MECHANISM OF ACTION OF ETHANOL ON RAT BRAIN ENKEPHALINASE A ACTIVITY

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In recent times research workers have paid the closest attention to mechanisms of the action of ethanol on brain opiate systems. Administration of alcohol to animals is known to lead to changes in the enkephalin concentration in different parts of the brain and to disturb the binding characteristics of opiate receptors [5, 14]. The writers showed previously that in acute and chronic ethanol consumption by animals there is a significant increase in enkaphalinase A activity in the striatum, hypothalamus, and mesencephalon. It has been suggested that disturbances of enkephalin degradation play the key role in realization of the action of ethanol on the enkephalinergic system of the brain [1, 2].

It can be tentatively suggested that ethanol activates enkephalinase A either directly, by its effect on the enzyme molecule or the membrane microenvironment, or indirectly through its metabolic products, including those active against opiate receptors. The aim of this investigation was to study this problem.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-200 g were used. The animals received ethanol with their diet for six weeks; experimental (7 rats) and control (8 rats) groups received a diet of equal calorific value [8].

The rats were decapitated. The mesencephalon (including the hypothalamus) was isolated by the method in [12] and frozen at −70°C.

The membrane fraction was obtained by the method in [6] and kept at −70°C.

Enkephalinase A activity was measured by quantitative determination of ^{3}H-Tyr-Gly-Gly, using thin-layer chromatography [1] to separate products of enzymic hydrolysis of ^{3}H-Leu-enkephalin. The experimental samples (final volume 50 μl) contained 600 nM of ^{3}H-Leu-enkephalin, 0.5 mg/ml of protein of membrane suspension, 10^{-3} M puromycin, in 10 mM Tris HCl buffer, pH 7.7. To determine the contribution of angiotensin-converting enzyme to recorded activity in the control samples, 10^{-4} M Gly-Gly-Phe-Met, an inhibitor of enkephalinase A [11], also was added. Samples were incubated at 30°C. The reaction was stopped by the addition of 5 μl of 0.2 M HCl. Thin layer chromatography of the reaction products was carried out on silica-gel 60 plates with a layer 0.2 mm thick (Merck, West Germany, No. 5748) in a system of ethyl acetate:isopropanol:acetic acid:water (40 : 40 : 1 : 19). Markers were chromatographed concurrently and developed with fluorexamine [7]. After chromatography, zones corresponding to the markers were cut out and their radioactivity measured in toluene scintillator. Values of radioactivity of ^{3}H-Tyr-Gly-Gly were converted to its quantity in the samples and expressed in moles. Activity was determined as the difference between the quantity of tripeptide in the experimental and control samples. The unit of activity of enkephalinase A was taken to be that amount of it which, under the conditions described above, forms 10^{-12} mole of ^{3}H-Tyr-Gly-Gly per minute.

The effect of different substances on enkephalinase A activity was studied by adding them to the reaction mixture 15 min before addition of the membrane preparation.

Protein was determined by the method in [10].

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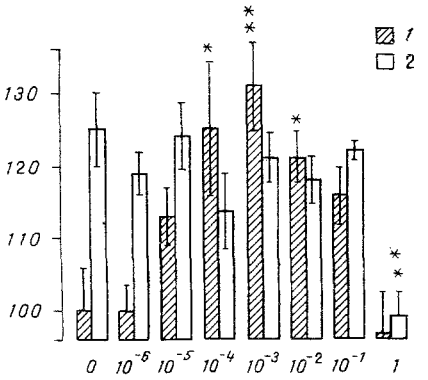

Fig. 1. Effect of ethanol on enkephalinase A activity in rat mesencephalon and hypothalamus in vitro. 1) Enzyme preparation isolated from brain of control group of animals; 2) experimental group. Abscissa, ethanol concentration in vitro (in M); ordinate, enkephalinase A activity, in percent of activity of enzyme preparation of control animals in absence of ethanol in incubation mixture; 100% equals 1.055 ± 0.093 activity unit/mg protein. Results of 6 experiments are shown. * $P < 0.05$, ** $P < 0.01$, compared with activity of corresponding enzyme preparation in absence of ethanol in incubation mixture.

The following reagents were used: ^{3}H-Leu-enkephalin (39 Ci/mmole) was from Amersham Corporation, England; Tyr-Gly, Tyr-Gly-Gly, Gly-Gly-Phe-Met, and Leu-enkephalin were from Serva, West Germany; fluorexamine, Tris-buffer, and puromycin were from Sigma, USA. The remaining reagents were of Soviet manufacture, of the highest available degree of purity.

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

EXPERIMENTAL RESULTS

Data showing the effect of ethanol in vitro on enkephalinase A activity in the rat hypothalamus and mesencephalon are shown in Fig. 1. During incubation of membrane fractions from the brain of animals not receiving alcohol with ethanol, dose-dependent activation of the enzyme clearly took place. The maximal effect was observed with ethanol in a concentration of 10^{-3} M in the incubation mixture. It can be concluded from data in the literature that this same ethanol concentration is attained in vivo in parts of the brain of rats receiving alcohol [4].

Washing membrane fractions of the rat mesencephalon and hypothalamus, preincubated with ethanol (10^{-3} M, 30 min, 30°C), 5 times in 10 mM Tris HCl buffer, pH 7.7, did not lead to any significant reduction in the activating action of ethanol.

It is interesting to note that incubation of brain membrane fractions of rats chronically consuming ethanol with increasing concentrations of alcohol caused no further increase in enzyme activity (Fig. 1). The absence of effect in this case was perhaps due to the development of adaptive changes in cell membranes observed during prolonged administration of alcohol [9]. On the other hand, the results can be explained by the presence of ethanol in brain preparations from chronically alcoholized animals in sufficient concentration to realize an activating action on enkephalinase A, so that the addition of ethanol in vitro did not lead to further activation of the enzyme.

Addition of acetaldehyde, the principal product of enzymic oxidation of ethanol in the body, to the reaction mixture in concentrations of between 10^{-8} and 10^{-2} M did not lead to any increase in enkephalinase A activity of the hypothalamus and mesencephalon. Natural condensation products of acetaldehyde with biogenic amines (1-methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline, 1 methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline, and salsolinol), formed in brain tissues after administration of alcohol to animals [13], which have affinity for opiate receptors [3], caused no significant changes in a concentration of 10^{-4} M in enzyme activity. It is interesting to

note that morphine and its antagonist naltrexone, in a concentration of 10^{-4} M in the reaction mixture, likewise inactivated enkephalinase A in parts of the rat brain studied.

The results of this investigation thus suggest that the activating effect of ethanol on enkephalinase A in the rat mesencephalon and hypothalamus is the result of the direct effect of alcohol on the enzyme molecule or on its membrane microenvironment, and not to any indirect effect of metabolic products of ethanol or the state of the enkephalin receptors. These findings are in good agreement with the writers' previous results showing no correlation between specific binding of Leu-enkephalin and enkephalinase A activity in the hypothalamus and mesencephalon of control rats and of animals receiving alcohol for a long time.

There are grounds for supposing at the present time that some of the pharmacological effects of alcohol are associated with its membranotropic action [9]. However, it is not yet clear whether this disturbance of enkephalin metabolism in the brain in alcoholism is observed as the result of the direct or indirect effect of ethanol on the CNS. The results suggest that one cause of these disturbances is the direct action of ethanol on enkephalinase A.

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