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EFFECT OF ETHANOL ON BRAIN ENKEPHALIN CONCENTRATION IN RATS WITH DIFFERENT LEVELS OF ALCOHOL MOTIVATION

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UDC 612.822.1:[547.95:547.94].014.46:547.262

KEY WORDS: alcoholism; enkephalins; parts of the brain.

Current hypotheses put forward to explain interaction between alcohol and opiates in the body accept both the possibility that substances with opiate activity may be formed in response to administration of ethanol and the direct action of alcohol on functional activity of the enkephalinergic system [5]. To investigate the presence of common mechanisms of action of ethanol and opiates it is necessary to study the effect of ethanol on the concentrations of met- and leu-enkephalin during chronic alcohol intake. The aim of the present investigation was therefore to measure concentrations of enkephalins at different stages of development of chronic experimental alcoholism and in rats with different alcohol motivation.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing initially 200-250 g. The duration of alcohol narcosis was determined by the time in the side position after intraperitoneal injection of 25% ethanol solution in a dose of 4.5 g/kg. The peptide concentrations were measured 15 days after determination of the duration of ethanol narcosis. Chronic alcoholization of the animals was carried out by allowing them free choice between water and 15% ethanol solution. During the period of daily determination of alcohol intake the animals were kept in individual cages. Rats alcoholized for 10 days and 3 and 10 months were used in the experiments. A state of abstinence was simulated by depriving rats of ethanol for 24 h after 10 months of alcoholization. At each stage of chronic experimental alcoholism two groups of animals were selected: one group consisted of rats consuming ethanol in a volume of not more than 10% of the total fluid intake (light drinkers), the other of animals consuming not less than 70% of ethanol solution (heavy drinkers). The animals were decapitated and the brain divided into parts by the method in [8]. Brain tissue was quickly frozen in liquid nitrogen, weighed, and placed in 0.1 M acetic acid, previously warmed, treated on a water bath for 5-7 min, cooled on ice, and homogenized in a homogenizer with Teflon pestle. The samples were then centrifuged at 10,000g and the residue was again homogenized in acetic acid and centrifuged. The two supernatants were pooled and lyophilized. The residue was dissolved in 50 mM Tris-HCl buffer, pH 8.0, containing 0.05% bovine serum albumin and 0.05% sodium azide (RIA buffer). The samples were centrifuged for 5 min at 8000g, divided into aliquots, and frozen. Concentrations of enkephalins were determined radioimmunologically. Antibodies and iodinated peptides were

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 48-51, July, 1983. Original article submitted December 13, 1982.

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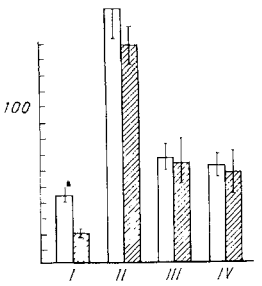

Fig. 1

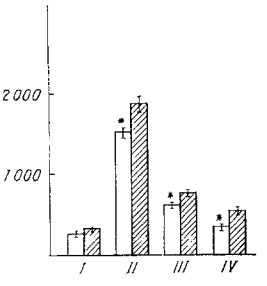

Fig. 2

Fig. 1. Concentrations of leu-enkephalin (in fmole/mg tissue) in rats differing in predisposition to ethanol consumption. Unshaded columns – short-sleeping rats, shaded columns – long sleepers. I) Cortex; II) striatum; III) thalamus; IV) medulla + pons.

Fig. 2. Concentrations of met-enkephalin (in fmole/mg tissue) in rats differing in predisposition to ethanol consumption. Legend as to Fig. 1.

generously supplied by A. D. Dmitriev (Institute of Psychiatry, Academy of Medical Sciences of the USSR). For radioimmunologic analysis (RIA) antiserum against met-enkephalin was taken in a final dilution of 1:2000, and antiserum against leu-enkephalin in a final dilution of 1:5000. To each sample were added 10 μl of a standard solution of peptides or the unknown sample, 100 μl of iodinated peptide (10,000 cpm), and 100 μl antiserum and the volume was made up to 0.5 ml with RIA buffer. Incubation proceeded for 24 h, after which the antigen–antibody complex was precipitated with ammonium sulfate and centrifuged for 30 min at 3000g. The radioactivity of the precipitates was determined. The concentrations of peptides in the unknown sample were determined by means of an inhibition curve. The sensitivity of the method for leu-enkephalin was 15-20 fmole per sample and for met-enkephalin 100-120 fmole per sample. Crossed immunoreactivity between met- and leu-enkephalins did not exceed 1.5%. The experimental results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The duration of ethanol narcosis, reflected in the time in the side position, is a convenient indicator for distinguishing between animals differing in their attitude toward ethanol consumption [2]. For instance, rats with a short duration of ethanol sleep (short-sleepers – 81 ± 14 min in our experiments) are characterized by high alcohol motivation, whereas animals with a long duration of alcohol sleep (long sleepers, 195 ± 24 min) are characterized by a low tendency toward alcohol consumption. Determination of the enkephalin concentrations in long- and short-sleepers showed that the leu-enkephalin concentration in the cerebral cortex of the short-sleeping rats was more than twice as high as in long-sleepers (Fig. 1); in the remaining regions of the brain studied no difference could be found in the peptide concentrations between the two groups of animals. The met-enkephalin concentration, on the other hand, did not differ in the cerebral cortex of long- and short-sleepers but was significantly reduced in the striatum, thalamus, and medulla of the short-sleepers (Fig. 2). Such specific differences in peptide concentrations between the two groups of animals, in our opinion, may be attributable to differences in functions of the enkephalins in different parts of the brain. In the cerebral cortex, for instance, where opiate receptors of delta-type are most frequently found, exogenous enkephalins may induce myoclonic convulsions and epileptiform and enhanced motor activity [6, 7], whereas in the thalamus and striatum they give rise to the development of analgesia, catalepsy, and hypothermia. The low met-enkephalin concentrations which we found in these parts of the brain of short-sleeping rats reflect a deficiency of these functional systems in these animals, whereas the increased leu-enkephalin concentration in the cerebral cortex of the short-sleeping rats can explain the greater predisposition to stress found in the animals of this group. In addition, these differences may be of significance in the pathogenesis of alcohol intoxication.

To test this hypothesis we investigated the enkephalin concentrations at different stages of experimental alcoholism, distinguished by the use of a model developed in the writers' laboratory [1]. In stage I, when an inclination for ethanol consumption is being formed no differences were found in leu-enkephalin concentrations

TABLE 1. Effect of Chronic Alcoholization on Enkephalin Concentrations (in fmoles/mg tissue) in Rats' Brain (M ± m)

Peptide	Duration of contact with ethanol	Group of rats	Cortex	Striatum	Thalamus	Medulla and pons
Leu-enkephalin	10 days	Heavy drinkers	37,3±6,3 (4)	296±24 (4)	99,2±5,6 (4)	90,5±15,7 (4)
		Light drinkers	35,0±6,5 (4)	222,0±26,8 (4)	131,8±22,7 (4)	134±22 (4)
	3 months	Heavy drinkers	35,1±1,2** (3)	105,5±11,8 (3)	55,0±12,1 (3)	81,5±3,8 (3)
		Light drinkers	58,5±3,2 (3)	150,0±19,8 (3)	63,0±10,4 (3)	93,5±10,7 (3)
	10 months	Heavy drinkers	50,5±2,1** (6)	102±13,4** (6)	82,5±5,2** (6)	87,5±3,7** (6)
		Light drinkers	29,5±3,1 (6)	192±11,3 (6)	123,2±4,9 (6)	111,5±2,1 (6)
		Heavy drinkers after withholding ethanol for 24 h	41,6±6,2 (3)	173,0±12,1*** (4)	866,5±10,4* (4)	94,0±1,3** (3)
Met-enkephalin	10 days	Heavy drinkers	450±23 (4)	2540±126* (4)	930±23 (4)	940±77 (4)
		Light drinkers	444±13 (4)	3200±180 (4)	1210±125 (4)	1130±98 (4)
	3 months	Heavy drinkers	370±16 (3)	750±40 (3)	470±35 (3)	540±32 (3)
		Light drinkers	310±20 (3)	610±76 (3)	495±11 (3)	600±55 (3)
	10 months	Heavy drinkers	450±30 (6)	630±14** (6)	400±35** (7)	485±28** (7)
		Light drinkers	390±38 (5)	1250±155 (7)	850±56 (6)	810±48 (6)
		Heavy drinkers after withholding ethanol for 24 h	475±27 (3)	950±80 (3)	530±72** (3)	640±72 (3)

Legend. Number of animals shown in parentheses. *P < 0.05, **P < 0.01 compared with light drinkers, ***P < 0.05 compared with heavy drinkers.

between light and heavy drinking animals in all parts of the brain studied, whereas the met-enkephalin concentration was lowered in the striatum of the heavy drinkers. Meanwhile a considerable increase in the concentrations of both peptides was found in animals after 10 days of alcoholization compared with their level in rats with an initially different predisposition toward ethanol consumption, which may have been due to stress produced in the animals by placing them in individual cages. After 3 months of alcoholization, in stage II of experimental alcoholism, a decrease in the leu-enkephalin concentration was observed in the cerebral cortex of the heavy-drinking rats (Table 1). The most marked changes in concentrations of the peptides were found after 10 months of alcoholization, when physical dependence on ethanol was formed [1, 3]. The concentrations of leu- and met-enkephalins in the striatium, thalamus, and medulla of the light drinkers were reduced in this case (Table 1).

In our view the fall in concentrations of the neuropeptides after long-term alcoholization may be instrumental in one of the mechanisms of formation of ethanol dependence. In a state of abstinence, after withdrawal of ethanol for 24 h, the leu-enkephalin concentration rises in the striatum whereas in the thalamus and medulla the concentration of this peptide remains much lower in animals in a state of abstinence than in the light-drinking rats (Table 1). The fall in the concentrations of met- and leu-enkephalins in the striatum, thalamus, and medulla may be due to exhaustion of the compensatory powers of the enkephalinergic system in these regions of the brain, which is activated by ethanol. A similar decrease in concentration during prolonged alcoholization also was found for catecholamines [4], and enkephalins are known to be modulators of activity of noradrenergic and dopaminergic neurons [10]. It can be tentatively suggested that exhaustion of the catecholamine reserves in the brain is due to depression of functional activity of the enkephalinergic system. Meanwhile the increase in leu-enkephalin concentration which we found in the cerebral cortex in stage III of experimental alcoholism and the increased concentration of this peptide in rats predisposed to ethanol consumption indicate the possibility of differential changes in activity of the enkephalinergic system in different parts of the brain. There is reason to suppose that these differences are linked with the existence of two enkephalinergic systems with different functions in the brain. It has been shown that leu-enkephalin interacts mainly with δ-receptors, whose concentration is highest in the cerebral cortex [9]; whereas in the striatum and thalamus the highest concentration of μ-opiate receptors has been found; the possible endogenous ligand for μ-receptors is met-enkephalin [4, 9]. The results now obtained thus suggest an increase in activity of the δ-enkephalinergic system and a decrease

in activity of the μ-enkephalinergic system in the course of chronic alcoholization, and these changes may play an important role in the development of dependence on ethanol.

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MOTOR ACTIVITY AND NEUROACTIVE AMINO ACID CONCENTRATIONS IN BRAIN TISSUES

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UDC 612.822.1:547.466]-06:612.766

KEY WORDS: neuroactive amino acids; gamma-aminobutyric acid; cerebral blood flow.

The discovery of gamma-aminobutyric acids (GABA) and enzymes of its metabolism in the walls of the cerebral blood vessels [4-6] and the discovery of changes in neuroactive amino-acid levels in cerebrovascular insufficiency [1, 7] led to the establishment of a link between elevation of the GABA concentration in the brain and its vessels and manifestations of compensation of disturbances of the cerebral hemodynamics. Another basis for this conclusion was the results of experiments [2, 5] which demonstrated the dynamics of changes in concentrations of neuroactive amino acids in brain tissues and vessels with age and also under the influence of vasoactive drugs.

The object of the present investigation was to study the effect of forced motor activity on quantitative changes in neuroactive amino-acid levels in the brain tissues of normal rats and rats with experimental disturbances of the cerebral blood flow. For comparison, changes in neuroactive amino-acid levels also were investigated in the cerebrospinal fluid (CSF) of cats.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature rats of both sexes (120 animals) weighing 180-240 g, under ether anesthesia, and on 18 cats weighing 3-4 kg, under pentobarbital anesthesia (50 mg/kg, intraperitoneally).

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 7, pp. 51-54, July, 1983. Original article submitted January 31, 1983.