EFFECT OF ANTENATAL EXPOSURE TO ETHANOL ON BLOOD-BRAIN BARRIER FUNCTION IN ANIMALS

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The state of the blood-brain barrier (BBB) during acute and chronic exposure to ethyl alcohol has been studied by radioisotope and other modern methods of investigation [2, 7]. However, existing data on the effect of acute and chronic ethanol poisoining on permeability of the BBB are contradictory [1, 3, 9, 12]. The effect of prenatal exposure to ethanol on BBB function in ontogeny has not been studied. In view of the importance of this question for the understanding of the mechanisms of development of the "fetal alcoholic syndrome" [10, 11], it was decided to study the state of the BBB in rats exposed to ethanol in the antenatal period.

EXPERIMENTAL METHOD

Female rats were given 2.5-3 ml of 40% ethanol (4-5 g/kg body weight) daily by gastric tube from the 5th through the 21st day of pregnancy. The progeny of the experimental and control animals were kept under standard conditions in the animal house. Tests were carried out on 88 rats aged 20 and 60 days. The indicators used were ^{32}P ($Na_2H^{32}PO_4$), ^{3}H-valine, ^{3}H-tyrosine, and ^{35}S-methionine. One hour after intraperitoneal injection of the compounds in indicator doses with a total activity of 10, 75, and 25 μCi/100 g body weight of ^{32}P, ^{3}H, and ^{35}S, respectively, the rats were anesthetized with hexobarbital and exsanguinated, after which the cerebral vessels were perfused with physiological saline and weighed samples taken from six brain structures. The tissues were solubilized in 0.5 N NaOH solution and transferred into Bray's scintillator in counting flasks. Radioactivity was counted on a RackBeta liquid scintillation counter (LKB). The relative activity (RA) — the ratio of specific radioactivities of the tissue and of blood taken at the same time, per unit weight or volume, expressed as a percentage — was determined. After injection of the isotopes separate series of animals were exposed to functional loads: combined exposure to noise and vibration for 50 min.

EXPERIMENTAL RESULTS

In experiments on 20-day-old rats, using ^{32}P as indicator, maximal accumulation of the isotope in the brain of the control animals was observed in the cerebellum, brain stem, and hypothalamus and minimal in the hippocampus. In rats exposed antenatally to ethanol, radioactivity in all brain structures tested was lower than in the control. A fall in incorporation of ^{32}P by 37-43% was observed in the cerebellum, basal ganglia, and medulla, and a smaller fall (by 13-19%) in the hypothalamus (Fig. 1).

In experiments on sexually mature animals, labeled amino acid and also ^{32}P were used. In animals exposed antenatally to ethanol, penetration of ^{3}H-valine into all brain structures was reduced compared with the control (Fig. 2). The reduction of RA was greatest in the medulla, hypothalamus, and cerebellum, and minimal changes were observed in the hippocampus. When the other amino acids were used as indicators, similar changes were observed. In the experimental animals a tendency for penetration of ^{3}H-tyrosine to fall (by 14-17%) was observed in the cerebellum, brain stem, hypothalamus, and cerebral cortex. The changes for ^{55}S-methionine which, under normal conditions, penetrates easily into the brain, were very small and did not exceed 5-7% compared with the control.

In the experiments in which ^{32}P was used penetration of the indicator into different parts of the brain showed unequal changes in the experimental rats. Whereas incorporation of the

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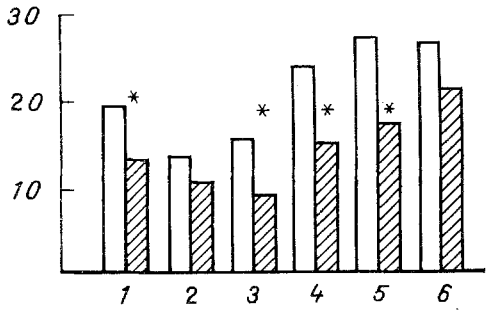

Fig. 1

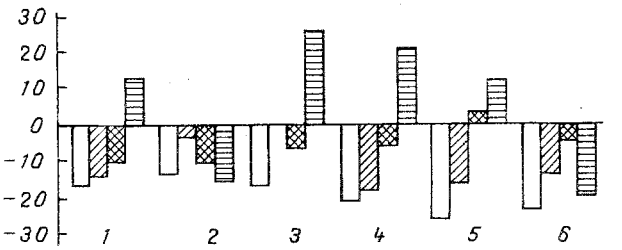

Fig. 2

Fig. 1. Penetration of ^{32}P into parts of the brain in 20-day-old rats exposed antenatally to ethanol. Ordinate, RA (in %). 1) Cortex, 2) hippocampus, 3) basal ganglia, 4) cerebellum, 5) medulla, 6) hippocampus. Unshaded column — control, shaded — experiment. *P < 0.05.

Fig. 2. Changes in permeability of BBB in sexually mature rats exposed antenatally to ethanol (in % of control). Unshaded column — ^{3}H-valine, obliquely shaded — ^{3}H-tyrosine, cross-hatched — ^{35}S-methionine, horizontally shaded — ^{32}P. Remainder of legend as to Fig. 1.

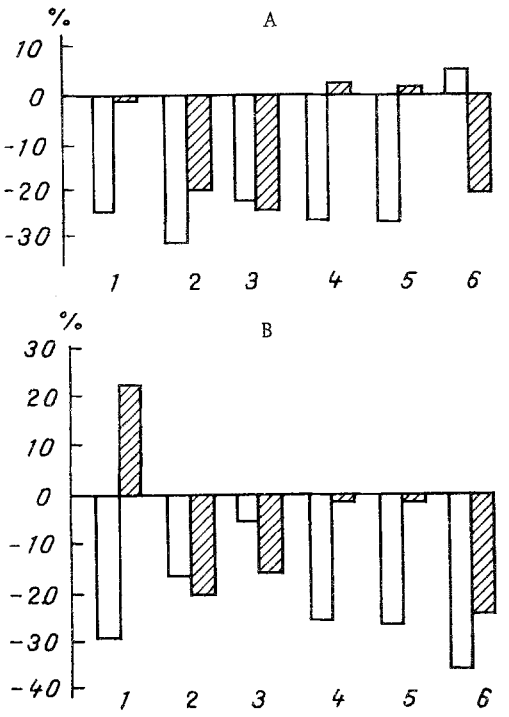

Fig. 3. Changes in penetration of ^{3}H-valine (A) and ^{32}P (B) into parts of the brain of experimental and control rats under the influence of stress factors. Legend as to Fig. 1.

isotope into the hippocampus and hypothalamus was reduced, in other brain structures an increase in accumulation of ^{32}P was observed, which varied in degree and was maximal in the basal ganglia (Fig. 2).

In rats exposed antenatally to ethanol significant differences were found in the changes in permeability of BBB under the influence of extremal stimuli. Exposure of the control animals to noise and vibration caused significant inhibition of ^{32}P and ^{3}H-valine uptake into nearly all the brain structures studied. Similar exposure of the experimental animals to ethanol was not accompanied by any changes in penetration of the indicators into tissues of the cerebellum and medulla. Accumulation of ^{3}H-valine in the cerebral cortex did not differ from the control, but accumulation of ^{32}P was increased. Incorporation of ^{3}H-valine into the hypothalamus in animals of the experimental and control group likewise showed opposite changes un-

der the influence of stress (Fig. 3). Thus changes in ^{32}P uptake in certain parts of the brain (cerebral cortex, hippocampus, cerebellum, brain stem, hypothalamus) of animals exposed to intrauterine alcoholization, under the influence of vibration and noise, were less marked than or opposite in direction to the control.

These investigations indicate distinguishing features of the barrier functions in the brain of rats exposed *in utero* to ethanol. At different age periods in such animals changes in penetration of the indicators into brain structures (mainly a decrease) compared with control rats were observed. This diversity of response of the barrier mechanisms in the brain of the experimental animals on the reaction of stress factors also was characteristic.

Considering modern views on the functions of BBB [3, 8] and also that the indicators used (^{32}P, amino acids) participate in metabolism of phosphorus compounds and brain proteins, it can be tentatively suggested that the changes in BBB permeability thus revealed reflect the state of processes of tissue metabolism in different parts of the brain in animals exposed antenatally to ethanol. Possible confirmation of this is given by the changes in protein synthesis, discovered by the writer previously, in brain structures (also predominantly a decrease) of animals exposed to ethanol *in utero* [4-6]. An essential role in the mechanism of these disturbances of protein metabolism in the brain is evidently played by a decrease in penetration of amino-acid substrates essential for protein synthesis into the brain. This gives further confirmation of the connection found between barrier functions and tissue metabolism in the CNS.

LITERATURE CITED

1. G. M. Butenko, in: Tissue-Blood Barriers [in Russian], Moscow (1961), pp. 222-227.
2. G. N. Kassil' and É. S. Lokshina, in: The Blood-Brain Barrier [in Russian], Moscow–Leningrad (1935), pp. 263-268.
3. M. Ya. Maizelis, in: Problems in Experimental Psychiatry [in Russian], Moscow (1965), pp. 77-83.
4. M. Ya. Maizelis, The Blood-Brain Barrier and Its Regulation [in Russian], Moscow (1973).
5. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Zh. Nevropatol. Psikhiat., No. 3, 394 (1983).
6. M. Ya. Maizelis, A. L. Zabludovskii, and S. N. Shikhov, Neirokhimiya, 2, 288 (1983).
7. L. S. Shtern and É. S. Lokshina, in: The Blood-Brain Barrier [in Russian], Moscow–Leningrad (1935), pp. 269-270.
8. J. Dobbing, Physiol. Rev., 41, 130 (1961).
9. M. Hillbom and T. Tervo, Experientia, 37, 936 (1981).
10. K. L. Jones and D. W. Smith, Lancet, 2, 999 (1973).
11. R. Little, I. Graham, and H. Samson, in: The Effect of Maternal Alcohol and Drug Abuse on the Newborn, New York (1982), pp. 103-125.
12. F. B. McGillon, C. Thompson, M. Moore, and A. Goldberg, Biochem. Pharmacol., 23, 472 (1974).