BINDING OF ^{3}H-MUSCIMOL* BY NEOCORTICAL MEMBRANES OF RATS EXPOSED PRENATALLY TO ETHANOL

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Parental alcoholism frequently gives rise to the so-called fetal alcohol syndrome in the children — a disease accompanied by malformations, behavioral disturbances, and retarded mental development [2].

Experiments on animals have shown that intrauterine exposure to alcohol leads to retardation in height and weight [13], delay in the development of various parts of the brain [7], and impairment of conditioned reflex formation and preservation [1]. Metabolic processes in the brain also are disturbed [10].

In the modern view an important role in the realization of the effects of ethanol is played by GABA-ergic system of the brain [4]. Ethanol stimulates transport of Cl^- ions by its action on the postsynaptic receptor complex, which includes receptors for GABA, benzodiazepines, and barbiturates [8]. By passing through the placental barrier, ethanol can induce a disturbance of normal development of the GABA-ergic receptor system.

The aim of this investigation was to test this hypothesis by studying the effect of prenatal exposure to ethanol on binding of ^{3}H-muscimol (a GABA agonist) with neocortical synaptic membranes in rats.

EXPERIMENTAL METHOD

Female albino rats were given 2.5 ml of a 40% solution of ethanol by gastric tube daily from the 5th to the 20th days of pregnancy. Instead of drinking water, the animal also received 7% ethanol to drink, and the average daily consumption was 24 ml per rat. Instead of alcohol, control animals received sucrose solution equivalent in calorific value. After birth of the young rats, the litters were equalized for the number of animals.

When the male offspring of the two groups reached the age of 2 months and a weight of 200-250 g they were decapitated, and the neocortex was removed and homogenized in 20 ml of isolation medium (0.32 M sucrose, 0.05 M Tris-HCl buffer, pH 7.4, and 0.01 M EDTA). The homogenate was centrifuged at 1000g for 10 min and the supernatant was then recentrifuged at 20,000g for 20 min. The residue (R_2) was suspended in 20 ml of 0.05 M Tris-HCl buffer, pH 7.4, centrifuged at 20,000g (20 min), and the residue thus obtained was frozen for 16 h at −40°C. The thawed residue was suspended in 15 ml of 0.05 M Tris-HCl buffer, pH 7.4, with 0.02% Triton X-100, incubated for 20 min at 37°C, after which 10 ml of cold 0.05 M Tris-HCl buffer, pH 7.4, was added and the sample was centrifuged at 20,000g for 30 min. The residue now obtained was resuspended in 20 ml of 0.05 M Tris-HCl buffer, pH 7.4, and centrifuged at 20,000g for 30 min. The residue of the fraction of washed, unpurified synaptic membranes was then suspended in 8 ml of 0.05 M Tris-HCl buffer, pH 7.4. At all stages of isolation except freezing and incubation with Triton X-100, the temperature was kept at 4°C.

The reaction of binding ^{3}H-muscimol with GABA-receptors was started by adding 0.25 ml of membrane suspension to 0.25 ml of an aqueous solution of ^{3}H-muscimol (8 Ci/mmole, "Amersham,"

*Muscimol is 5-aminomethyl-3-isoxazolol — Translator.

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England) and it was stopped 30 min later by filtering the sample through a GF/B filter ("Whatman," England) and by washing four times with 2 ml of 0.05 M Tris-HCl buffer, pH 7.4 (6 points were chosen within the concentration range of ^{3}H-muscimol from 2 to 100 nM; at each point there were three parallel tests; the duration of filtration of one sample was 10 sec and incubation was carried out at 1-2°C). Radioactivity on the filters was counted with the aid of ZhS-8 scintillation fluid ("Reakhim," USSR).

To analyze nonspecific binding, dependence of binding of ^{3}H-muscimol with the synaptic membrane fraction on the presence of 10 μM GABA was studied. Specific binding was calculated as the difference between total binding and nonspecific. The protein concentration determined by the method in [5] was 0.15-0.25 mg per sample. Each experimental and control group consisted of 6 animals. The data were averaged and a binding saturation isotherm plotted.

EXPERIMENTAL RESULTS

To study the properties of $GABA_A$-receptors muscimol, a structural analog of GABA with physiological activity and affinity for receptors closely similar to those of GABA [9], is often used. Values which we obtained for the level of ^{3}H-muscimol binding with neocortical synaptic membranes were close to those given in the literature for binding both of ^{3}H-muscimol and of ^{3}H-GABA [3, 6]. The relationship between the maximal number of binding sites (B_{max}) and affinity of the receptor (K_d) between the control and experimental groups of animals was complex in character (control: B_{max} = 4.381 ± 0.227 pmole/mg protein, K_d = 5.00 ± 0.46 mM; experiment: B_{max} = 5.547 ± 0.483 pmole/mg protein, K_d = 7.47 ± 1.09 nM, $p < 0.05$).

Chronic exposure to ethanol during pregnancy led to a significant (27%, $p < 0.05$) increase in the level of binding of ^{3}H-muscimol with the neocortical synaptic membranes of the young rats.

An increase in binding of ^{3}H-muscimol, incidentally, was found in experiments on brain tissue obtained postmortem from persons suffering from alcoholism [12]. It can accordingly be suggested that ethanol, on entering the blood stream in the fetus–mother system induces synergic changes in the properties of GABA-receptors of the maternal and fetal brain. However, we investigated the animals 2 months after the end of exposure to alcohol, and for that reason the cause of the increase in ^{3}H-muscimol binding could be both reorganization of the fetal GABA-ergic system and disturbance of the development of that system during postnatal ontogeny.

Intrauterine exposure to ethanol thus causes changes in the normal functioning of the GABA-ergic system, and this may be one cause of disturbance of various forms of behavior.

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