment the weight of the animals was checked and they were killed by exsanguination. The liver was removed promptly and placed in 4 vols of 0.1 M KCl solution at 4°C. The liver tissue was homogenised with a Biomix teflon-glass homogeniser at 10000 rpm for 20 min, at 4°C filtered through gauze filter and centrifuged at 21000 × *g* for 30 min [7]. The determinations were made using the resulting supernatant. The activity of ADH was determined by the method of Bonnichsen [2] in a Spectromom 195 spectrophotometer at 340 nm. Final NAD^+ and ethanol concentrations were 2.0 and 0.54 mM, respectively. The enzyme was assayed in 76 mM sodium pyrophosphate, 22 mM glycine buffer (pH 8.8) at 25°C in 1.0 ml total volume. The control cuvette contained the reaction mixture without alcohol. Enzyme activity was expressed as μmol NAD^+ reduced/min. The ADH activity was referred to the DNA content of liver tissue. The DNA content was measured according to Schmidt and Tanhauser [9], and protein content according to Lowry et al. [6]. The significance of the difference between two groups of values was determined by Student's *t*-test.

3. RESULTS

The ADH activity of liver tissue increased significantly after 5 weeks alcohol consumption. In the control animals the ADH activity, referred to the DNA, was 3.623 μmol/min per μg DNA. At the end of 5 weeks alcohol intake the ADH activity increased to 7.24 μmol/min per μg DNA. The body weight of treated animals was lower than that

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

Table 1

Protein content and ADH activity of liver tissue

	No.of rats	Protein (μg/μg DNA)	ADH activity (μmol/min per μg DNA)
Control	8	1186	3.623
Experimental	20	1623	7.240
		$p < 0.02$	$p < 0.001$

of the untreated group. The protein content of liver tissue increased under the influence of alcohol treatment. The protein content of liver tissue was 1186 μg/μg DNA for the control group, and the protein content increased to 1623 μg/μg DNA in the treated animals.

4. DISCUSSION

The increased tolerance to alcohol is a well-known phenomenon of chronic alcohol addicts. The increase in tolerance is not supposed to be connected with an increased activity of ADH.

Authors in [4,5,11] attributed a more significant role to the increase of mitochondrial oxidation of NADH, arising from catabolism of alcohol or the activation of MEOS. They referred changes of ADH activity to the altered protein content of liver tissue.

According to our postulate one may find an increased production after long-term alcohol consumption in the group of enzymes which play an active role in the catabolism of alcohol, an adaptation of the electron transport chain. In the latter process, taking place in mitochondria, the significance of the malate-aspartate shuttle is unquestionable [10]. Similarly we found increased activity of alcohol dehydrogenase, which is a key enzyme in alcohol catabolism. The increase of protein content is most significant in the cytosolic and microsomal fractions of liver tissue [1].

Owing to the above-mentioned fact we calculated the ADH and protein values on the basis of the DNA content because, according to our microscopical examination, there is no increase in the number of hepatocytes [3], which could influence the DNA content of liver tissue. In the experiments [1] the DNA content remained unchanged in alcoholic hepatomegaly which was found to be due only to increased cell size. Calculating ADH activity on the basis of liver DNA demonstrates a significant increase after chronic alcohol treatment in rats, which may be related to the increased tolerance of chronic alcohol addicts.

Besides the increased ADH activity we found an increased concentration of total protein in the supernatant. This explains the diverging results of former examinations, which did not show an increased ADH activity of chronic alcohol addicts, when activity of ADH was calculated on the basis of liver protein content.

REFERENCES

[1] Baraona, E., Leo, M.A., Borowsky, S.A. and Lieber, C.S. (1975) Science 190, 794–795.
[2] Bonnichsen, R.K. (1963) Academic Press, New York.
[3] Buris, L., Törőcsik, I. and Csabai, G. (1982) Acta Morphol. Acad. Sci. Hung. 30, 89–95.
[4] Guerry, C., Wallace, R. and Grisolia, S. (1978) Eur. J. Biochem. 86, 581–587.
[5] Lieber, C.S. and De Carli, L.M. (1970) J. Biol. Chem. 245, 2505–2512.
[6] Lowry, O.H., Rosebrough, M.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–273.
[7] Messika, F.S. and Sproat, H.F. (1979) Proc. West Pharmacol. Soc. 22, 339–342.
[8] Porta, E.A., Cesar, L.A. and Gomez-Dumm, S.A. (1968) Lab. Invest. 18, 352–364.
[9] Schmidt, C. and Tanhauser, S.J. (1945) J. Biol. Chem. 161, 83–89.
[10] Videla, L., Bernstein, J. and Israel, Y. (1973) Biochem. J. 134, 507–514.
[11] Wadstein, J. and Skude, G. (1979) Acta Med. Scand. 205, 313–316.