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Alcohol dehydrogenase (ADH)

Influence of homo- and heterologous ADH administration on albino rats craving for alcohol and on ADH isozyme activity in the liver

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The effects of homo- and heterologous alcohol dehydrogenase (ADH) administration into albino rats were investigated. It was found that homologous ADH increases and heterologous ADH decreases the craving for ethanol. The latter effect was accompanied by the appearance of anti-ADH-3 antibodies and by a decrease in ADH-3 activity in the liver. Craving for alcohol decreased after both active and passive immunization against ADH.

Alcohol dehydrogenase isozyme; Alcohol abuse

1. INTRODUCTION

The role of alcohol dehydrogenase (ADH) in alcohol abuse mechanisms has not yet been clarified. It has been proposed that the transformation of ethanol into acetaldehyde by ADH can suppress alcohol abuse as an increase in the acetaldehyde level results in the appearance of some unpleasant subjective feelings [1]. There is, however, indirect evidence of an opposite rol for ADH. The reduced level of endogenous ethanol in blood is characteristic of alcoholics deprived of ethanol [2]. Thus an increase in ADH activity, promoting an endogenous enthanol deficit, should stimulate craving for alcohol. Previously we reported the suppression of alcohol consumption after injection of horse ADH into albino rats [3]. Here we investigated the influence of homo- and heterologous ADH administration on craving for alcohol and on endogenous ADH activity.

2. MATERIALS AND METHODS

Horse liver ADH from Reanal was additionally purified by DEAE-Sepharose chromatography in order to obtain S-isozyme which is partially similar to rat liver ADH-3 isozyme (4,5,7]. Rat liver isozymes, ADH-2 and ADH-3 (classification after Pares et al. [4,5]), were purified by subsequent ammonium sulfate fractionation [6], DEAE-Sepharose, 5'AMP-Sepharose and Sephadex G-200 chromatography [7,8]. All final enzyme preparations were found to be homogeneous by SDS-PAGE. The specific activity of purified horse ADH was 3.2–3.5 U/mg, ADH-3 isozyme 0.9 U/mg (33 mM ethanol, 2 mM NAD^+), and ADH-2 isozyme 0.65 U/mg (1 mM octanol, 2 mM NAD^+). ADH activity in tissues toward ethanol and octanol was determined spectrophotometrically by NADH formation [5]. Anti-ADH antibodies were purified by affinity chromatography on immobilized liver ADH. For active immunization, 200 μg of ADH were injected subcutaneously into rats, three times with 10 day intervals (the first two times with complete Freund's adjuvant, CFA). The titres of antibodies were determined by ELISA.

The alcoholization of albino rats (170–200 g) was performed by a free choice method between water and 15% ethanol in individual cages. The time for preliminary alcoholization was no less than 8–10 weeks. The rats chosen for experiments consumed more than 3 g ethanol/kg body weight per day.

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3. RESULTS AND DISCUSSION

The i.v. injections of 150 μg homologous ADH into alcoholized rats resulted in a significant increase in alcohol consumption over 2–3 weeks (Fig. 1). On the other hand injections of heterologous horse ADH lead to the gradual suppression of alcohol consumption, which became statistically significant on the 10th day and continued up to the 40–70th day [3]. This effect was accompanied by the appearance of anti-ADH antibodies. In the next series of experiments, the effects of immunization against ADH were studied in detail. Active immunization (with CFA) was performed in three groups of rats: (i) alcoholized before the immunization, (ii) alcoholized after immunization (one week after the last injection of ADH with CFA) and (iii) alcoholized and then deprived of alcohol for two weeks before immunization and during the whole period of immunization.

The second and the third regimes of the experiment were the most effective, namely a very significant decrease in alcohol consumption for about 90–120 days (Fig. 2). The changes in alcohol consumption at the first

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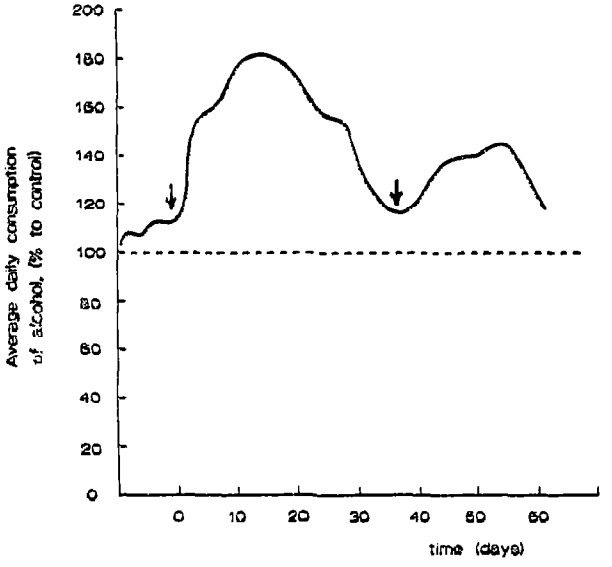

Fig. 1. Average daily consumption of alcohol (percent to control) after intravenous injection of homologous ADH into rats. Arrows indicate the days of injections. The differences between experimental and control data in intervals 2–26th and 45–56th days are statistically significant by the non-parametric Wilcoxon's test ($P<0.001$).

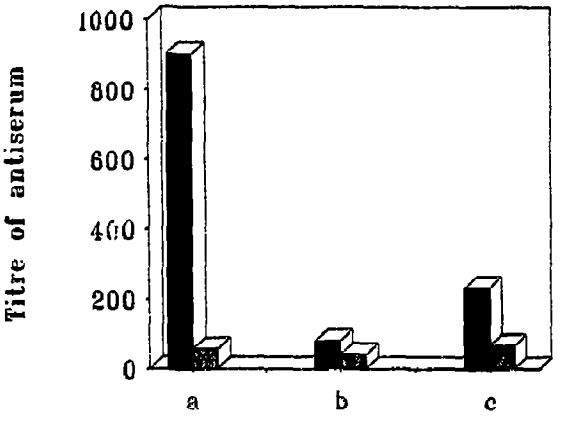

Fig. 3. Titres of antibodies in the serum of rats against ADH from horse liver (a), ADH-2 from rat liver (b), and ADH-3 from rat liver (c). Filled bars, rats immunized against horse ADH; hatched bars, control rats. For procedures of immunization and titres determination, see text. The measurements were done on the 30th day after the 1st injection.

experimental regime were not statistically significant. Immunization was accompanied by the appearance of anti-horse ADH antibodies. The antibodies had significant cross-reactivity with rat ADH-3 isozyme (Fig. 3).

In the last series of experiments, we measured the activity of ADH isozymes in the liver of the rats immunized against horse ADH. A significant decrease in ADH-3 activity was found (Fig. 4). Finally, we purified anti-horse ADH antibodies and performed passive immunization on the alcoholized rats by the intravenous administration of these antibodies (Fig. 5). The character of the changes of alcohol consumption was the same as after active immunization, but the duration of the suppression was much lower, namely 1–2 days.

It is difficult to explain the increase in alcohol consumption after homologous ADH injection by the temporal increment of ADH activity in blood or tissues and the rapid fall in the ethanol concentration: the effect is too prolonged to be accounted for in such a way. Perhaps, the injected ADH acts as a trigger for a chain of biochemical and physiological reactions. For example, a short-term rise in acetaldehyde concentration after ADH injection may cause the formation or modification of more stable regulatory compounds (for instance, the various products of acetaldehyde condensation with amines and some peptides).

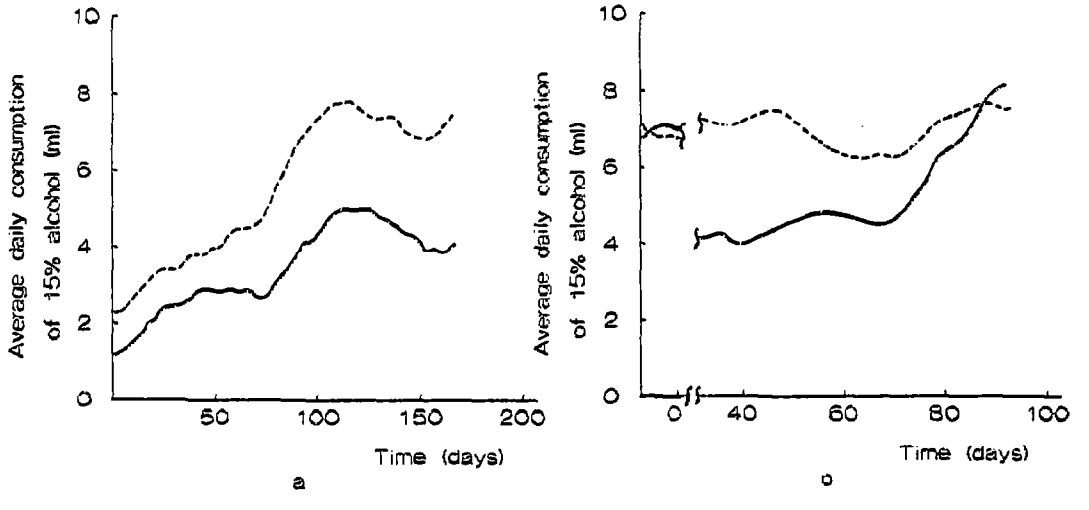

Fig. 2. Average daily consumption of 15% ethanol (ml) by rats after the immunization against horse ADH. (a) Alcoholization is started one week after the last injection of ADH with CFA; (b) immunization after alcoholization and two weeks of deprivation (period of deprivation and immunization corresponds to the gaps on curves). The differences between experimental (continuous line) and control data for periods 50–170th day in (a) and 30–70th day in (b) are statistically significant ($P<0.001$).

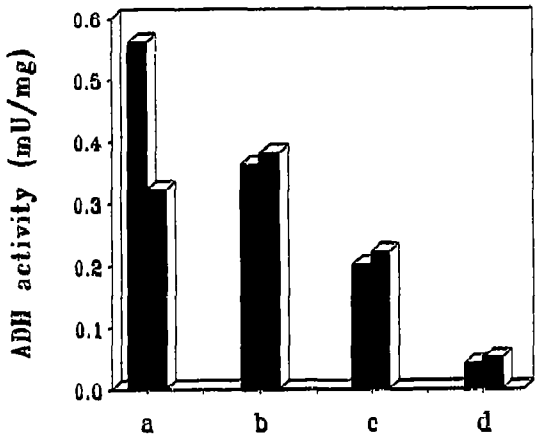

Fig. 4. Activity (mU per 1 mg of protein in supernatant) of different ADH isozymes in tissues of control rats (filled bars) and rats immunized against horse liver ADH (hatched bars). (a) ADH-3 in liver, (b) ADH-2 in liver, (c) ADH-1 in stomach mucosa, (d) ADH-2 in brain. Experimental conditions, 25°C, pH 9.25, 0.1 M sodium borate buffer, 2 mM NAD; ADH-3 and ADH-1 activities were measured with 33 mM ethanol as a substrate, and ADH-2 activity with 1 mM octanol. For other experimental details, see section 2. For the immunization procedure, see text.

Concerning the immunization against heterologous ADH we can conclude that the mechanisms of the first stage of alcohol abuse suppression consists of a rise of anti-ADH-3 antibodies and a decrease in ADH-3 isozyme activity in the liver. Perhaps diminished alcohol oxidation is the next stage toward a behavioral effect. The subsequent stages of the process which result in the change of craving for alcohol need further investigation. It is also important to estimate the influence of all possible anti-isozyme antibodies on craving for alcohol.

Thus a provocative role of endogenous ADH-3 in the formation of craving for alcohol and the participation of immune mechanisms in anti-alcoholic effects of heterologous ADH described above seems probable.

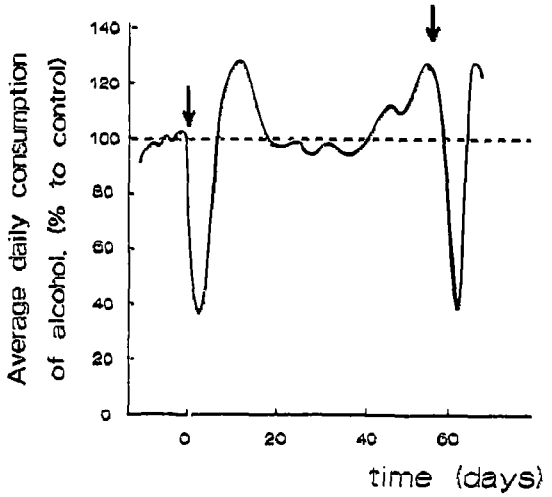

Fig. 5. The average daily consumption of 15% ethanol by rats after injection of purified rat antibodies against horse ADH. The arrows indicate the time of injection (15 μg in saline into tail vein).

REFERENCES

[1] Amit, Z., Brown, Z.W. and Rockman, G.E. (1977) Drug Alcohol Dependence 2, 495–513.
[2] Ostrovsky, Yu.M. (1986) Alcohol 3, 239–247.
[3] Ashmarin, I.P., Danilova, R.A., Pshezhetsky, A.V., Sagimbaeva, Sh.K., Fedorova, I.M., Obukhova, M.F. and Antonenkov, V.D. (1990) Biomed. Sci. 1, 308.
[4] Julia, P., Farres, J. and Pares, X. (1987) Eur. J. Biochem. 162, 179–189.
[5] Boleda, M.D., Julia, P., Moreno, A. and Pares, X. (1989) Arch. Biochem. Biophys. 274, 74–81.
[6] Bonichen, R.K. and Brink, N.G., (1953) Methods Enzymol. 1, 495–500.
[7] Pietruszko, R. (1982) Methods Enzymol. 89, 428–435.
[8] Anderson, L. and Mosbach, K. (1982) Methods Enzymol. 89, 436–445.