EFFECT OF ETHANOL in utero ON HIGHER NERVOUS ACTIVITY AND PROTEIN AND LIPID METABOLISM IN THE RAT BRAIN

A. L. Zabludovskii and V. V. Zhulin

UDC 616.831-008.939.15+616.831-008.939.6]-053.1-02:547.262]092.9-07

KEY WORDS: phosphorylation of proteins; synthesis of glycoproteins and phospholipids; intrauterine exposure to ethanol; conditioned reflex.

According to clinical observations, alcoholism in parents may lead to cerebral developmental defects in the children: oligophrenia and delayed mental development [1, 6]. Experimental studies have shown that development of the CNS is delayed in the progeny of animals receiving ethanol during pregnancy [5, 8, 9] and that abnormalities of behavior – in motor activity and in conditioned reflex formation – appear in late ontogeny [4, 7].

The writers showed previously that one consequence of intrauterine alcoholization in mature rats is depression of protein synthesis in various parts of the brain, corresponding to a definite degree to a disturbance of conditioned-reflex activity [2]. On the basis of these facts it was decided to study parameters of protein phosphorylation and glycoprotein and phospholipid synthesis in the neocortex and hippocampus of adult rats and to compare these findings with the results of an investigation of formation and preservation of defensive conditioned reflexes. One aspect of the work was to study the pattern of changes in these metabolic parameters in response to stress.

EXPERIMENTAL METHOD

Noninbred albino rats were given ethanol internally in a daily dose of 5-7 g/kg body weight from the 1st day of pregnancy until parturition. The male progeny of the experimental and control animals were investigated on reaching the age of 2 months. During formation of a conditioned passive avoidance reflex (CPAR) the rats were placed in the illuminated half of a chamber and the time before crossing into the darkened compartment, where they received electric shocks, was recorded. The parameter of preservation of CPAR was the time from being placed in the illuminated compartment until crossing into the dark compartment of the chamber, measured 24 h after conditioning. A conditioned active avoidance reflex (CAAR) was formed in an automated shuttle box. Electrical stimulation was applied at the 6th section of action of the conditioned stimulus (interrupted light and sound). Fifty combinations of conditioned and unconditioned stimuli were presented. Preservation of CAAR was tested after 7 days by repeated presentation of 50 combinations of conditioned and unconditioned stimuli and calculation of the increase in the number of conditioned avoidance responses from the first experimental session to the second. For the biochemical tests, the animals were lightly anesthetized with ether, fixed in a stereotaxic apparatus, and a mixture of sodium [^{32}P]orthophosphate (Medradiopreparat, USSR) and [^{3}H]fucose (Amersham Corporation, England), of 0.4 and 1.9 MBq in a volume of 50 μl respectively, was injected into the lateral cerebral ventricle. Anesthesia continued for 8-12 min. The rats were decapitated 90 min after injection of the labeled precursors. In some experiments, animals were subjected to stress for 1 h before sacrifice, in the form of vibration and noise in a metal box fixed to a moving platform. After removal of the brain, weighed samples of cerebral cortex and hippocampus were homogenized in distilled water at 4°C. Water-soluble and water-insoluble proteins were precipitated with 10% TCA. Lipids were extracted with a mixture of chloroform and methanol. Proteins were freed from nucleic acids and dissolved in 0.5 N NaOH. The pooled lipid extract was treated with chloroform and 0.1 M KCl. After separation into layers, the chloroform layers were evaporated on a rotary vaporizer, and the lipid residue dissolved in benzene and

Group for Radioisotope Investigations, Laboratory of Brain Pathology, Moscow Research Institute of Psychiatry, Ministry of Health of the RSFSR. Laboratory of Neurochemical Mechanisms of the Conditioned Reflex, Institute of Higher Nervous Activity and Neurophysiology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Rusinov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 5, pp. 545-548, May, 1985. Original article submitted July 19, 1984.

TABLE 1. Formation of Defensive Conditioned Reflexes in Rats Exposed to Ethanol in utero

Experimental conditions	CPAR		CAAR		r
	formation, sec	preservation, sec	formation number of avoidances	preservation, number of avoidances	
Control	9,1±1,07	249±13,4	5,95±1,81	12,3±2,8	0,47**
Ethanol	13,3±2,41	245,5±12,9	2,9±0,72*	6,9±1,98*	0,24

Legend. *P < 0.05, **)Level of significance of coefficient of correlation < 0.05.

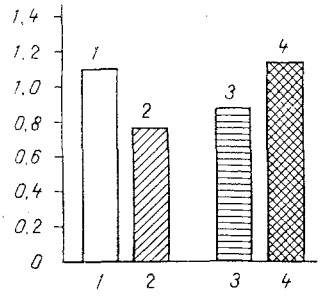

Fig. 1

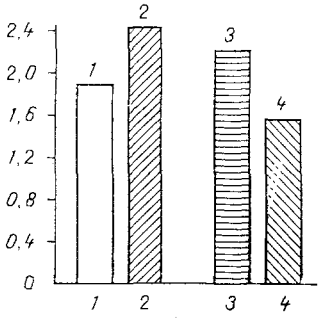

Fig. 2

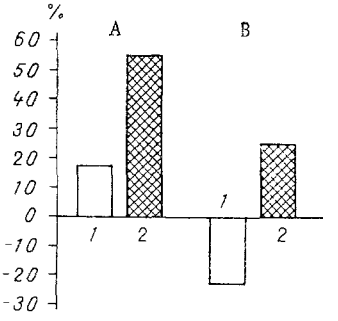

Fig. 3

Fig. 1. Phosphorylation of water-insoluble proteins of cerebral cortex in rats exposed to ethanol in utero. 1) Control; 2) control + stress; 3) ethanol; 4) ethanol + stress. Ordinate, RSA ($\cdot 10^{-2}$).

Fig. 2. Incorporation of ^{3}H-fucose into water-soluble hippocampal glycoproteins of rats exposed to ethanol in utero. Legend as in Fig. 1.

Fig. 3. Changes in incorporation of ^{32}P-orthophosphate into brain phosphatidylcholine of rats exposed to ethanol in utero. A) Cortex; B) hippocampus. 1) Ethanol; 2) ethanol + stress. Ordinate, difference between control and exposure to ethanol (in %).

fractionated by thin-layer chromatography on Silufol (Czechoslovakia) plates. Phospholipids were identified with molybdate reagent. Radioactivity of the protein digest and lipids was measured in Bray's scintillator on a Rack-Beta liquid scintillation counter (LKB, Sweden). The relative specific activity (RSA) was calculated as the ratio of radioactivity of the fractions to total radioactivity of the corresponding homogenates. The significance of differences between the results was determined by the Wilcoxon – Mann – Whitney test. Coefficients of correlation was calculated by Spearman's method, with Kendall's correction for tied ranks. Experiments were conducted on 92 rats.

EXPERIMENTAL RESULTS

Intrauterine alcoholization did not affect CPAR but disturbed the formation and preservation of CAAR (Table 1). In the initial training session the experimental rats performed half as many avoidances as the control animals, whereas during testing of preservation, they made 1.8 times fewer than the controls. Significant correlation between CAAR formation and preservation was observed in the control rats ($r = 0.47$, $P < 0.05$) but not in the experimental animals. This disparity between formation and preservation of the reflex in animals with inborn brain pathology may indicate that different mechanisms exist in the CNS for the formation of temporary connections, and that they may be selectively damaged.

The study of phosphorylation of brain proteins showed (Fig. 1) that incorporation of [^{32}P]orthophosphate into the fraction of water-soluble neocortical proteins was reduced by 20% ($P < 0.05$) in rats exposed to ethanol in utero. Stress evoked an increase in the intensity of phosphorylation of water-insoluble proteins in these animals compared to the initial level, whereas in intact animals exposed to stress incorporation of the indicator was inhibited by 30% ($P < 0.05$). Differences in RSA between the control and experiment in this case increased to 48% ($P < 0.05$). In the initial experiment no changes were found in phosphorylation of water-insoluble hippocampal proteins in the experimental rats, whereas under the influence of stress some degree of

activation was observed, although it was less marked than in the neocortex. Incorporation of [^{32}P]orthophosphate into proteins of the water-soluble fraction of the cerebral cortex and hippocampus of the experimental animals was not significantly changed compared with the control either in the original experiments or under the influence of stress.

Data on incorporation of ^{3}H-fucose into glycoproteins extracted in the water-soluble fraction of hippocampal proteins are given in Fig. 2. Some increase in synthesis of water-soluble glycoproteins was found in the progeny of rats receiving ethanol during pregnancy. Exposure to stress caused opposite changes in the groups studied: synthesis was increased by 22% in the control animals but reduced by 30% ($P < 0.05$) in the experimental rats. As a result of this the differences between the experimental and control series amounted to 36% ($P < 0.05$). No significant changes in synthesis in the experimental animals compared with the controls could be found in the other glycoprotein fractions studied (water-insoluble cortical and hippocampal glycoproteins, water-soluble cortical glycoproteins). Stress did not affect incorporation of fucose into these fractions.

The study of brain phospholipid synthesis showed (Fig. 3) that incorporation of [^{32}P]orthophosphate into phosphatidylcholine was increased by 17% in the cerebral cortex of rats exposed to alcohol in utero, but reduced by 23% in the hippocampus ($P < 0.05$). Under the influence of stress an increase in phosphatidylcholine synthesis in the cortex by 55% ($P < 0.05$) and in the hippocampus by 25% was observed compared with intact rats exposed to equal stress.

The use of stress thus brought metabolic differences between the brain of the experimental and control rats more clearly to light.

Considering data on the possible role of glycoproteins in the mechanisms of temporary connection consolidation [10], coefficients of correlation were calculated between parameters of glycoprotein synthesis, on the one hand, and of formation and preservation of active avoidance, on the other hand. Statistically significant correlation was found in the control animals between incorporation of ^{3}H-fucose into water-soluble cortical ($r = 0.77$, $P < 0.05$) and hippocampal glycoproteins ($r = -0.88$, $P < 0.05$) and preservation of CAAR (number of avoidances). No significant values of the coefficient of correlation were obtained in the experimental group. This suggests that disturbance of glycoprotein synthesis in rats exposed to alcohol in utero can be regarded as the probable cause of the shorter preservation of CAAR. These experiments confirmed previous findings [3] showing that exposure to ethanol in utero causes disturbance of conditioned-reflex activity in rats in late ontogeny; more complex forms of learning are affected the most. The experimental results indicate a definite role of disturbances of phosphorylation of individual groups of proteins and of glycoprotein and phospholipid synthesis in the development of cerebral pathology due to antenatal alcoholization. These changes may be an important factor in the mechanisms of injury to higher integrative brain functions.

LITERATURE CITED

1. L. S. Kunikovskaya, Zh. Nevropatol. Psikhiat., 80, 417 (1980).
2. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Neirokhimiya, 2, 288 (1983).
3. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Zh. Nevropatal. Psikhiat., 83, 394 (1983).
4. O. G. Malakhovskii and V. B. Prozorovskii, Farmakol. Toksikol., No. 1, 88 (1975).
5. É. N. Popova and L. E. Frumkina, Zh. Nevropatol. Psikhiat., 60, 1059 (1960).
6. E. V. Usova, N. S. Sazonova, and Z. A. Rassadina, Zh. Nevropatol. Psikhiat., 81, 1552 (1981).
7. E. L. Abel, Pharmacol. Biochem. Behav., 10, 239 (1979).
8. T. Majdecki, M. Marciniak, and M. Beskid, Neuropath. Pol., 11, 411 (1973).
9. B. Rasmussen and N. Christensen, Acta Path. Microbiol. Scand., A-88, 258 (1980).
10. W. Wetzel, N. Popov, B. Lössner, et al., Pharmacol. Biochem. Behav., 13, 765 (1980).