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EFFECT OF CYCLOHEXIMIDE BLOCKADE OF PROTEIN SYNTHESIS IN RATS ON ALCOHOL MOTIVATION

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It has been shown that cycloheximide, which blocks ribosomal protein synthesis [2], when injected into the lateral ventricles, depresses food-motivated and defensive behavior and self-stimulation in rabbits [1, 5, 6]. Against the background of the action of cycloheximide, food-motivated behavior is restored by administration of pentagastrin [5], self-stimulation by $ACTH_{4-10}$ [1], and defensive behavior by bradykinin [6]. These findings are evidence that biological motivations are realized as the corresponding behavior as a result of expression of special protein molecules by the genome of brain neurons.

Alcohol motivations are formed in animals on the basis of biological motivations of fear, thirst, etc., when artificially replaced by the taking of ethanol [4]. It can be tentatively suggested that realization of the taking of alcohol by animals with artificially formed alcohol motivation also is determined by expression of specific protein molecules by the genome of brain neurons.

To solve this problem, in the investigation described below alcohol motivation, formed artificially in rats on the basis of water deprivation, was studied during administration of cycloheximide, a blocker of protein synthesis. In view of data in the literature pointing to the initiating role of the perifornical region of the hypothalamus in the mechanisms of alcohol motivation, formed on the basis of water deprivation in animals [3], the investigative behavior of rats and their ethanol consumption also were investigated during blockade of protein synthesis by cycloheximide, injected directly into this region.

EXPERIMENTAL METHOD

Experiments were carried out on 31 noninbred rats (males weighing 200-300 g). Alcohol motivations were formed in the rats by providing them with a 20% solution of ethanol for 4-5 months as the sole source of fluid. The rats were then transferred to individual cages and the daily consumption of 20% ethanol solution, food, and water was recorded. Animals with a formed

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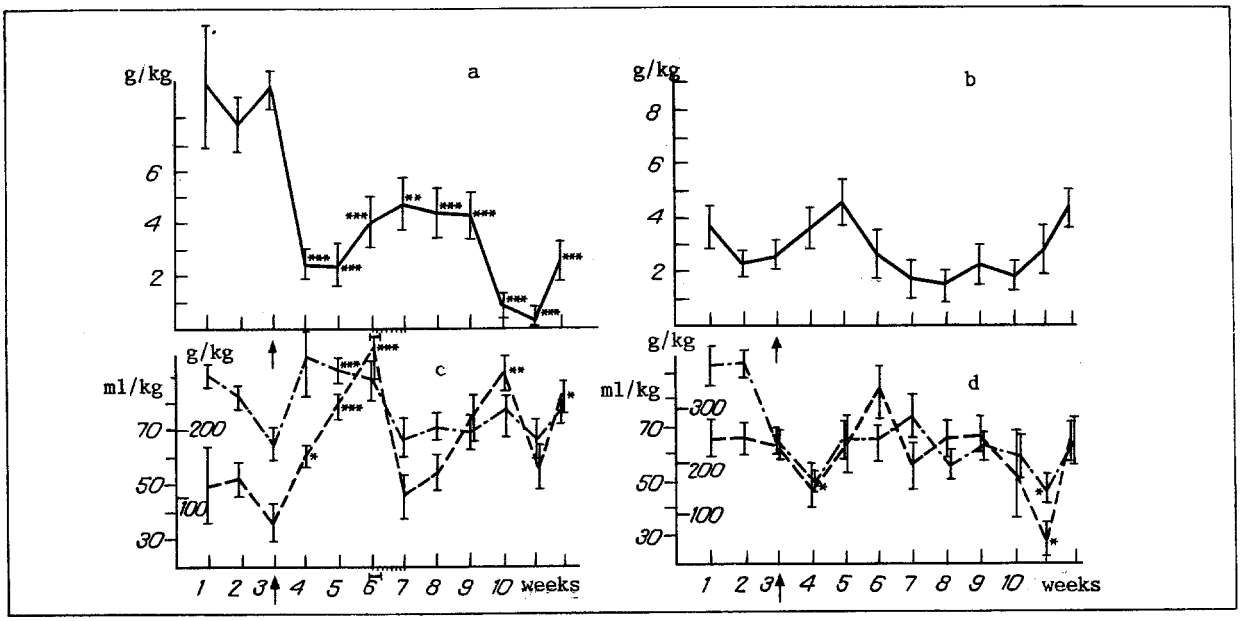

Fig. 1. Dynamics of mean daily consumption of 20% ethanol solution (a and b), food, and water (c and d) in rats with established physical dependence on ethanol (a and c) and without physical dependence on ethanol (b and d) after intraventricular injection of 10.9 μg of cycloheximide. Legend (here and subsequent figures): arrow indicates injection of cycloheximide – alcohol deprivation for 2 days. Solid line) ethanol; dashed line) water; dotted and dashed line) food.

*$p < 0.05$.

**$p < 0.01$ compared with previous week.

***$p < 0.001$ before injection of cycloheximide.

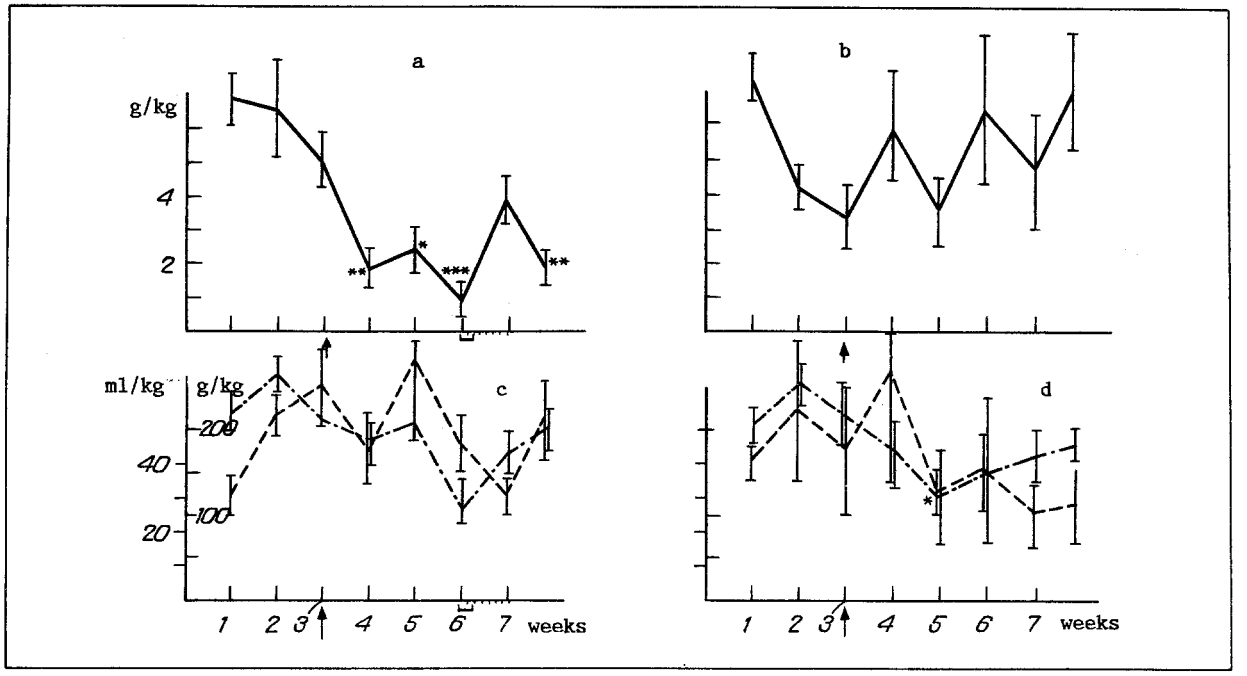

Fig. 2. Dynamics of mean daily consumption of 20% ethanol solution (a and b), food, and water (c and d) in rats with established physical dependence on ethanol (a and c) and without physical dependence on ethanol (b and d), after injection of 1.5 μg cycloheximide into perifornical region of hypothalamus.

TABLE 1. Dynamics of Food and Water Consumption by Control Rats after Injection of Cycloheximide into Lateral Ventricle or Perifornical Region of Hypothalamus

	Mode of administration	Before injection of cycloheximide			After injection of cycloheximide				
		1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Quantity of food consumed, g/kg	I/V	52,9±3,4	62,8±4,6	65,5±8,05	*45,6±5,1	61,5±4,2	60,5±4,9	58,2±3,9	51,2±3,7
	I/H	**32,25±5,6	46,9±4,8	65,2±5,9	**45,0±4,0	56,1±3,7	60,25±5,2	52,5±4,2	*49,6±4,4
Quantity of water consumed, mg/kg	I/V	43,4±4,0	38,7±3,37	43,6±4,8	42,9±5,1	39,6±4,0	40,7±4,4	38,5±3,2	41,5±4,1
	I/H	*32,9±3,7	31,0±2,9	49,1±6,01	***26,3±4,9	46,9±4,1	40,1±3,7	*38,5±3,9	40,2±2,9

Legend. *$p < 0.05$ Compared with last week before injection of cycloheximide; **$p < 0.01$, ***$p < 0.001$. I/V) Intraventricular injection, I/H) intrahypothalamic injection.

alcohol motivation, in which, given free choice, preference was expressed for ethanol consumption, were chosen for the experiments; this quantity of alcohol exceeded its narcotic doses (3.5 g/kg body weight). Dependence on ethanol was found in 12 of the 19 rats. During alcohol deprivation, on the subsequent days of observation ethanol consumption increased by 30-40%.

In the first series of experiments on 11 rats with established alcohol motivation, seven of which were found to be dependent on alcohol, after recording the dynamics of the daily consumption of 20% ethanol solution, water, and food in the animals for 2 weeks, single injection of 10.9 μg cycloheximide in 5 ml physiological saline was given through cannulas implanted into the lateral ventricles. The diameter of each cannula was 0.8 mm. The dose of cycloheximide was chosen on the basis of data of Sudakov [5], indicating that cycloheximide, if injected into the lateral ventricles in the above dose, suppressed the food behavior of rabbits. Observations on consumption of alcohol, water, and food by the rats after injection of cycloheximide continued for 4-9 weeks.

In the next series of experiments the dynamics of consumption of ethanol, water, and food in eight rats with established alcohol motivation was compared (in five of them ethanol dependence was detected) after injection of cycloheximide through cannulas implanted into the perifornical region of the hypothalamus. Cycloheximide was injected in a dose of 1.5 μg in 3 μl of physiological saline. The animals remained under observation after injection of cycloheximide into the perifornical region of the hypothalamus for 4 weeks. As the control, the dynamics of water and food consumption was studied in intact rats after injection of cycloheximide in the same doses into the lateral ventricles (six rats) and into the perifornical region of the hypothalamus (six rats).

The location of the tips of the cannulas in the lateral ventricles and perifornical region of the hypothalamus was verified against De Groot's atlas. The results were subjected to statistical analysis by determination of the arithmetic mean by Student's test.

EXPERIMENTAL RESULTS

The experiments showed that injection of cycloheximide into the lateral ventricles during the first 3 weeks of observation on six animals dependent on ethanol led to depression of ethanol consumption on average by 65-72.3%. Alcohol deprivation caused a small temporary increase in ethanol consumption in the animals of this group. Meanwhile, in the course of 9 weeks after injection of cycloheximide, ethanol consumption remained significantly depressed in these animals (Fig. 1a). In one animal with alcohol dependence, no changes were observed in ethanol consumption. During the first 3 weeks of observation on animals of this group, food consumption increased significantly by 60-87% and water consumption increased by 74-195%. With an increase in ethanol consumption caused by deprivation, consumption of food and water decreased, and then increased once again, with a decrease in the animals' ethanol consumption (Fig. 1c). In four rats not exhibiting alcohol dependence, during the 1st and 8th week of observation after administration of cycloheximide, a tendency was observed for ethanol consumption to increase, and this correlated with a marked decrease in food and water consumption on average by 25-27% and 23-50%, respectively (Fig. 1b, d).

After injection of cycloheximide into the perifornical region of the hypothalamus of eight rats, in five animals with alcohol dependence a marked decrease in ethanol consumption, on average by 51-81%, was observed during the first 3 weeks of observation. Alcohol deprivation in these animals also led to a transient increase in ethanol consumption. The ethanol consumption then again decreased (Fig. 2a). Food and water consumption in animals of this group did not change significantly. Only a tendency was noted for the water consumption to increase (Fig. 2c). In three rats, not dependent on ethanol, injection of

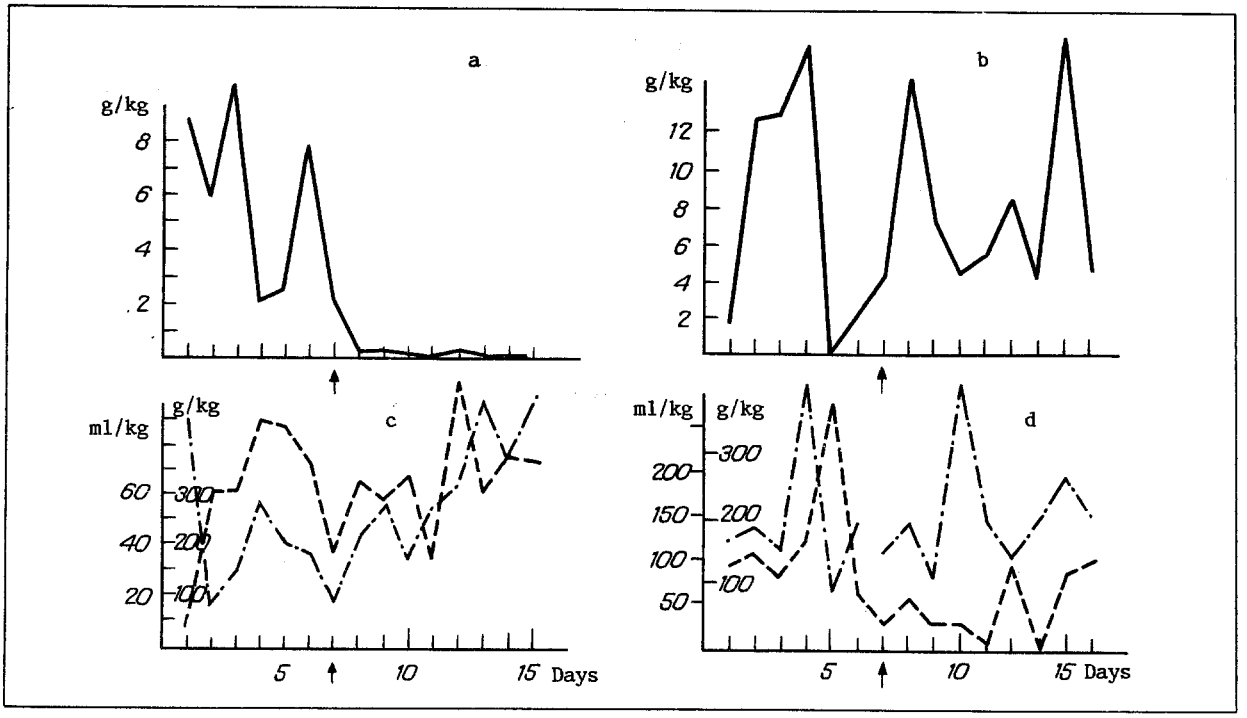

Fig. 3. Individual values of mean daily consumption of 20% ethanol solution (a and b), food, and water (c and d) in rat No. 22 (a and c) after intraventricular injection of cycloheximide in phase of reduced ethanol consumption, and in rat No. 13 (b and d) after injection of cycloheximide into perifornical region in phase of increased ethanol consumption.

cycloheximide did not change ethanol and water consumption. The food consumption of these animals was significantly reduced in the 2nd week of observation on average by 42.8% (Fig. 2b, d).

The study of the dynamics of food and water consumption of the control animals which, like the experimental rats, did not exhibit ethanol dependence, revealed a significant decrease in food consumption after injection of cycloheximide both into the lateral ventricles and into the perifornical region of the hypothalamus. Reduction of water consumption in rats of the control group was observed only after injection of cycloheximide into the perifornical region of the hypothalamus (Table 1).

As our previous investigations showed, changes in the daily ethanol consumption lasting 3-4 days were observed in rats [3].

The present investigation showed that if cycloheximide was injected into rats with established alcohol dependence, in the phase of increased ethanol consumption, its blocking action was manifested only to a weak degree. Reduction of ethanol consumption was observed on average by 12-40% during the first week of observation. After injection of cycloheximide into rats in the phase of reduced ethanol consumption, a marked blocking action of the compound on ethanol consumption was observed. A decrease in ethanol consumption in the course of the first week of observation was noted on average by 70-100% (Fig. 3).

We thus demonstrated the blocking action of cycloheximide when injected into the lateral ventricles or directly into the perifornical region of the hypothalamus on ethanol consumption in rats with experimentally established alcohol dependence.

The results indicate that an important role in the formation of alcohol motivation and dependence is played by a factor of protein nature, which begins to be expressed by the genome of the brain neurons after repeated substitution of ethanol for water reinforcement of the animals, i. e., during formation of artificial alcohol dependence. Characteristically, against this background of cycloheximide blockade of ethanol consumption, after its injection into the perifornical region of the hypothalamus and, in particular, into the lateral ventricles, reciprocal relations between ethanol consumption and food and water consumption were observed in the rats: a decrease in ethanol consumption corresponded to an increase in food and water consumption, and vice versa. In rats in which cycloheximide did not depress ethanol consumption, food and water consumption decreased. All this indicates that during blocking of alcohol motivation formed on the basis of water deprivation by cycloheximide in animals, motivation of another biological quality becomes dominant: hunger and thirst. A similar situation is observed in

"alcoholic" rats when ethanol consumption is reduced as a result of injection of several oligopeptides (antiotensin II, bradykinin, enkephalins, delta sleep-inducing peptide, etc.) or under conditions of alcohol deprivation [4].

Characteristically, the blocking action of cycloheximide is revealed more strongly in the phase of reduced ethanol consumption. The mechanism of this phenomenon we have discovered is not yet easy to explain. It can be tentatively suggested that, since reduced consumption of food and water by animals is observed in the phase of increased ethanol consumption, under these circumstances the tonic activating influence of some hypothalamic centers of these motivations on neurons in other parts of the brain is reduced, and expression of protein molecules initiating alcohol-motivated behavior is depressed.

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