EFFECT OF BLOCKING OF PROTEIN SYNTHESIS BY CYCLOHEXIMIDE ON DEVELOPMENT OF ALCOHOL MOTIVATION

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The authors showed previously that injection of cycloheximide, a blocker of ribosomal protein synthesis, into the lateral cerebral ventricles and directly into the perifornical region of the hypothalamus, which initiates alcohol motivation, inhibits alcohol-related behavior in rats with developed alcohol dependence [3]. It has been suggested on the basis of these observations that during repeated reinforcement of animals with ethanol, changes may perhaps take place in the genome of the neurons as a result of which the genome begins to express new protein molecules essential for formation of alcohol motivation. According to this hypothesis, blockage of ribosomal protein synthesis in the initial stages of formation of alcoholism may inhibit the subsequent development of alcohol motivation.

The aim of this investigation was to study the action of cycloheximide, which blocks protein synthesis on the formation of alcohol motivation in rats with constant free access to ethanol.

METHODS

Experiments were carried out on 16 noninbred male rats weighing 200-300 g. Alcohol motivation was formed in the animals, which were allowed free choice between food, water, and 20% ethanol solution. The animals were divided into two groups, with eight rats in each group. After daily consumption of food and water by the rats, kept in individual cages, had been recorded for 1 week, the rats were allowed free access to 20% ethanol solution (with food and water ad libitum). Next, through cannulas implanted into the lateral cerebral ventricles, the animals of group 1 received a single injection of 10.9 μg cycloheximide in 5 μliters of physiological saline, whereas rats of group 2 received 5 μliters of physiological saline alone. The diameter of the cannula was 0.8 mm. The choice of cycloheximide as blocker of protein synthesis was based on the results of a previous study [3], which showed that the dose of cycloheximide injected into the lateral cerebral ventricles was effective for inhibiting alcohol consumption by rats with developed alcohol dependence. Observations on consumption of alcohol, water, and food by the rats after injection of cycloheximide or physiological saline was maintained for 3 weeks. The location of the cannula tip in the lateral ventricles was verified by reference to De Groet's atlas. The results were subjected to statistical analysis by determination of the arithmetic mean by Student's test.

RESULTS

A single intraventricular microinjection of cycloheximide into the rats of group 1, allowed free access to 20% ethanol solution for 7 days previously (food and water ad libitum), led to depression of ethanol consumption by the animals for the next 3 days of observation (Fig. 1a). The mean daily ethanol consumption of all rats of this group during the first week after injection of cycloheximide was reduced by 41.6%, in the second week by 60.6%, and in the third week by 75.5% (Table 1). In the animals of group 2, after injection of equivalent volumes of physiological saline into the lateral ventricles, the time course of the mean daily ethanol consumption on the whole showed no significant change.

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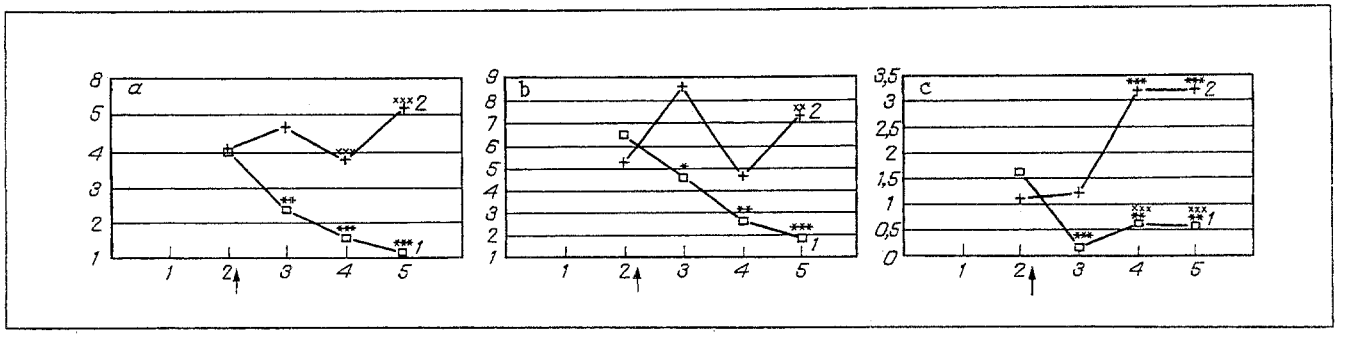

Fig. 1. Time course of mean daily ethanol consumption by rats of groups 1 (1) and 2 (2) together (a), and with preference (b) and without preference (c) for ethanol. Arrow indicates injection of cycloheximide; *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$ compared with initial data; *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$ compared with each other.

TABLE 1. Changes in Mean Daily Consumption of 20% Ethanol Solution, Food, and Water by Rats After Intraventricular Injection of Cycloheximide (group 1) or Physiological Saline (group 2)

Weeks after injection	Mean daily consumption of 20% ethanol solution, food, and water, %								
	group 1			Rats of group 1 preferring ethanol			Rats of group 1 not preferring ethanol		
	ethanol	food	water	ethanol	food	water	ethanol	food	water
1-	41,6** ↓	18,9 ↓	9,4 ↓	29,0*	10,9 ↓	16,6 ↓	90,0*** ↓	23,9 ↓	5,9 ↓
2-	60,6*** ↓	35,5*** ↓	19,0 ↓	60,0**	31,1*** ↓	2,1 ↑	63,8** ↓	38,5*** ↓	26,7** ↓
3-	70,5*** ↓	36,5*** ↓	13,9 ↓	70,8***	23,2** ↓	13,1 ↑	66,2** ↓	45,4*** ↓	26,1** ↓
	Group 1			Rats of group 2 preferring ethanol			Rats of group 1 not preferring ethanol		
1-	12,6 ↑	7,2 ↑	1,1 ↑	61,7 ↑	19,0 ↑	11,6 ↑	9,0 ↑	16,8 ↓	29,5** ↓
2-	7,6 ↓	29,1*** ↓	6,5 ↓	13,2 ↓	32,8* ↓	2,4* ↓	189*** ↑	40,0** ↓	25,5** ↓
3-	26,5 ↑	34,7*** ↓	16,8* ↓	37,0 ↑	26,8* ↓	12,5 ↓	190*** ↑	49,1*** ↓	38,8* ↓

Note. Arrow indicates direction of change. *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$ compared with initial data.

The results indicate that blocking protein synthesis by cycloheximide inhibits the formation of alcohol motivation in rats allowed free access to ethanol, water, and food.

The study of individual differences in the changes in ethanol consumption by animals after intraventricular injection of cycloheximide showed that the intensity of the inhibitory action of cycloheximide on ethanol consumption during the development of alcohol addiction depends on the degree of initial preference for ethanol by the animals. On that basis, all the rats of groups 1 and 2 were divided into animals preferring (initial mean daily ethanol consumption above 2.5 g/kg body weight) and those not preferring (initial mean daily consumption of ethanol under 2.5 g/kg body weight) ethanol.

Injection of cycloheximide into rats preferring ethanol led to a significant decrease in their ethanol consumption, which was particularly marked during the 2nd and 3rd weeks after microinjection of cycloheximide (Table 1). Meanwhile, injection of physiological saline into the animals of group 2, initially preferring ethanol, caused no change in their alcohol consumption (Fig. 1b).

In rats with an initially low level of ethanol consumption, a significantly greater decrease in ethanol consumption (on average by 90%) was observed compared with animals preferring alcohol, and it was exhibited during the first week after injection of cycloheximide (Table 1). The ethanol consumption of these rats remained at a significantly lower level in the subsequent period of observation (Fig. 1c). Meanwhile, injection of physiological saline into animals with initially low ethanol consumption did not prevent the formation of alcohol motivation. It is characteristic that, unlike animals preferring ethanol, increase their uptake by a greater degree during the formation of alcohol addiction.

These results indicate a stronger inhibitory action of cycloheximide on the development of alcohol motivation above all in animals with an initially low ethanol consumption.

Considering the close association of alcohol motivation in animals with natural biological needs — for drinking water and food [1], and also data showing the inhibitory action of cycloheximide on the realization of food-getting behavior [3], we studied interaction between a change in the consumption of alcohol, food, and water in the rats of groups 1 and 2.

It was found that after injection of cycloheximide, a decrease in ethanol consumption by the animals of group 1 was accompanied by a decrease in consumption of food and water, exhibited during the 2nd and 3rd weeks after injection of the compound. However, a similar reduction of food and water consumption during the formation of alcohol addiction also was observed in animals receiving physiological saline as the control (Table 1).

Thus, injection of cycloheximide, a blocker of protein synthesis, into the lateral cerebral ventricles of rats inhibits the formation of alcohol motivation. The intensity of the blocking action of cycloheximide on the formation of alcohol motivation was found to depend on the level of initial preference for ethanol by the rats, and it was most marked in animals with an initially low level of alcohol consumption.

LITERATURE CITED

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