CONTENT OF DELTA-SLEEP-INDUCING PEPTIDE IN THE BRAIN OF RATS WITH DIFFERENT LEVELS OF ALCOHOL MOTIVATION

Yu. V. Burov, R. Yu. Yukhananov, and A. I. Maiskii

UDC 616.89-008.441.13-092.9-07:616.154.95:547.262]-033.1

KEY WORDS: delta-sleep-inducing peptide; rat brain; alcohol motivation; experimental alcoholism; ethanol.

One of the endogenous regulators of circadian rhythms and of the functional state of the serotoninergic, noradrenergic, and dopaminergic systems of the brain is the delta-sleep-inducing peptide (DSIP) [4, 6]. In turn, these neurotransmitters evidently take part in the formation of fondness for and dependence on ethanol [1, 3, 5].

The aim of the investigation described below was accordingly to study the DSIP content in different parts of the brain of rats predisposed and not predisposed to the development of experimental alcoholism, and changes in its content under the influence of a single dose of alcohol.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats, weighing initially 200-250 g. The duration of alcohol anesthesia was determined from the time taken for the animal to adopt the side position after intraperitoneal injection of a 25% solution of ethanol in a dose of 4.5 g/kg body weight. An initial predisposition toward ethanol consumption of individuals in the population of rats was determined by the method in [2]. The neuropeptide level in these experiments was measured in the brain 10 days after testing. It was shown previously that animals with a short period of sleep as a rule had a higher level of ethanol consumption and a higher rate of elimination of alcohol from the blood [2]. They could thus be characterized as predisposed to develop experimental alcoholism. The DSIP concentration after a single intraperitoneal injection of ethanol in doses of 1.0, 2.5, and 4.5 g/kg was determined in the general population of animals 30 min after injection. Antibodies against DSIP, synthesized in the Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, were obtained by immunizing rabbits with a conjugate of this peptide and ovalbumin together with Freud's adjuvant. The conjugate, formed with the aid of glutaraldehyde, contained 9-10 molecules of neuropeptide to 1 ovalbumin molecule. The antibody titer was determined by Ouchterlony's immunodiffusion method and by binding tritiated DSIP. For radioimmunologic analysis (RIA), ^{125}I-tyr^{6}-DSIP (7×10^3 to 10×10^3 cpm) was used; it was iodinated by means of lactoperoxidase and hydrogen peroxide. The ^{125}I-labeled preparation was purified on a column with DEAE-cellulose in a molarity gradient of ammonium acetate buffer. The specific activity of the compound thus obtained was 1 Ci/μmole. RIA was carried out in 50 mM Tris-buffer, pH 8.0, containing 0.05% bovine serum albumin and 0.05% sodium azide. Each sample, containing ^{125}I-tyr^{6}-DSIP, and 0.1 ml of a solution of DSIP or of the test material, was added to serum containing antibodies against DSIP in a final dilution of 1:25,000. Incubation was carried out at 4° for 42 h. The labeled analog bound with antibodies was separated by the addition of 0.1% α-globulin solution and 0.6 ml of saturated ammonium sulfate followed by centrifugation for 30 min at 3000g and 4°C. Radioactivity of the residue was measured in γ-flasks on an SL-9000 counter. For quantitative determination of DSIP a calibration curve of displacement of ^{125}I-tyr^{6}-DSIP by the unlabeled analog was plotted. The DSIP content in the unknown sample was determined on the graph between semilogarithmic coordinates, where the logarithm of the standard DSIP concentration was plotted along

Laboratory for the Search for and the Study of Substances for Prevention and Treatment of Drug Addictions, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (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. 94, No. 9, pp. 67-70, September, 1982. Original article submitted April 8, 1982.

 0007-4888/82/9409-1240$07.50

TABLE 1. Effect of Ethanol on DSIP Concentration in Rat Brain (in femtomoles/mg tissue)

Experimental conditions	Cerebral cortex	Medulla	Thalamus	Striatum
Rats with different durations of ethanol anesthesia:				
short sleepers	0,94±0,14* (5)	1,32±0,19 (5)	1,18±0,5 (5)	0,85±0,24* (5)
long sleepers	1,23±0,10 (5)	1,36±0,28 (5)	0,85±0,33 (5)	1,23±0,14 (5)
Single injection of ethanol in different doses:				
physiological saline	1,21±0,25 (4)	1,46±0,3 (4)	1,33±0,29 (4)	0,99±0,22 (4)
ethanol:				
1 g/kg	2,73±1,73 (4)	3,25±0,26* (4)	3,35±0,41* (4)	2,64±0,52* (4)
2,5 g/kg	2,6 ±0,9 (3)	3,33±1,2* (4)	2,51±0,13* (4)	2,4 ±0,33* (4)
4,5 g/kg	1,24±0,35 (4)	2,03±0,34* (4)	1,94±0,15 (3)	1,27±0,34 (4)
Rats with different periods of exposure to ethanol under free choice conditions				
10 days:				
heavy drinkers	1,0 ±0,23 (4)	1,1 ±0,31 (4)	1,04±0,07 (3)	0,75±0,31 (4)
light drinkers	1,03±0,47 (4)	1,68±0,4 (4)	1,15±0,05 (4)	1,35±0,21 (4)
2 months:				
heavy drinkers	0,92±0,18 (4)	1,16±0,33 (3)	0,8 ±0,41 (3)	1,62±0,29 (4)
light drinkers	1,2 ±0,3 (4)	1,4 ±0,17 (5)	0,9 ±0,37 (3)	1,31±0,24 (3)
12 months:				
heavy drinkers	0,75±0,42 (7)	0,68±0,35* (7)	0,57±0,07* (7)	0,54±0,12* (7)
heavy drinkers 24 h after ethanol deprivation	0,69±0,23* (3)	0,72±0,28 (3)	0,84±0,16 (3)	0,61±0,11* (3)
light drinkers	1,45±0,38 (7)	1,23±0,13 (8)	1,3 ±0,4 (8)	1,0 ±0,08 (8)

*Statistically significant differences, values given represent x ± σ, number of animals in parentheses.

the abscissa and the percentage of binding along the ordinate. The quantity of ^{125}I-tyr^{6}-DSIP in the sample in the absence of unlabeled analog was taken as 100%. To determine the DSIP concentration in the unknown sample the degree of binding was determined as a percentage of maximal and the equivalent quantity of DSIP was read off on the graph. The method is able to determine as little as 5 femtomoles DSIP in the sample, and the region of greatest confidence is that from 20 to 200 femtomoles, i.e., from 80 to 20% binding. Verification of the specificity of the antiserum with several other peptides shows a very low percentage of cross reactions, as a rule not more than 0.01%. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Analysis of the duration of alcohol anesthesia in the rats revealed individuals with a short period of stay in the side position (85.3 ± 19.1 min), and these were described as short sleepers, whereas those with a longer period of ethanol anesthesia (191.7 ± 27.3 min) were called long sleepers. Measurement of the DSIP concentration in homogenates of whole brain showed that the short sleepers had a significantly lower level of this neuropeptide (0.69 ± 0.15 femtomole/mg tissue) compared with the long sleepers (1.16 ± 0.31 femtomole/mg tissue) ($P < 0.05$). Determination of its concentration in different parts of the brain showed that in the cerebral cortex of the short sleepers it amounted to 0.94 femtomole/mg tissue, which is significantly lower than in rats with a long period of alcohol sleep (Table 1). A lowered DSIP level also was found in the striatum of the short sleepers. Meanwhile the differences found in other parts of the brain were not statistically significant.

Definite changes in the DSIP concentration also were observed after a single injection of ethyl alcohol in rats without preliminary determination of alcohol motivation. The maximal increase in the neuropeptide level was observed 30 min after injection of ethanol in a dose of 1 g/kg body weight (Table 1). The concentration of the neuropeptide in the striatum of these animals was increased by 167% and in the thalamus by 152%, whereas the increase in the medulla was rather less. Injection of ethanol in a dose of 2.5 g/kg was accompanied by a marked increase in the DSIP concentration in the medulla and also by elevation of its level in the striatum and thalamus. A single injection of ethanol in a dose of 4.5 g/kg caused only a small rise in the concentration of the neuropeptide in the medulla.

The differences observed in the changes in DSIP concentration on the brain under the influence of ethanol in rats which differed in the duration of ethanol anesthesia and also after a single injection of ethyl alcohol served as the starting point for a study of the concentration of the neuropeptide during chronic voluntary consumption of 15% ethanol solution over a period of time. Since a population of noninbred albino rats includes individuals with different levels of ethanol consumption [2], two groups of animals were selected for the experiments. One group consisted of rats consuming 6.2 ± 1.4 ml/kg body weight of 15% ethanol solution daily (little drinkers), the other group consisted of big drinkers, with a mean daily alcohol consumption of 56.3 ± 8.7 ml/kg. The study of the dynamics of changes in the DSIP concentration in animals with different lengths of exposure to alcohol under free choice conditions showed that during the first 2 months of alcoholization there was no difference between the concentrations of the neuropeptide in the various brain regions in the heavy and light drinkers. This first can be regarded as reflecting a compensatory rise in the DSIP level in rats predisposed to the development of experimental alcoholism. In fact, during subsequent alcoholization, when physical dependence on ethanol was formed, the compensatory system was exhausted and the DSIP concentration fell in the striatum, medulla, thalamus, and cerebral cortex (Table 1). It may be that the increase in the concentration of the neuropeptide after injection of a low dose of ethanol is responsible for the tranquilizing effect of the latter. The fall in the DSIP concentration, however, during prolonged chronic alcoholization of rats may play a role in the disturbance of the sleep structure which accompanies the development of alcoholism.

The authors are grateful to V. V. Rozhanets (Institute of Pharmacology, Academy of Medical Sciences of the USSR), with whom the method of determination of DSIP was worked out jointly.

LITERATURE CITED

1. I. P. Anokhina, Drug Alcohol Depend., 4, 215 (1979).
2. Yu. V. Burov, T. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
3. Yu. V. Burov and I. V. Viglinskaya, Byull. Éksp. Biol. Med., No. 6, 689 (1981).
4. M. Graf, H. Christen, H. J. Tobler, et al., Experientia, 37, 624 (1981).
5. S. Liljeguist, Behavioral and Biochemical Effects of Chronic Ethanol Administration, Gothenburg (1979).
6. D. Schneider-Helmert, F. Gnirss, M. Monnier, et al., Int. J. Clin. Pharmacol. Ther. Toxicol., 19, 341 (1981).