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PHARMACOLOGIC ANALYSIS OF THE ROLE OF INDIVIDUAL ETHANOL-OXIDIZING ENZYME SYSTEMS IN ETHANOL METABOLISM AT DIFFERENT STAGES OF EXPERIMENTAL ALCOHOLISM

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Investigations of the pharmacokinetics of ethanol in the blood of rats at different stages of experimental alcoholism have shed light on the character of the pharmacokinetic parameters reflecting the quantitative aspect of the course of ethanol metabolism [3].

However, the degree of involvement of each of the ethanol-oxidizing enzyme systems has received little study.

In the investigation described below a pharmacologic analysis was made of the role of ethanol-oxidizing enzyme systems in the metabolism of ethanol (by recording its elimination from the blood) at different stages of experimental alcoholism.

EXPERIMENTAL METHOD

There were four series of experiments on 78 noninbred male albino rats. The animals were selected by the stage of experimental alcoholism by the method in [1]; the weight of the rats with stage I of alcoholism was 180-200 g and their mean daily ethanol intake was 17.8 ± 1.3 ml; the corresponding figures for rats in stages II and II were 450-535 g and 26.2 ± 5.3 ml, and 500-600 g and 38.2 ± 3.8 ml.

The first three series of experiments were conducted on rats in the three different stages of experimental alcoholism. The scheme of all the series was the same: the animals were divided into four groups with six rats in each group and the pharmacokinetics of ethanol in the

TABLE 1. Pharmacologic Parameters of Ethanol in Blood of Rats Treated with Pyrazole at Different Stages of Experimental Alcoholism (M ± m)

Period of contact with ethanol	Experimental conditions n = 6	Elimination constant (K_e)	Absorption constant (K_a)	Maximal Time (T_{max}), h	Maximal concentration (C_{max}), μ moles/ml	Partition volume (V_p), ml/kg	Clearance (CIT) ml/kg/h	Area beneath curve, μmoles/ml/h
10 days	Expt.	0,19 ± 0,09***	0,88 ± 0,1***	3,4 ± 0,8***	11,7 ± 0,9***	1327 ± 216***	180 ± 23***	230 ± 89**
	Control	0,36 ± 0,04	3,0 ± 0,2	0,8 ± 0,02	5,4 ± 0,7	3036 ± 360	967 ± 83	22,3 ± 1,8
4 mo.	Expt.	0,2 ± 0,08*	3,2 ± 0,6***	1,13 ± 0,4**	9,93 ± 0,6*	1807 ± 264	233 ± 78***	145 ± 46***
	Control	0,8 ± 0,09	3,0 ± 0,1	0,56 ± 0,05	7,2 ± 0,5	1870 ± 122	1552 ± 309	15,0 ± 2,3
8 mo.	Expt.	0,05 ± 0,01***	2,2 ± 0,4	1,98 ± 0,1***	16,5 ± 0,12**	1156 ± 31*	54,4 ± 17,2***	633 ± 282***
	Control	0,34 ± 0,02	3,3 ± 0,4	0,8 ± 0,04	11,4 ± 1,2	1476 ± 195	430 ± 56	45,5 ± 4,7

Legend. Here and in Table 3: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control.

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TABLE 2. Pharmacokinetic Parameters of Ethanol in Blood of Rats Receiving 3-amino-1,2,4-triazole at Different Stages of Experimental Alcoholism (M ± m)

Period of contact with ethanol	Experimental conditions n = 6	K_e	K_a	T_{max}, h	C_{max}, μmoles/ml	V_p	CIT, ml/kg/h	Area beneath curve, μmoles/ml/h
10 days	Expt.	0,4±0,03	2,5±0,4	0,81±0,04	8,4±0,8	1607±790	800±25	26,4±7,9
	Control	0,36±0604	3,0±0,2	0,88±0,15	5,4±0,7	3036±360	967±362	22,3±1,8
4 months	Expt.	0,31±0,1	2,0±0,4	0,4±0,7	6,2±0.6	2202±298	1066±201	22,8±2,2
	control	0,8±0,09	3,0±0,1	0,56±0,05	7,2±0,5	1870±122	1522±309	15,0±2,3
8 months	Expt.	0,36±0,04	3,3±0,4	0,78±0,06	12±0,77	1355±147	488±53	45±4,8
	Control	0,34±0,02	3,3±0,4	0,8±0,04	11,4±1,2	1476±194	430±56	45,5±4,7

Legend. *p < 0.05 compared with control.

TABLE 3. Pharmacokinetic Parameters of Ethanol after Combined Inhibition of ADH and Catalase at Different Stages of Experimental Alcoholism (M ± m)

Period of contact with ethanol	Experimental conditions n = 6	K_e	K_a	T_{max}, h	C_{max}, μmoles/ml	V_p, ml/kg	CIT, ml/kg/h	Area beneath curve, μmoles/ml/h
10 days	Expt.	0,06±0,02***	1,8±0,4**	2,53±0,5***	11,4±1,0***	1707±225***	83,7±32***	778±390***
	Control	0,36±0,04	3,0±0,2	0,88±0,15	5,4±0,7	3036±360	967±83	22,3±1,8
4 mo.	Expt.	0,07±0,01***	2,58±0,4	1,59±0,4**	11,2±0,3*	1708±359	109±21***	226±48***
	Control	0,8±0,09	3,0±0,1	0,56±0,05	7,2±0,5	1870±127	1552±309	15±2,3
8 mo.	Expt.	0,05±0,004***	2,8±0,7	1,9±0,3**	16,8±0,2*	1158±51	45,2±15,4***	672±185***
	Control	0,34±0,02	3,3±0,4	0,8±0,04	11,4±1,2	1472±194	430±56	45,5±4,7
	Intact rats	0,27±0,16	4,7±0,27	0,65±0,16	14,4±2,1	1097±98	325±49	59±3,8

blood was studied 15 min after its intraperitoneal injection; for 15 h before the experiment the animals had no access to ethanol [3]. The animals of group 1 served as the control, rats of group 2 received pyrazole — an alcohol dehydrogenase (ADH) inhibitor — in a dose of 290 mg/kg intraperitoneally 5 h before administration of ethanol [7], rats of group 3 received an injection of 3-amino-1,2,4-triazole (a catalase inhibitor) in a dose of 1 g/kg 30 min before administration of ethanol [7], and rats of group 4 received a combination of 3-amino-1,2,4-triazole, pyrazole, and ethanol, as indicated above. In the experiments of series IV, 12 intact rats were kept in common cages for 8 months, then divided into two groups with 6 rats in each group: the rats of group 1 received 3-amino-1,2,4-triazole preceded by pyrazole and ethanol, whereas the animals of group 2 served as the control and were used to study the pharmacokinetics of ethanol alone.

The ethanol concentration was determined by vapor-phase gas-chromatographic analysis [3, 5]. The pharmacokinetic parameters of ethanol were calculated by computer, using a first order kinetic equation and allowing for absorption [3].

EXPERIMENTAL RESULTS

Inhibition of ADH by pyrazole led to a marked reduction of ethanol elimination from the blood of the experimental rats at all three stages of experimental alcoholism by comparison with normal animals. Analysis of the pharmacokinetic parameters of ethanol before and after administration of pyrazole showed an increase in the maximum of the ethanol concentration and the marked inhibitory effect of pyrazole on the rate of its elimination in the experimental series compared with the control. The effect described above was confirmed by a decrease in K_e, a decrease in V_p, and delay of total ethanol clearance (Table 1).

Specific inhibition of catalase by 3-amino-1,2,4-triazole at all three stages of experimental alcoholism in rats did not reveal any essential role for it in ethanol metabolism: the pharmacokinetic parameters of the experimental and control animals, describing resorption and elimination of ethanol, are given in Table 2.

Inhibition of catalase against the background of inhibition of ADH activity by pyrazole at all three stages of experimental alcoholism led to a considerable increase in ethanol concentration, a decrease in the rate of elimination, and a corresponding change in the pharmacokinetic parameters compared with the control (Table 3).

In view of reports [13, 14] of the important role of the microsomal ethanol-oxidizing system (MEOS) in chronic alcohol consumption, and also the absence of a specific inhibitor for this pathway of ethanol metabolism, an additional investigation of the role of this system in alcohol oxidation was carried out on intact rats compared with animals with stage III of experimental alcoholism (of the same age). The experiment showed that the pharmacokinetics of ethanol in the presence of inhibition of catalase by 3-amino-1,2,4-triazole, and of inhibition of ADH activity by pyrazole was the same in alcoholized and intact rats (Table 3).

The results of this investigation are in agreement with those obtained by other workers [4, 8] and, what is particularly important, with data in [2] showing that the serum ADH activity of men addicted to alcohol in the period of acute alcoholic intoxication is three times higher than in the control, which may be evidence of high ADH activity at all stages of experimental alcoholism in rats.

The passive role of catalase in the pharmacokinetics of ethanol thus revealed is largely in agreement with data obtained by other workers [11, 15] showing that the non-ADH pathways of ethanol metabolism are unimportant.

The results of the study of the role of MEOS in alcohol metabolism with combined inhibition of ADH and catalase in rats at all stages of experimental alcoholism and in intact animals are in agreement with data obtained by other workers [4, 10, 11, 15] and they may be evidence that the role of MEOS in ethanol metabolism in experiments on animals is unimportant.

It can be concluded from these experimental results that the rate of elimination of ethanol from the blood of animals at all three stages of experimental alcoholism is determined principally by ADH activity. Changes in activity of catalase and MEOS evidently do not play a significant role under these conditions.

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