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EFFECT OF INTRAUTERINE EXPOSURE TO ETHANOL ON POSTNATAL SYNTHESIS OF SOME RAT BRAIN PHOSPHOLIPIDS

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According to clinical observations, a fetal alcoholic syndrome, a disease accompanied by disturbances of physical and mental development and by impairment of the mental faculties, is observed frequently in children whose mothers abuse alcohol during pregnancy [5]. It has been shown that animals exposed to alcohol in the intrauterine period are retarded in height and weight [14], their ability to form and maintain conditioned reflexes is reduced [6], and energy and protein metabolism in the brain is disturbed [3, 11]. Meanwhile brain lipid metabolism in inborn cerebral pathology of alcoholic genesis has so far received little study. We know that ethanol, on penetrating from the blood stream into the brain, acts on the lipid components of the cell membranes and modifies their permeability [12]. It is very probable that chronic exposure of the undeveloped brain to ethanol may prevent the normal formation of the lipid composition of the membrane. It was accordingly decided to study parameters of phospholipid synthesis in the brain of rats exposed antenatally to ethanol.

We know that when the aftereffects of intrauterine damage to the CNS are studied, the use of additional provocative factors can lead to the detection of latent metabolic shifts [1]. One aspect of the present investigation was therefore a study of the features of phospholipid synthesis in response to functional loading.

EXPERIMENTAL METHOD

Female albino rats were given ethanol perorally in a daily dose of 5-7 g/kg from the 1st day of pregnancy and until giving birth, whereas control animals received water. The male progeny of these mother rats, at the age of 2 months, were lightly anesthetized with ether and given an injection of sodium ^{32}P-orthophosphate (0.4 MBq in a volume of 50 µl) into the left lateral cerebral ventrical. The action of the anesthetic continued for 10-12 min. The rats were decapitated 90 min after injection of labeled phosphate. In some experiments, for 1 h before sacrifice the animals were subjected to functional loading in the form of vibration and noise in a metal box.

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After removal of the brain, weighed samples of the sensomotor cortex and hippocampus (50 mg on average) from the right hemisphere were homogenized in 1 ml water. The complex of water-insoluble proteins and lipids was then precipitated by centrifugation and the residue suspended in 2 ml of 5% TCA. The suspension of coagulated proteins and lipids was again precipitated and aliquots of the supernatant after the first centrifugation and of the TCA-supernatant were used for measurement of radioactivity in order to determine the sodium ^{32}P-orthophosphate pool.

The residue thus obtained was treated with 1.8 ml of a chloroform–methanol mixture in the ratio of 2:1, shaken for 15 min, after which a further 0.6 ml of methanol was added and the insoluble part was sedimented by centrifugation. The supernatant with the extracted lipids was preserved and the residue treated with 1.8 ml of chloroform–methanol mixture in the ratio of 1:2, shaken for 15 min, the proteins were precipitated, and the lipid supernatants pooled. The pooled supernatant was treated with 3 ml of chloroform and 1.44 ml of 0.1 M KCl. After separation into layers the chloroform layer was removed and evaporated on a rotary evaporator. Lipids were dissolved in 200 µl benzene and kept at –10°C for 2-3 days.

Phospholipids were fractionated by the use of "Silufol" plates (Czechoslovakia) measuring 20 × 20 cm. An aliquot of total lipids was applied to the bottom left hand corner of the plate, previously activated in a thermostat, and this was then placed in the first chamber with a chloroform–methanol–ammonia (65:35:5) mixture. When the front reached the opposite edge the plate was removed, dried in a current of air, and placed at an angle of 90° to the first direction in the second chamber, containing a chloroform–acetone–methanol–acetic acid–water (5:2:1:1:0.5) mixture. At the end of fractionation the dry plate was dipped for 15-20 sec into a solution of molybdenum blue [8], rinsed with water, and the stained blue spots were scraped off with a spatula into scintillation flasks containing 1 ml of methanol. After 1 h, 9 ml of ZhS-8 scintillation fluid ("Reakhim," USSR) was added, the mixture was kept for 24 h, and readioactivity was counted on a "RackBeta" counter (LKB, Sweden). Phosphatidylethanolamine (PEA), phosphatidylcholine (PCh), and phosphatidylserine (PS) were identified by the use of purified phospholipid preparations. The relative specific radioactivity (RSA) was calculated as the ratio of the radioactivity of the test phospholipids to that of the labeled sodium orthophosphate pool. The significance of differences was estimated by Student's test. Altogether 40 rats were used.

EXPERIMENTAL RESULTS

After development of the chromatographic plates with the molybdenum reagent eight zones with different intensity of color were found, but only in four of them was the radioactive count significantly above the background level. By the use of external standards PEA (R_{f_1} = 0.60 ± 0.06, R_{f_2} = 0.51 ± 0.05), PCh (R_{f_1} = 0.24 ± 0.02, R_{f_2} = 0.22 ± 0.02), and PS (R_{f_1} = 0.35 ± 0.04, R_{f_2} = 0.23 ± 0.02; R_f denotes retention factor) were identified. The RSA for phospholipids of the cerebral cortex of intact rats was: PEA 0.0168 ± 0.0013, PCh 0.0529 ± 0.0030, and PS 0.0338 ± 0.0037. The level of incorporation of sodium ^{32}P-orthophosphate into hippocampal phospholipids was lower for these animals: PEA 0.0151 ± 0.0014, PCh 0.0442 ± 0.0027, and PS 0.0194 ± 0.0014.

Intrauterine exposure to alcohol caused a significant increase of 30% in incorporation of labeled sodium orthophosphate into PCh of the cerebral cortex relative to the control. No significant deviations in phospholipid synthesis were found in the hippocampus of the experimental animals.

Functional loading led to a significant reduction of PCh synthesis in the cerebral cortex by 13% and of PCh and PEA synthesis in the hippocampus by 26 and 32% respectively. Incorporation of radioactive phosphorus into PS also was somewhat depressed in both structures.

In animals exposed in utero to alcohol, stress induced opposite changes: a significant increase in PCh and PS synthesis in the cortex by 53 and 48% respectively and of PCh synthesis in the hippocampus by 41%, the amplitude of the shift being increased.

Phospholipids constitute the greater part of the lipid bilayer of brain cell membranes [7]. They play an important role in the permeability of biomembranes and in conduction of impulses along nerve fibers [13]. During maturation (phospholipid metabolism, measured as incorporation of ^{32}P into the individual phospholipid families, is lowered [4]. Meanwhile

the content of certain phospholipids falls sharply. In particular, for instance, it has been shown that the PCh content in the brain of sexually mature animals is much lower than in neonates [2]. In the light of these data, the raised level of PCh synthesis in animals exposed in utero to alcohol may indicate delayed development of the brain, and this is confirmed by the results of morphological investigations [15].

It was shown previously that antenatal exposure to ethanol leads to an increase in the incorporation of glucose into myelin and to an increase in its content [10]. This effect may be assumed to be largely due to increased synthesis of PCh, for the latter is the principal phospholipid component of myelin [9].

The results are thus evidence of a significant disturbance of phospholipid synthesis in the brain as a result of intrauterine exposure to ethanol. These disturbances may be latent and may be brought to light by the use of additional functional loads and, in particular, by the use of factors inducing stress.

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