13. W. Haefely, Actions and Interactions of GABA and Benzodiazepines, ed. by N.Y. Bowery, New York (1984), pp. 263-286.
14. G. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther., **96**, 99 (1949).
15. K. G. Lloyd, Therapie, **38**, No. 4, 355 (1983).
16. B. Lown, R. A. Desilva, P. Reich, and B. J. Murawski, Am. J. Psychiat., **137**, 1325 (1980).
17. B. S. Meldrum, Antiepileptic Drugs, H. Frey and G. Jauz (eds.), New York (1985), pp. 153-188.
18. J. E. Skinner, Stress and Heart Disease, R. E. Beamish et al. (eds.), Boston (1985), pp. 44-59.
19. H. J. Villareal, B. J. Wilder, L. J. Willmore, et al., Neurology (Minneapolis), **28**, No. 9, 886 (1978).

ETHANOL- AND ACETALDEHYDE-METABOLIZING SYSTEM OF RAT LIVER DURING DEVELOPMENT OF TOLERANCE TO ETHANOL

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KEY WORDS: narcotic sleep; aldehyde dehydrogenase; alcohol dehydrogenase

During single and repeated contact of the body with ethanol, the latter is metabolized by many different enzymes: alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AldDH), etc. The duration of narcotic sleep induced by ethanol, used as a criterion to assess tolerance, depends on the levels of both ethanol and acetaldehyde in the body. The ratio between them is determined by the state of the metabolic systems for the two compounds [2, 5].

The aim of this investigation was to study the time course of changes in activity of ADH, AldDH, and other enzymes in the rat liver during the development of the initial stages of alcohol tolerance.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male rats weighing 140-200 g, kept on a standard animal house diet. A 25% solution of ethanol was injected intraperitoneally into the animals in a dose of 3.5 g/kg body weight. The injections were given daily in the morning (10-11 a.m.) by the same persons in the same room. The time for loss of the turning reflex, namely 2-4 min after injection of ethanol, and the time for recovery of this reflex were recorded. The time spent by the rats in the side position reflected the duration of narcotic sleep. The animals were divided into four groups (20 in each group): in group 1 a single injection of ethanol was given, three injections in group 2, seven in group 3, and 10 in group 4. The rats were decapitated 24 h after the last injection of ethanol. The tissues were quickly frozen in liquid nitrogen. ADH activity with ethanol and acetaldehyde as substrates [1], AldDH activity [8], and protein [7] and DNA [3] concentrations in the liver were determined. Activity of the NADPH-dependent ethanol-metabolizing system (EMS) was estimated in liver microsomes [6].

EXPERIMENTAL RESULTS

During repeated administration of ethanol at intervals of 24 h tolerance developed to it, as shown by a sharp fall in the duration of ethanol-induced sleep. The following groups of rats were found: those not sleeping, classed as highly tolerant (HT); those sleeping under 30 min, i.e., short-sleepers (SS), and those sleeping more than 60 min, i.e., long sleepers (LS). The general reduction in the duration of sleep was accompanied by changes in the number of HT, SS, and LS rats. The data given in Fig. 1 indicate a sharp decrease in the number of LS individuals and their moving into the SS and HT categories, reflecting an increase in tolerance of the population as a whole to ethanol. There was also a decrease in the duration of sleep in the LS from 130-100

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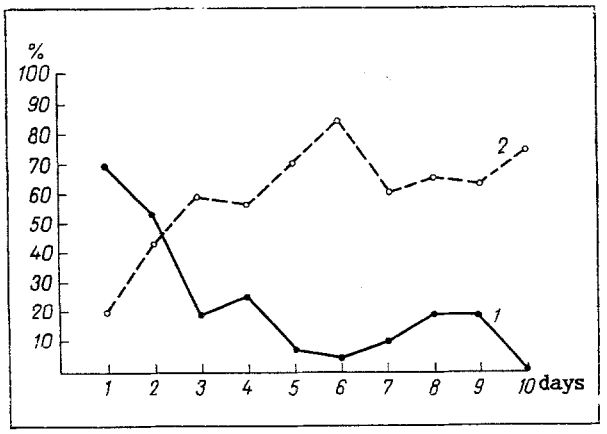

Fig. 1. Number of LS (1) and SS + HT (2) rats (in % of total number of animals) during daily intraperitoneal injections of ethanol in a dose of 3.5 g/kg.

TABLE 1. Activity of Rat Liver ADH during Development of Tolerance ($M \pm m$)

Parameters	Group of rats			
	1	2	3	4
ADH-E, μmoles NADH/min/g tissue	0,86±0,03	1,13±0,03*	1,34±0,03*	1,22±0,04*
ADH-AA, μmoles NAD/min/g tissue	2,04±0,09	2,38±0,03*	2,48±0,03*	2,48±0,03*
ADH-E, nmoles NADH/min/mg protein	11,30±0,82	16,20±0,83*	14,40±0,45*	15,30±0,84*
ADH-AA, nmoles NADH/min/mg protein	26,10±1,60	34,20±1,75*	26,70±0,90	30,70±1,20*
Soluble protein, mg/g tissue	81,40±4,00	73,00±3,00	95,00±3,00*	83,00±3,00

Legend. Here and in Table 2: E) substrate ethanol; AA) substrate acetaldehyde; *$p < 0.05$ compared with group 1.

TABLE 2. Activity of ADH (in μmoles NADH/min/mg DNA) and DNA Content (in mg/g liver tissue; $M \pm m$; $n = 20$)

Parameters	Group of rats			
	1	2	3	4
ADH-E	0,24±0,02	0,32±0,01*	0,36±0,02*	0,32±0,01*
ADH-AA	0,55±0,03	0,69±0,03*	0,67±0,03*	0,65±0,03*
DNA	3,85±0,17	3,55±0,15*	3,86±0,22	3,92±0,19

min after the first to 64-88 min after the 9th injection of ethanol. In connection with the developing tolerance to ethanol it was interesting to assess adaptive changes in activity of the enzymes of ethanol and acetaldehyde metabolism in the liver after single and repeated contact with alcohol.

A decrease in activity of NAD-dependent AldDH in the liver was found in groups 2-4 compared with group 1, mainly on account of a decrease in activity of an enzyme with low K_m for aldehydes. Activation of ADH was found when the rate of oxidation of ethanol or reduction of acetaldehyde was estimated (Table 1). The DNA level in the liver reflects the number of hepatocytes and is a relatively stable parameter compared with protein and the mass of the organ [3]. Some decrease was found in the DNA content in the animals of group 2. However, on calculation of ADH activity per milligram DNA, activation of the enzyme was confirmed (Table 2). Thus during repeated administration of ethanol ADH activity was increased, whether calculated relative to protein, tissue, or DNA, thus reflecting the development of an adaptive process aimed at stimulating the elimination of ethanol from the body. Under these circumstances activity of EMS was unchanged.

The assess the change in the contribution of the enzyme systems studied to metabolism of ethanol and acetaldehyde in the course of development of the initial stages of tolerance to the narcotic effect of alcohol, coefficients of correlation of their activity were analyzed. Maintenance of proportion between forward and reverse reactions of ADH is evidence that they both belong to the same enzyme protein, and the increase in the activity of these reactions which was found evidently reflects an increasing

quantity of the enzyme. A fall of the AldDH/ADH-E ratio may lead to increased production of acetaldehyde accompanied by its delayed utilization to acetic acid. However, a fall in the AldDH/ADH-AA ratio can be regarded as evidence of stimulation of the reductase pathway of utilization of acetaldehyde into ethanol. As was shown previously [4], in the liver perfused with ethanol only 2% of acetaldehyde is reduced to ethanol. On the basis of our observations it is logical to suggest a more significant contribution of the fall in AldDH activity than of activation of the reductase reaction of ADH in the process of acetaldehyde accumulation. The biphasic action of alcohol on the brain can be explained by antagonism of the inhibitory effect of alcohol itself and the excitatory effect of acetaldehyde [9]. The degree of narcotic action of ethanol will evidently depend on the ethanol/acetaldehyde ratio.

During repeated contact of the body with ethanol conversion of alcohol is thus intensified and utilization of acetaldehyde is delayed in the liver; this may be one mechanism of the development of metabolic tolerance, which is accompanied by diminution of the narcotic action of ethanol on the brain.

LITERATURE CITED

1. R. K. Bonnichsen and N. Brink, Meth. Enzymol., **1**, 495 (1955).
2. L. Buris, G. Csabai, M. Fodor, and M. Varga, FEBS Lett., **183**, 143 (1985).
3. A. Fleck and H. N. Munro, Biochim. Biophys. Acta, **65**, 571 (1962).
4. R. D. Hawkins and H. Kalant, Pharmacol. Rev., **24**, 67 (1972).
5. H. Kalant, Alcohol and Alcoholism, Suppl. 1, 1 (1987).
6. C. S. Lieber and M. de Carlil, J. Biol. Chem., **245**, 2505 (1970).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
8. S. O. C. Tottmar, H. Pettersson, and K. H. Kiessling, Biochem. J., **135**, 577 (1973).
9. J. P. Wartburg, R. Buhler, J.-A. Maring, and D. Pestalozzi, Pharmacol. Biochem. Behav., **18**, Suppl. 1 (1983).

EFFECT OF PIRACETAM ON SENSITIVITY TO PAIN, BLOOD β-ENDORPHIN LEVEL, AND CEREBRAL CORTICAL *c*AMP LEVEL IN RATS

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Piracetam is a classical member of the nootropic group, whose antiamnesic effect has been well studied [12]. However, the mechanism of this effect is not yet clear. An important role in the regulation of learning processes is played by cyclic nucleotides. It has been shown, for example, that administration of dibutyryl-*c*AMP or of the adenylate cyclase stimulator, forskolin, improves defensive conditioning in poorly trained animals [3] and prevents memory disturbances in hypoxia [7]. Data on the effect of piracetam on the cyclic nucleotide system are virtually absent. One of the aims of this investigation was accordingly to study the effect of piracetam on the *c*AMP level in the cerebral cortex of rats.

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