EFFECT OF INTRAUTREINE HYPOXIA ON PROTEIN SYNTHESIS IN DIFFERENT PARTS OF THE BRAIN AND ON TISSUE–BLOOD BARRIER FUNCTION IN LATER STAGES OF DEVELOPMENT IN RATS

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Penetration of six labeled amino acids into the tissues of different parts of the brain and their incorporation into proteins were investigated in sexually mature rats exposed to acute hypoxia at a certain stage of antenatal development. Depression of protein synthesis, mainly in the hypothalamus and cerebral cortex, was characteristic of the experimental animals, and correlated with disturbances of their conditioned-reflex activity. Disturbances of the function of the blood-brain barrier were found in the experimental animals with the aid of labeled amino acids and labeled phosphate, but permeability of the other tissue-blood barriers was unchanged.

KEY WORDS: *intrauterine hypoxia; brain; protein synthesis; tissue–blood barriers.*

Fetal hypoxia is one of the pathogenetic factors leading to disturbance of brain development[5, 6, 9, 13]. An important stage in the mechanism of hypoxic lesions in the fetus is disturbance of amino acid transport and of protein synthesis in the brain [3, 12], but there are no experimental data in the literature to confirm this hypothesis. In previous experiments on animals exposed to acute hypoxia in the antenatal period, the writers found significant changes in the incorporation of [^{35}S]methionine and [^{14}C]leucine into tissue homogenates and proteins from different parts of the brain [8]. These results are evidence of a disturbance of protein metabolism and of the function of the blood–brain barrier (BBB) as a result of intrauterine hypoxic brain damage.

In order to develop this line of research, in the present investigation protein renewal in different parts of the brain and the state of the tissue-blood barriers were studied with the aid of a wide spectrum of labeled amino acids in rats exposed to acute intrauterine hypoxia.

EXPERIMENTAL METHOD

Acute hypoxia was induced in fetuses by keeping the mother rats at the 15th-16th day of pregnancy in a pressure chamber at a pressure equivalent to an altitude of 8000-8500 m for 2 h. The experiments were carried out on the animals at the age of 2-3 months. Protein synthesis was studied by the use of [^{3}H]valine, [^{3}H]tyrosine, [^{35}S]methionine, [^{14}C]leucine, ^{14}C-labeled chlorella protein hydrolyzate, and [^{14}C]phenylalanine. The labeled amino acids (total activity 25-150 μCi) were injected intraperitoneally. The animals were decapitated 1 h later, protein was precipitated with 10% TCA, and lipids were subsequently extracted by the usual method. The radioactivity of the alkaline protein hydrolyzates and tissues from different parts of the brain was determined with the Soviet SBS-1 liquid scintillation counter in Bray's dioxan scintillator [10]. In the experiments with labeled phosphate (^{32}P) the radioactivity of the tissues of the brain and internal organs and of the blood was determined with the PP-8 apparatus and BFL-25 end-window counter. The ratio of the radioactivity of the tissues to that of the blood and also to the injected activity was determined; the relative specific activity of protein was calculated, allowing for the radioactivity of the TCA-supernatant. The results were subjected to statistical analysis with the aid of the Wilcoxon–Mann–Whitney criterion. Altogether 160 noninbred albino rats were used in the experiments.

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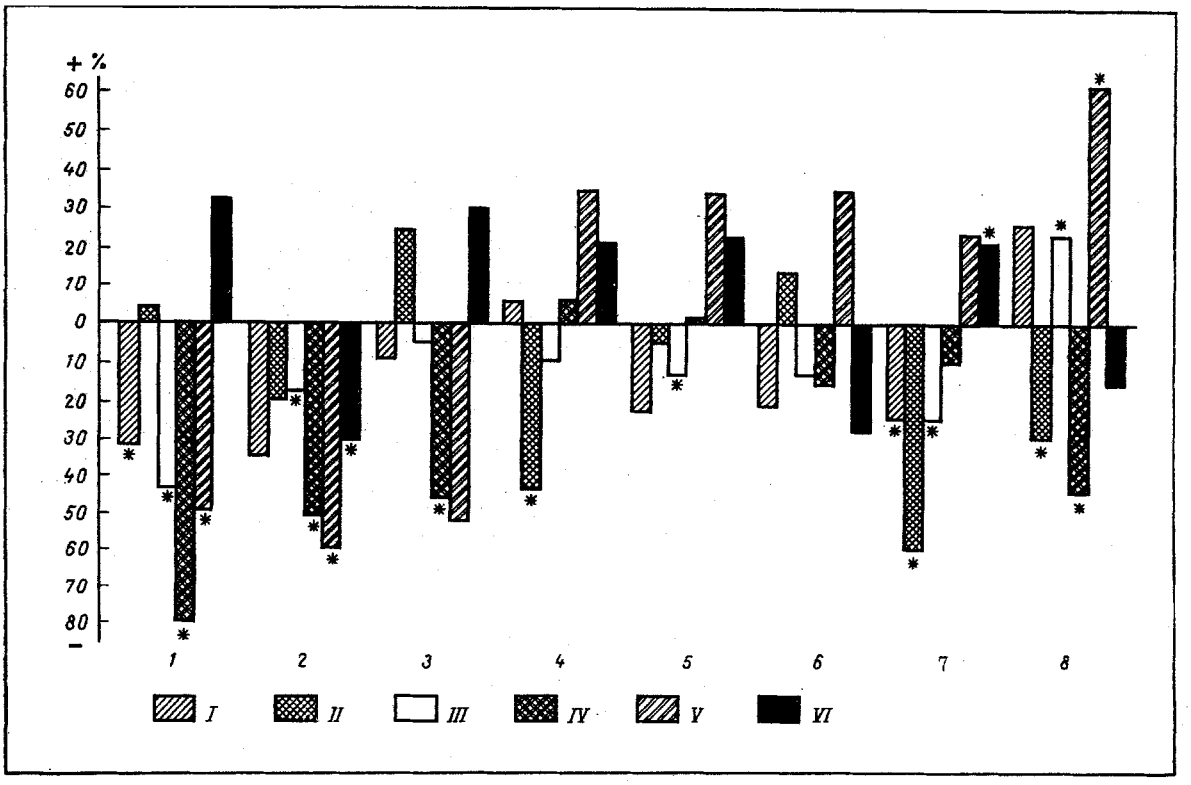

Fig. 1. Changes in incorporation of labeled amino acids into proteins in different parts of brain of animals exposed to intrauterine hypoxia (in % of control). I) [^{3}H]-valine; II) [^{14}C]phenylalanine; III) [^{3}H]tyrosine; IV)^{14} C-labeled chlorella protein hydrolyzate; V) [^{35}S]methionine; VI) [^{14}C]leucine. 1) Cerebral cortex; 2) hippocampus; 3) basal ganglia; 4) cerebellum; 5) corpora quadrigemina; 6) medulla; 7) spinal cord; 8) hypothalamus. Significant differences ($P < 0.05$) are marked by an asterisk.

EXPERIMENTAL RESULTS

Data on the change in incorporation of labeled amino acids into proteins from various brain structures of the experimental animals are compared in Fig. 1 with the corresponding data for the controls. In animals exposed to intrauterine hypoxia, depression of protein synthesis to a varied degree was observed in many parts of the brain. In some structures these changes were statistically significant. The clearest changes in the experimental animals were observed in the hippocampus, where incorporation of all the amino acids used was characteristically reduced. In the sensory-motor cortex incorporation of all the labeled amino acids except leucine and phenylalanine into protein also was considerably reduced. In the experiments with [^{14}C]leucine, incidentally, besides an increase in radioactivity in the proteins of the sensory-motor complex of the experimental animals, a decrease in incorporation into protein was found in other zones — the temporal and olfactory cortex. In the other parts of the brain studied, changes in incorporation of the various amino acids into protein were not always in the same direction. In some structures no significant changes were found or there was a tendency toward activation of incorporation of certain amino acids into protein.

Simultaneously with the investigation of protein synthesis in brain tissue, the accumulation of labeled amino acids also was determined in tissues of various brain structures of experimental and control animals (relative to the radioactivity of the blood). Comparison of the results given in Figs. 1 and 2 shows that the patterns of incorporation of labeled amino acids into proteins in different parts of the brain of the experimental animals were not always accompanied by analogous changes in the accumulation of the amino acids in the tissues of the same structures, but often the changes were actually in the opposite direction. In particular, relationships of this sort were found in the sensory-motor cortex and hippocampus, where inhibition of protein synthesis was a characteristic feature (experiments with [^{3}H]valine, [^{14}C]leucine, and [^{35}S]methionine). These results show no parallel between changes in protein synthesis and the state of the BBB in different parts of the brain.

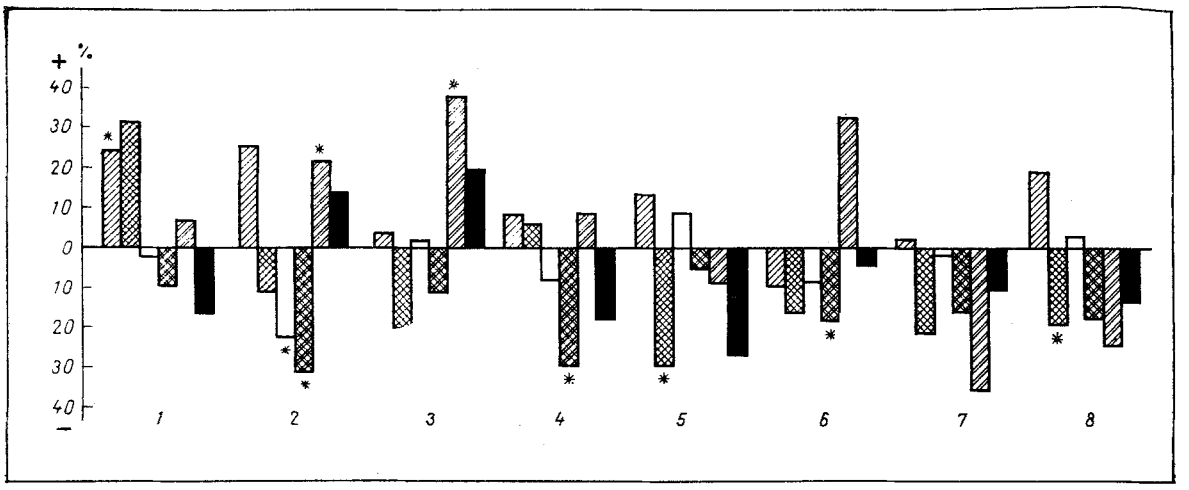

Fig. 2. Changes in accumulation of labeled amino acids into brain tissues of experimental animals (in % of control). Legend as in Fig. 1.

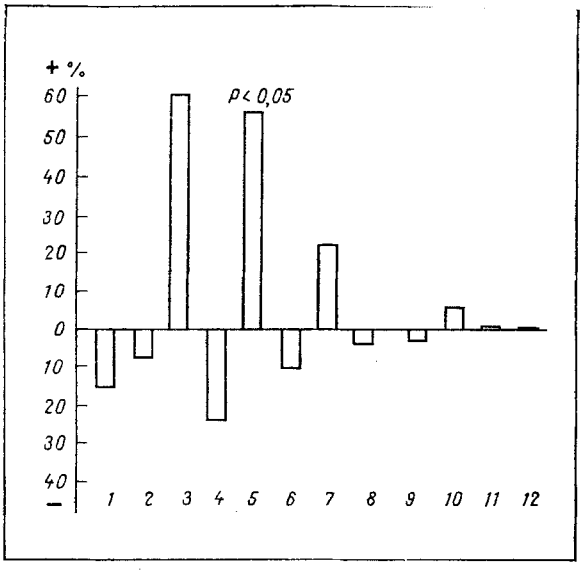

Fig. 3. Changes in penetration of ^{32}P into parts of the brain and internal organs of experimental rats (in % of control). Values of 1-8 the same as in Fig. 1; 9) pituitary; 10) liver; 11) kidney; 12) adrenal.

The use of labeled amino acids, most of which easily penetrate from the blood into the brain, is not sufficiently adequate for the investigation of BBB function. In a separate series of experiments on rats exposed to intrauterine hypoxia, and on control rats, the function of BBB and the other tissue–blood barriers was investigated with the aid of the traditional indicator, ^{32}P. The results given in Fig. 3 show that in the experimental animals the penetration of ^{32}P was reduced in most brain structures, although statistically significant changes were observed only in the corpora quadrigemina. Accumulation of ^{32}P in the subcortical regions and spinal cord was increased a little. In the hypothalamus the changes in the ^{32}P concentration were not significant.

During the investigation of radioactivity of the pituitary, adrenals, liver, and kidneys of the experimental and control animals no significant changes were found and the mean differences between the series did not exceed 3-6%. The results show that the state of the tissue–blood barriers of these organs in the experimental rats was undisturbed.

Definite changes in protein structure are thus observed in various brain structures of sexually mature animals exposed to intrauterine hypoxia. These changes are most marked in the hippocampus and cerebral cortex, where protein synthesis is characteristically depressed. These data correlate to some extent with the results of many investigations which have indicated the important role of the hippocampus in the mechanisms of memory [1, 2, 11].

In the study of changes in the higher nervous activity of rats exposed to hypoxia in the antenatal period, a sharp decrease was found in the index of preservation of conditioned reflexes in such animals [4]. Comparison of the results suggests that inhibition of protein synthesis in certain brain structures and, in particular, in the hippocampus of animals exposed to intrauterine hypoxia may have a definite connection with disturbance of the fixation of temporary connections and their transition into long-term memory.

In the modern view of BBB one of the main factors determining its function is the intensity of tissue metabolism in the CNS [7]. In the present experiments on animals exposed to intrauterine hypoxia no parallel was found between the changes in incorporation of labeled amino acids into brain tissue and into proteins isolated from the same brain structures. The discrepancy between these indices could point to a disturbance of the function of BBB in the experimental animals. This conclusion is confirmed by the results of investigations showing changes in the penetration of ^{32}P into individual brain structures of the experimental rats compared with the controls. Characteristically the function of the other tissue–blood barriers (pituitary, liver, kidney, adrenal) of these animals was unchanged.

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