EFFECT OF ACUTE AND CHRONIC ALCOHOL INTOXICATION ON ENKEPHALINASE A ACTIVITY IN RAT BRAIN

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UDC 616.89-003.441.13-092.9-07:616.831-008.939.5:547.943]-074

KEY WORDS: enkephalinase A; alcohol intoxication; rat brain; enkephalins.

Data showing that the opiate systems of the brain participate in pharmacological effects following administration of alcohol has recently been published [3, 14]. However, the concrete mechanisms of involvement of these systems in the development of acute and chronic alcohol intoxication have not yet been discovered. There is reason to suppose that the enzyme enkephalinase A participates in the pathogenesis of alcohol intoxication. This membrane-bound enzyme splits enkephalins (Tyr-Gly-Phe-Leu/Met) at the Gly-Phe bond. We know that it is activated during chronic administration of morphine and that it perhaps causes degradation of enkephalins within the synaptic space [12].

To study the role of enkephalinase A in the development of alcohol intoxication the activity of this enzyme was studied in the midbrain (including the hypothalamus) and striatum of rats during acute and chronic administration of ethanol to the animals.

EXPERIMENTAL METHOD

Noninbred male rats weighing 80-120 g were used. Animals of group 1 (n = 30) received an intraperitoneal injection of a 20% solution of ethanol in 0.9% NaCl. After 15 min, when the ethanol concentration in the midbrain and hypothalamus reached a maximum, [2], the rats were decapitated. The control animals of this group (n = 6) received an intraperitoneal injection of 1 ml of 0.9% NaCl.

The animals of group 2 received ethanol with the diet for 6 weeks: the experimental (n = 7) and control (n = 8) rats of this group received a diet of equal calorific value. The animals of group 2 were decapitated after 6 weeks.

The midbrain (including the hypothalamus) and corpus striatum were removed by the method in [10] and frozen at −70°C. The membrane fraction was obtained by the method in [4] and preserved at −70°C.

Enkephalinase A activity was measured (25 min, 30°C) by quantitative determination of [^{3}H]-Tyr-Gly-Gly, using thin-layer chromatography to separate products of enzymic degradation of [^{3}H]-Leu-enkephalin [4]. The experimental samples (final volume 50 µl) contained 600 nM [^{3}H]-Leu-enkephalin, 0.5 mg/ml protein of membrane suspension, and 10^{-3} M puromycin in 10 mM Tris-HCl buffer, pH 7.7. To determine the contribution of the angiotensin-converting enzyme to the recorded activity, 10^{-4} M of Gly-Gly-Phe-Met, an inhibitor of enkephalinase A [9], also was added to the control samples. The reaction was stopped by the addition of 5 µl of 0.2 M HCl. Thin-layer chromatography of the reaction product was carried out on silica-gel-60 plates with a layer 0.2 mm thick (from Merck, West Germany) in a system of ethyl acetate—isopropanol—acetic acid—water (40:40:1:18). The reaction mixture was applied to the lane in a volume of 10 µl. Markers were chromatographed simultaneously and developed with fluorescamine [5]. The developed markers had the following R_f values: Leu-enkephalin 0.54, Tyr-Gly-Gly 0.16, Tyr-Gly 0.24, Tyr 0.34. After chromatography zones corresponding to the markers were cut out and their radioactivity was measured in toluene scintillator. Data for radioactivity of [^{3}H]-Tyr-Gly-Gly were recalculated for its content in the samples and expressed

Laboratory of Neurochemistry of Alcoholism and Drug Addiction, V. P. Serbaskii All-Union Research Institute of General and Forensic Psychiatry, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 6, pp. 84-85, June, 1983. Original article submitted November 2, 1982.

 0007-4888/83/9506-0822$07.50

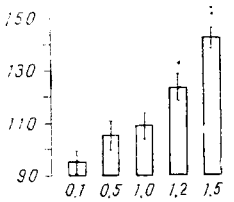

Fig. 1. Effect of a single intraperitoneal injection of ethanol on enkephalinase A activity in rat midbrain and hypothalamus. Abscissa, dose of ethanol (per kg body weight); ordinate, enkephalinase A activity (in % of control); 100% = 2.130 ± 0.096 unit/mg protein in sample. Results of six experiments are shown. *P < 0.01, **P < 0.001 compared with control.

TABLE 1. Effect of Chronic Alcohol Poisoning on Enkephalinase A Activity (in units/mg protein) in Rat Brain (M ± m)

Part of brain	Experiment (n = 14)	Control (n = 16)
Midbrain and hypothalamus	1,304±0,044**	1,024±0,036
Striatum	3,538±0,132*	3,186±0,064

Legend. *P < 0.05, **P < 0.001 compared with control; n) number of measurements.

in moles. Activity was determined as the difference between the quantity of the tripeptide in the experimental and control samples. The unit of activity of enkephalinase A was taken to be that quantity of it which, under the conditions described above, formed 10^{-12} moles of [^{3}H]-Tyr-Gly-Gly in 1 min. Protein was determined by the method in [8].

The following reagents were used: [^{3}H]-Leu-enkephalin (39 Ci/mmole, from Amersham Corporation, England); Tyr-Gly-Gly (synthesized and generously supplied by V. F. Pozdnev, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR); Tyr-Gly and Leu-enkephalin (from Serva, West Germany); fluorescamine, Tris, and puromycin (from Sigma, USA); the remaining reagents were of Soviet origin with the highest possible degree of purity.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Dependence of enkephalinase A activity of the combined fraction of rat midbrain and hypothalamus on the dose of ethanol is shown in Fig. 1. A single injection of ethanol caused dose-dependent activation of enkephalinase A and the effect was maximal with a dose of 1.5 g/kg of ethanol. The ethanol concentration in the midbrain and hypothalamus of the rats after one such injection was 0.2-10 mM [2]. The presence of alcohol in the brain tissue in such a high concentration may lead to changes in the physicochemical properties of neuronal cell membranes [7]. The increase in enkephalinase A activity thus found could be the result of a disturbance of the state of the lipid microenvironment of the enzyme. However, the possibility of the existence of other mechanisms of enzyme activation cannot be ruled out, including through modification of opiate receptors, such as is observed during acute and chronic ethanol administration [13].

It will be clear from Table 1 that during chronic alcohol administration for 6 weeks activation of enkephalinase A also was observed in the parts of the brain tested (by about 25% in the combined fraction of midbrain and hypothalamus and by 10% in the corpus striatum). The amount of enkephalinase A activation during chronic alcohol poisoning, however, was considerably less than during acute ethanol administration, probably as a result of adaptive changes

in the body (in particular, in the enkephalinergic synapses) in response to the long-term action of ethanol.

Chronic administration of alcohol to rats is known to induce a marked decrease in the Met-enkephalin concentration in their midbrain and striatum [11]. Activation of enkephalinase A found in these parts of the brain in the present experiments may therefore be one cause of the lowering of the enkephalin concentration in chronic alcohol poisoning. Considering modern views on the special importance of injuries to structures of the midbrain and hypothalamus in the development of dependence on and tolerance to alcohol [1], the much greater activation of the enzyme (in chronic alcohol poisoning) in the combined fraction of midbrain and hypothalamus than in the corpus striatum of the rats also is interesting.

The authors are grateful to Dr. Chem. Sci. L. M. Ginodman (M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR) for valuable remarks during the course of this investigation and of its discussion.

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