EFFECT OF SINGLE AND CHRONIC ETHANOL ADMINISTRATION ON BRAIN β-ENDORPHIN CONCENTRATION OF RATS DIFFERING IN ALCOHOL MOTIVATION

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Endogenous ligands of opiate receptors may perhaps participate in the pathogenesis of alcoholism [3]. A mutual effect of opiates on the development of ethanol dependence and an effect of ethanol on the formation of morphine dependence have been found [7]. One of the mechanisms through which alcohol acts in the body may be its effect on the concentration of endogenous opiates, as has been shown in the case of neurotransmitters [9].

The object of this investigation was to determine the β-endorphin concentration after a single injection of ethanol into rats differing in their alcohol motivation and in the same animals during chronic voluntary alcoholization.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing initially 200-250 g. The animals were kept on a standard pellet diet and under natural daylight conditions. The duration of alcohol narcosis was determined by the time in the side position after intraperitoneal injection of ethanol in a dose of 4.5 g/kg. The β-endorphin concentration was determined 15 days after determination of the duration of ethanol narcosis. The effect of chronic alcoholization was studied on a model of chronic alcoholism in which animals consumed ethanol solution under conditions of free choice between water and 15% ethanol solution [1]. The alcoholized animals were kept in single cages and the level of consumption of the solution of water and ethanol was recorded daily at 9-10 a.m. Two groups of rats were chosen for the experiments: Group 1 consisted of animals consuming ethanol solution in a volume of not less than 70% of the total volume of fluid drunk (heavy drinkers), group 2 consisted of animals consuming ethanol solution in a volume of not more than 10% under the same experimental conditions (light drinkers). The animals were killed 60 min after a single intraperitoneal injection of ethanol solution in physiological saline. Control animals received an injection of physiological saline only. The rats were decapitated and the brain divided into parts by the method in [8]. Brain tissue was quickly frozen in liquid nitrogen, then weighed and placed in 0.2 M acetic acid, warmed beforehand on a waterbath. After heating on a waterbath for 5-7 min the samples were cooled on ice and homogenized. After centrifugation at 10,000g the supernatant was poured off and the residue rehomogenized in acetic acid and recentrifuged. The two supernatants were pooled and lyophilized. After lyophilization the residue was dissolved in 0.05 M Tris-HCl buffer, pH 8.0, at 4°C, containing 1% albumin and 0.05% sodium azide (RIA buffer). The samples were then centrifuged for 6 min at 8000g, frozen, and kept before use at −70°C. Before radioimmunologic analysis of the β-endorphin level the samples were thawed and again centrifuged for 5 min at 8000g. Antibodies and iodinated β-endorphin were generously provided by A. D. Dmitriev (Research Institute of Psychiatry, Academy of Medical Sciences of the USSR). Full details of the antibodies and method of iodination were described previously [5]. Crossed immunoreactivity of the serum with β-lipotropin was under 5%, and with met- and leu-enkephalins, under 0.01%. For radioimmunologic analysis 50 μl of the unknown sample or of a standard concentration of β-endorphin, antibodies against β-endorphin in a final dilution of 1:4000 and in a volume of 50 μl, and 250 μl of RIA buffer were added to siliconized test tubes. After incubation for 24 h, 9×10^3-10×10^3 cpm of [^{125}I]-β-endorphin in a

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TABLE 1. Effect of Chronic Alcoholization on β-Endorphin Concentration in Rat Brain (in fmoles/mg/mg tissue, M ± m)

Duration of contact with ethanol, months	Group of rats	Cortex	Striatum	Thalamus	Medulla + pons
3	Heavy drinkers	6,6±0,75 (4)	8,2±0,80 (3)	17,5±2,2 (4)	7,1±0,26 (3)
	Light drinkers	5,7±0,61 (4)	6,9±0,73 (4)	15,3±3,2 (4)	8,2±0,87 (4)
10	Heavy drinkers	2,2±0,22** (6)	1,9±0,2* (5)	4,7±0,65** (5)	12,9±1,48** (5)
	Light drinkers	4,6±0,6 (5)	3,0±0,4 (5)	12,2±1,6 (5)	8,5±0,74 (5)
	Heavy drinkers 24 h after deprivation of ethanol	1,2±0,1*** (3)	1,2±0,2*** (3)	5,9±0,8* (3)	17,0±3,2* (3)

Legend. Number of animals in parentheses. *P < 0.05, **P < 0.01 compared with light-drinking rats; ***P < 0.05 compared with heavy drinkers.

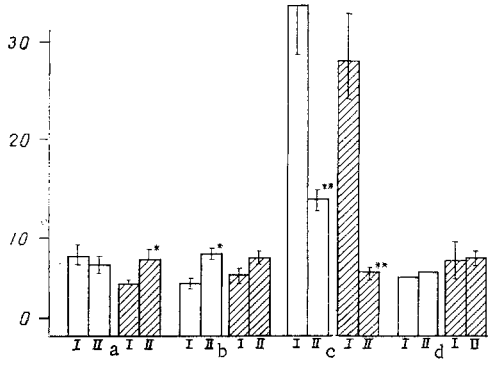

Fig. 1. Effect of a single injection of ethanol on β-endorphin concentration in animals differing in predisposition to alcohol consumption. Unshaded columns – short-sleeping rats, shaded columns – long sleepers. I) Physiological saline, II) ethanol in a dose of 2.5 g/kg. a) Cortex, b) striatum, c) thalamus, d) medulla + pons. *P < 0.05, **P < 0.01.

volume of 50 μl was added to the samples and incubation continued for a further 24 h at 4°C, after which antibodies against rabbit γ-globulin (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were added in a dilution of 1:5, together with 100 μl of rabbit γ-globulins in a concentration of 0.1 mg/ml and 200 μl of a 10% solution of polyethylene-glycol 6000. After incubation for 24 h the samples were centrifuged for 20 min at 3000g and the radioactivity of the residue determined. The β-endorphin concentration in the unknown sample was determined from the curve of inhibition of binding of labeled β-endorphin with antibodies of standard concentrations of unlabeled peptide. The sensitivity of the method was 20-25 fmoles per sample. The β-endorphin concentration in the blood plasma was determined by means of a standard kit of reagents from Amersham Corporation (England). The experimental results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

After determination of the duration of ethanol narcosis two groups of animals were selected: short sleepers, with 85 ± 9 min in the side position, and long sleepers, with 192 ± 15 min in the side position. It was shown previously that animals with a short duration of ethanol narcosis have a higher level of ethanol consumption than long-sleepers [2] and that they differ from the latter in several biochemical parameters [6] and behavioral features [10]. The experiments showed that concentrations of β-endorphin were statistically significantly higher in the cerebral cortex of the short-sleeping rats and did not differ in the striatum, thalamus, and medulla of short- and long-sleeping animals (Fig. 1). To determine the significance of these differences in realization of

the action of ethanol, the β-endorphin concentration was determined in animals of both groups after a single injection of ethanol in a dose of 2.5 g/kg. It was found that ethanol increases the β-endorphin concentration in the cerebral cortex of long-sleeping animals but does not affect the peptide concentration in this region of the brain in short-sleeping rats. There was also a marked increase in the β-endorphin concentration in the striatum of the short-sleepers. The level of the peptide in the thalamus fell very considerably in both short- and long-sleeping animals; under these circumstances the ratio of the β-endorphin concentrations in different parts of the brain also changed after injection of ethanol in the short- and long-sleeping rats. For instance, the peptide concentration was more than doubled in the thalamus of the short-sleeping rats but did not differ in the cortex, striatum, and medulla (Fig. 1). Considering that an increase in the β-endorphin concentration in the brain is characteristic of the response of the body to stress, it can be tentatively suggested that the observed increase in peptide concentration in the cerebral cortex of short-sleeping rats may reflect their greater sensitivity to stress. This also is confirmed by analysis of the concentration of the peptide in the blood plasma. In the present experiments the β-endorphin concentration in the plasma of short-sleeping rats was 207 ± 26 fmoles/ml, compared with 82 ± 16 fmoles/ml in long-sleepers. After 10 days of contact with ethanol, incidentally, the concentration of the peptide in the plasma of heavy- and light-drinking rats did not differ but was 209 ± 27 and 226 ± 59 fmoles/ml plasma respectively. Determination of the peptide concentration in the brain in chronic alcoholization likewise revealed no difference between heavy- and light-drinking animals at stage II of experimental alcoholism (Table 1), i.e., after contact with ethanol for 3 months. However, after contact with alcohol for 10 months a statistically significant decrease was found in the peptide level in the cerebral cortex, striatum, and medulla and an increase in the β-endorphin concentration was observed in the thalamus of the heavy-drinking rats. Under these circumstances, 24 h after deprivation of ethanol, when abstinence symptoms were most marked [4], a decrease was observed in the β-endorphin in the cerebral cortex and medulla, whereas a small increase in the concentration of the peptide was recorded in the thalamus (Table 1). The effects of β-endorphin are known to differ in specificity in different regions of the brain. For instance, after injection into the periaqueductal gray matter and striatum β-endorphin gives rise to sedation and muscular rigidity, whereas after injection into the third ventricle it stimulates locomotor activity [11].

The fall in β-endorphin concentration in the striatum after 10 months of contact with ethanol may thus determine the development of tolerance to the sedative effect of ethanol, whereas an increase in concentration of the peptide in the thalamus may play an important role in the pathogenetic mechanisms of the abstinence syndrome. Considering the divergent effects of ethanol when injected in a single dose, on the concentration of the neuropeptide in the thalamus and striatum of short-sleeping rats, we can postulate a differential action of ethanol on the state of the endorphinergic system in different parts of the brain in rats predisposed to ethanol consumption. The decrease in activity of this system in the striatum, medulla, and cerebral cortex is responsible for the development of tolerance to ethanol, whereas an increase in its activity in the thalamus may be connected with the formation of physical dependence on ethanol.

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