ENKEPHALIN AND CYCLIC NUCLEOTIDE LEVELS IN BRAIN STRUCTURES OF RATS AT DIFFERENT STAGES OF ALCOHOL DEPENDENCE

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Acute and chronic introduction of ethanol into an animal induces profound disturbances of activity of the principal neurotransmitter systems of the brain [1, 2], and this is collectively reflected by changes in the turnover and concentrations of cyclic nucleotides (CN): cAMP and cGMP [11]. The effect of ethanol on mediator and CN metabolism in the brain depends on the dose and duration of alcoholization and on several other factors [2]. In the case of chronic alcoholization of animals the cAMP [11] and cGMP [12] concentrations most frequencly fall. Metabolism of CN and brain mediators in the various stages of formation and development of alcohol dependence has received much less study.

The most important role in the mechanism of development of ethanol dependence is ascribed to a change in the state of the endogenous opiate system and to enkephalinergic brain structures [1, 3, 13] through which the regulatory influences on other neurotransmitter systems and on CN metabolism in various parts of the brain are realized [5, 7, 9, 10]. The predominant localization of enkephalinergic neurons in the striatum, and of their nerve endings in the hypothalamus (HT) and limbic cortex (LC) of the brain [7, 9], and also the important role of these structures in the control of the emotional-motivational sphere [13, 14] and the formation of the state of dependence on ethanol [3, 23] have attracted the attention of investigators to their study. However, no combined investigations, devoted to the comparative analysis of the state of the regulatory systems of the enkephalins and cyclic nucleotides have yet been undertaken in these brain structures at different stages of the formation and development of alcohol dependence.

It was accordingly decided to study the effects of chronic alcoholization on the concentrations of enkephalins and cAMP and cGMP in the basal ganglia (BD), LC, and HT of the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on 105 noninbred rats of both sexes weighing 160-180 g at the beginning of the experiment and up to 350 g in the late stages of alcoholization. The rats were kept on a dry diet. Animals of the control group drank water and had no contact with alcohol, whereas animals of the experimental groups had access to two individual drinking bowls containing water and a 15% solution of ethanol. Experimental animals preferring ethanol were chosen in accordance with the technical recommendations of the Pharmacologic Committee, Ministry of Health of the USSR [6]. During the formation of ethanol dependence (stage I of alcoholism) animals with different degrees of alcohol motivation were selected and studied: abstinent rats drinking 5-15 ml of 15% ethanol solution per kg body weight per dm after contact for 10 days with ethanol under free choice conditions, and heavy drinking rats, consuming 30-50 ml of 15% ethanol solution per dm per killogram body weight under the same conditions. The heavy drinking rats also were investigated after 4 months of voluntary consumption of ethanol (stage II of experimental alcoholism with a formed craving for

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TABLE 1. Enkephalin Concentrations in Brain Structures of Female Rats Differing in Alcohol Motivation during Period of Formation of Craving for Alcohol (M ± m)

Parameter studied	Brain structure	Group of animals: control	heavy drinking rats	abstinent rats
LE, fmoles/mg tissue	LC	41,80±3,89	64,70±0,93**	48,00±5,08
	BG	95,10±6,0	87,80±7,86	83,30±2,85
	HT	54,50±7,86	62,80±5,92	55,50±6,94
ME, pmoles/mg tissue	LC	0,442±0,040	0,599±0,056*	0,524±0,054
	BG	1,267±0,074	0,757±0,031**	1,022±0,084*
	HT	0,655±0,033	0,531±0,074	0,637±0,058
ME/LE, per cent	LC	100	86	104
	BG	100	65	92
	HT	100	70	96

Legend. *P < 0.05 compared with control, **P < 0.001 compared with control and P < 0.05 compared with abstinent rats. Here and in Table 2, in each group there were 5 animals.

ethanol (stage II of experimental alcoholism with a formed craving for ethanol) and after 9 months of voluntary ethanol consumption (stage III of experimental alcoholism with developed physical dependence on ethanol).

The rats were decapitated, the brain quickly removed in the cold, and cooled in ice-cold water. The brain structures were quickly separated and frozen in liquid nitrogen. The tissues were homogenized for enkephalin determination in the cold in 0.1M acetic acid, containing 0.15% EDTA, and centrifuged for 10 min at 10,000 g; 20 µl of a 10% solution of the supernatant was taken for radioimmunoassay of Met- and Leu-enkephalins (LE and ME respectively), using kits from Immuno Nuclear Corp. (USA). In stage I of alcoholism the enkephalins were assayed in brain structures of female rats (in the stage of diestrus), but all other data were obtained on male rats. cAMP and cGMP concentrations were determined, after alcoholic extraction from the tissues and preliminary purification, by radioimmunoassay using kits from Amersham Corporation (England).

EXPERIMENTAL RESULTS

Concentrations of LE and ME in LC were higher in heavy drinking female rats (by 55 and 35% respectively) than in controls not consuming ethanol (Table 1). The LE concentration in LC of heavy drinking rats also was higher than in abstinent animals. A marked fall in the ME concentration was found in BG compared with the control and with abstinent rats (by 41 and 26% respectively). No change was found in the LE level in BG or in concentrations of both enkephalins in HT. The ratio between concentrations of ME and LE in the heavy drinking animals was considerably lower than in abstinent and control rats in all brain structures studied (Table 1). This ratio is evidently an important criterion for the evaluation of alcohol motivation. A fall in the ratio is evidence of increased craving of the animals for alcohol. During chronic ethanol consumption for 4 months, a fall in the ME level in LC was observed down to 26% of the control value (Fig. 1A). After 9 months of voluntary alcohol consumption no changes were found in the ME concentration in the brain structures studied. No difference in the CN concentrations compared with the control likewise were found in the brain structures of heavy drinking animals (Table 2). However the cAMP level in abstinent rats was much lower in LC, and concentrations of both CN were low in BG (Table 2).

The ratio between the cAMP and cGMP concentrations in HT was lower in the heavy drinking rats than in the control or in the abstinent rats (Table 2). The most marked changes in the CN concentrations in the rats' brain structures were observed in stage II of experimental alcoholism. The cAMP level fell appreciably in BG and HT and the cGMP level fell in HT (Fig. 1C). During chronic alcoholization of the animals for 9 months the cGMP concentration in BG fell by 65%, whereas in HT it rose by 50% (Fig. 1C).

TABLE 2. cAMP and cGMP Level in Brain Structures (in pmoles/mg tissue) of Male Rats Differing in Alcohol Motivation During Formation of Craving for Ethanol (M ± m)

CN	Brain structure		Group of animals	
	Brain	control	heavy drinking rats	abstinent rats
cAMP	LC	1,38±0,18	1,02±0,07	0,84±0,08
	BG	1,43±0,09	1,51±0,18	1,19±0,04
	HT	1,18±0,17	1,24±0,13	1,21±0,13
c GMP	LC	0,030±0,003	0,035±0,003	0,037±0,004
	BG	0,028±0,005	0,028±0,004***	0,015±0,001*
	HT	0,034±0,004	0,042±0,005	0,034±0,004
cAMP/cGMP, per cent	LC	100	63	50
	BG	100	103	150
	HT	100	85	103

Legend. *P < 0.05, **P <0.01 compared with control, ***P < 0.01 compared with abstinent rats.

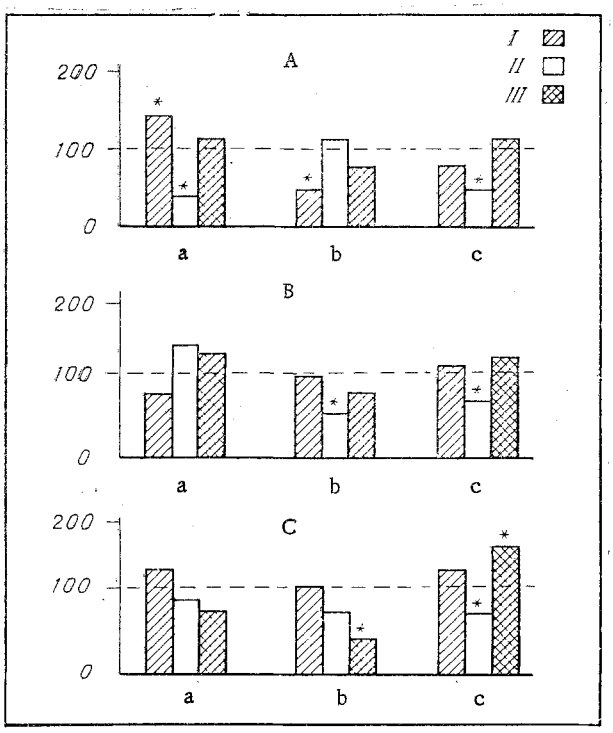

Fig. 1. Concentrations of ME (A), cAMP (B), and cGMP (C) in brain structures of male rats (in percent of control) at different stages of development of alcohol dependences: a) LC; b) BG; c) HT; I, II, III) stages of experimental alcoholism. *P < 0.05 compared with control.

Thus in animals with increased alcohol motivation changes are observed in the activity of the enkephalinergic system in the brain; a selective rise in the LE concentration in LC and a fall in the ME concentration in BG.

Considering that the highest concentration of σ-receptors, with which mainly LE interacts, is observed in the cerebral cortex, and that in the striatum ME is predominant in concentration and functional importance, having higher affinity for receptors of the μ-type [3, 7, 14], it can be tentatively suggested that animals with increased craving the ethanol consumption are distinguished by increased activity of the σ-enkephalinergic system and reduced activity of the μ-enkephalinergic system in these particular brain structures.

Similar views on increased activity of σ-receptor structures of the brain during the development of ethanol dependence have also been put forward by other workers [3].

The fall in the ME concentration, first in BG and later in LC and HT, during the development of alcohol dependence can be taken as evidence of weakening of positive emotional influence exerted by μ-opiate structures of the brain, which may cause the animal to strive to compensate for the insufficiency of the endogenous opiate system by consumption of ethanol, which can stimulate opiate receptors [10, 13]. Tolerance to the activating effect of ethanol on the positive reinforcement system, which develops gradually under these circumstances [4], ought to lead to the ever-increasing consumption of alcohol and to deepening and strengthening of the state of dependence on it. The formation of an alochol dominant focus is characterized by radical reorganization of the neurotransmitter and neurohormonal systems of the brain [1, 2], and this is accomplished by a decrease in the cAMP concentration and by contrary changes in the cAMP level in BG and HT. Changes in the CN levels in BG and HT of this kind, in agreement with data obtained by the writers previously [8], is evidence of marked disturbances of the activity of dopaminergic structures.

The results thus confirm the hypothesis of the important role of changes in the brain opiate system in the mechanisms both of the formation of alcohol motivation and of the development of dependence on alcohol. At the same time these data indicate the promising results of the search for effective antialcohol agents among compounds with a selective action on activity of μ- and σ-enkephalinergic structures in the brain.

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