EFFECT OF INMECARB ON ALCOHOL CONSUMPTION AND STATE OF THE CYTOCHROME P-450 SYSTEM OF THE LIVER AT DIFFERENT STAGES OF EXPERIMENTAL ALCOHOLISM IN RATS

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One possible mechanism of depression of alcohol consumption may be by inhibiting the system of liver ethanol-oxidizing enzymes. The aim of this investigation was to study the effect of inmecarb, a new drug suggested for the treatment of alcoholism, on the level of consumption of ethanol solution and on activity of the microsomal monooxygenases of the liver in different stages of experimental alcoholism in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male albino rats weighing initially 200-220 g and kept on a standard pellet diet. The animals were given alcohol under conditions of free access to two drinking bowls, one containing water, the other 15% ethanol solution. The animals' alcohol consumption was recorded daily before administration of the drug (background — 7 days) and during its administration; the results of its use were calculated per kilogram body weight per day. Inmecarb was injected intraperitoneally in a dose of 40 mg/kg twice a day in the form of a suspension with Tween-80 for 14 days. Control animals were kept under similar conditions of alcoholization and were given injections of the corresponding volumes of solvent. Depending on the duration of alcoholization the animals were divided into three groups (1, 4, and 8 months) depending on the stages of experimental alcoholism in rats [2]. The state of function of the cytochrome P-450 system of the liver was determined in intact and alcoholized rats. The animals were killed 24 h after withdrawal of ethanol and a last injection of inmecarb. The microsomal fraction was obtained from rat liver homogenate by differential centrifugation. The concentration of microsomal protein was determined by a modified Lowry's method [7]. The cytochrome P-450 and b_5 concentrations were measured by the method in [10] on an "Aminco" spectrophotometer. To estimate metabolism of the drugs in vitro, the rate of N-demethylation of aminopyrine and of hydroxylation of aniline was determined in the microsomal fraction of the liver [3]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Changes in consumption of ethanol solution under the influence of inmecarb in animals at different stages of experimental alcoholism are given in Table 1. The animals of Group 1, which received inmecarb starting 2 weeks after allowing the animals free access to ethanol solution, reduced ethanol consumption by 40%. In the stage of a formed addiction for ethanol (4 months of voluntary alcoholization) inmecarb depressed consumption of ethanol solution by an even greater degree (by 50%). Marked inhibition of the alcohol motivation in the rats was observed in the stage of physical dependence on ethanol (8 months of voluntary alcoholization), when the level of consumption of ethanol solution was 30% lower than in the background.

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TABLE 1. Effect of Inmecarb on Ethanol Consumption (in ml of 15% ethanol solution/kg body weight/day) at Different Stages of Experimental Alcoholism in Rats

Exptl. conditions	Duration of alcoholization, months	Background	Administration of preparation
Solvent	1 (n=9)	49,2±14,3	47,6±28,8
	4 (n=8)	37,6±13,4	38,8±16,3
	8 (n=10)	36,6±10,0	35,9±9,5
Inmecarb	1 (n=9)	48,1±8,4	27,4±15,5**
	4 (n=10)	36,3±12,9	18,4±6,5***
	8 (n=9)	34,5±6,8	11,6±5,7**

Legend. **p < 0.01, ***p < 0.001.

TABLE 2. Effect of Inmecarb on Liver Cytochrome P-450 System at Different Stages of Alcoholism in Rats

Duration of alcoholization, months	Experimental conditions	Cytochrome, nmole/mg protein		Enzyme, nmoles/min/mg protein	
		P-450	b_5	aminopyrine demethylase	aniline hydroxylase
1	Control (n = 9)	0,91±0,10	0,49±0,06	8,15±0,96	1,09±0,10
	Ethanol (n = 9)	0,88±0,10	0,49±0,07	7,20±1,36	1,00±0,17
	Ethanol + inmecarb (n = 9)	0,68±0,14**[b]	0,48±0,08	6,86±0,59	0,84±0,15*[b]
4	Control (n = 8)	1,00±0,07	0,49±0,03	12,12±1,82	1,11±0,07
	Ethanol (n = 8)	0,94±0,07	0,55±0,06	11,50±2,33	1,02±0,05
	Ethanol + inmecarb (n = 10)	0,82±0,15*[b]	0,53±0,06	11,45±2,32	0,89±0,10**[b]
8	Conrol (n = 8)	0,81±0,04	0,50±0,04	12,30±0,63	0,80±0,07
	Ethanol (n = 10)	0,91±0,17	0,51±0,06	11,00±1,67	1,06±0,09*[a]
	Ethanol + inmecarb (n = 9)	0,70±0,11**[b]	0,48±0,07	8,56±1,90**[b]	0,66±0,20***[b]

Legend. *P < 0.05 (b); **P < 0.01 (b); ***P < 0.001: Compared with control group (a), compared with "ethanol" group (b).

The effect on inmecarb in reducing ethanol consumption was intensified with an increase in the period of voluntary alcoholization of the rats.

At all stages of experimental alcoholism the state of function of the cytochrome P-450-dependent monooxygenase system of the liver was investigated. Data on the effect of ethanol on microsomal monooxygenase activity at different stages of alcoholization are given in Table 2. Chronic consumption of 15% ethanol solution had no effect on the cytochrome P-450 content in the liver and caused no change in aminopyrine N-demethylase activity at any time during the period of alcoholization studied. An increase of 32% in aniline hydroxylase activity was found in rats consuming ethanol for 8 months.

It will be clear from Table 2 that inmecarb reduced the cytochrome P-450 concentration at all stages of alcoholism by 15-24%. Aminopyrine N-demethylase activity was reduced by 22%, only after consumption of ethanol for 8 months. Inhibition of aniline hydroxylase activity was observed at all stages of chronic alcoholization, and it was most marked at the stage of physical dependence on ethanol (by 40%).

Information in the literature on the effect of chronic alcohol consumption on the microsomal monooxygenase system of the liver is extremely contradictory. Some workers report that chronic alcoholization of rats leads to an increase in cytochrome P-450 concentration and aniline hydroxylase activity [11]. An increase in the cytochrome P-450 concentration and liver microsomal monooxygenase activity during chronic ethanol consumption by hamsters has been demonstrated [6, 9]. Besides an increase in aniline hydroxylase activity, other workers [8] observed reduction of activity of enzymes responsible for metabolism of type I compounds (pentobarbital and benzphetamine).

In most such investigations, incidentally, the animals received ethanol either in a liquid diet (up to 36% ethanol) or in drinking water (10-20%), but without any free choice between alcohol and water. According to data in [1], obtained under conditions of a strictly controlled and balanced diet of rats, chronic administration of ethanol had no significant effect on activity of a wide spectrum of liver enzymes.

The present investigation was conducted on a model of chronic intake of moderate quantities of alcohol, with the possibility of choice between drinking water and 15% ethanol solution. The study of the state of the cytochrome P-450 system of the liver in the absence of enforced alcoholization revealed no differences compared with the group of intact animals. In a group of rats consuming ethanol for 8 months an increase in aniline hydroxylase activity was observed. Several workers have shown that a predisposition toward alcohol consumption is determined by the initial level of activity of the principal alcohol-metabolizing enzymes. During voluntary alcoholization, rats prefering 15% ethanol solution were shown to exhibit increased liver alcohol dehydrogenase activity compared with animals rejecting alcohol [4]. We noted that in rats predisposed toward the formation of experimental alcoholism, activity of the cytochrome P-450-dependent monooxygenase system of the liver was 36-48% higher than in rats not predisposed toward the development of alcoholism [5].

Slowing of alcohol catabolism in vivo by the aid of pharmacologic inhibition of ethanol-oxidizing enzymes leads to a decrease in the volume of alcohol consumed by the animals. Administration of inmecarb to rats led to a fall in the cytochrome P-450 concentration and to inhibition of activity of the enzyme hydroxylase, responsible for the metabolism of substrates such as aniline and ethanol. One possible mechanism of the effect of the decrease in voluntary alcohol consumption by rats at different stages of experimental alcoholism is inhibition of the first stage of ethanol metabolism by inmecarb, i.e., the action of the drug on ethanol-oxidizing enzyme systems and, in particular, on the cytochrome P-450 system of the liver.

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