EFFECT OF ACUTE HYPOXIA IN THE ANTENATAL PERIOD ON UPTAKE OF LABELED AMINO ACIDS INTO RAT BRAIN PROTEINS AND TISSUE HOMOGENATES

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Incorporation of leucine-^{14}C and methionine-^{35}S into tissue homogenates and protein isolated from various parts of the brain of rats exposed to acute hypoxia in the antenatal period was investigated. Besides active incorporation of amino acids into proteins in other parts of the brain, inhibition of protein synthesis also was observed in certain structures in the experimental animals, especially in the hippocampus. Changes in the uptake of labeled amino acids by the tissue homogenates did not correspond to the level of their incorporation into protein in the individual brain structures. The experimental results points to a disturbance of the intensity of protein metabolism and of the function of the blood–brain barrier in late ontogeny in rats exposed to intrauterine hypoxia.

KEY WORDS: rat brain; antenatal hypoxia; blood–brain barrier; protein synthesis.

Fetal hypoxia is one factor responsible for the development of brain pathology in postnatal ontogeny [1, 6, 8, 15]. There is evidence of disturbances of protein metabolism in vivo under the influence of antenatal hypoxia. Changes in the blood levels of free amino acids have been observed in infants exposed to hypoxia [10]. In animal experiments fetal hypoxia caused changes in the levels of free amino acids in brain tissue and a disturbance of the metabolism of certain amino acids; definite changes were found in the content of DNA, RNA, and protein in the brain [12, 13]. However, asphyxia of rat embryos was not accompanied by changes in the permeability of the brain capillaries for the dye Trypan Blue [7]. In the mechanism of the harmful action of hypoxia on the fetus an essential step may be inhibition of nucleic acid and protein synthesis, with consequent structural disturbances of the embryo [4].

Considering the important role of the blood–brain barrier (BBB) and of protein synthesis in the brain for the functions of the CNS, incorporation of labeled amino acids into proteins and into tissue homogenates of various parts of the brain was investigated in animals exposed to hypoxia in the antenatal period.

EXPERIMENTAL METHOD

Rats were kept in a pressure chamber on the 14th-16th day of pregnancy and exposed to a reduced pressure corresponding to an altitude of 8000-9000 m (220-250 mm Hg) for 2 h. The progeny of these animals were kept under observation for 2-3 months. Disturbances of growth and development, abnormalities of behavior, and disturbances of conditioned-reflex activity were observed in some of the young rats. The experimental and control rats at the age of 3 months were given an intraperitoneal injection of a solution of leucine-^{14}C or methionine-^{35}S with a total activity of 15 and 50 μCi respectively. The animals were decapitated 1 h later and pieces of tissue from different parts of the brain were isolated on ice. Some of the tissue (10-15 mg) was subjected to alkaline hydrolysis with heating. Parallel weighed samples of tissue were homogenized in 10% TCA, then washed with 5% TCA, and treated with ethanol and ether to extract lipids. The resulting dried protein was hydrolyzed in 1 M KOH. The radioactivity of the alkaline digests of tissue and protein was determined by means of the Soviet SBS-1 liquid scintillation counter in dioxan scintillator [11]. The protein content in the

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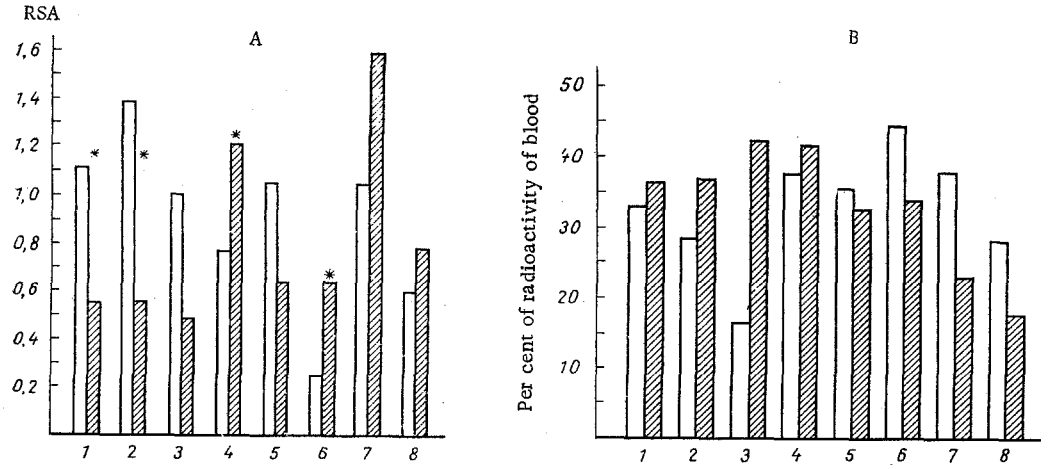

Fig. 1. Incorporation of methionine-^{35}S into proteins (A) of various rat brain structures and transport of methionine through BBB into brain tissue (B). Unshaded columns represent control, shaded columns experiment. 1) Sensomotor cortex; 2) hippocampus; 3) basal ganglia; 4) cerebellum; 5) corpora quadrigemina; 6) hypothalamus; 7) medulla; 8) spinal cord. Experiments for which $P < 0.05$ are indicated by an asterisk.

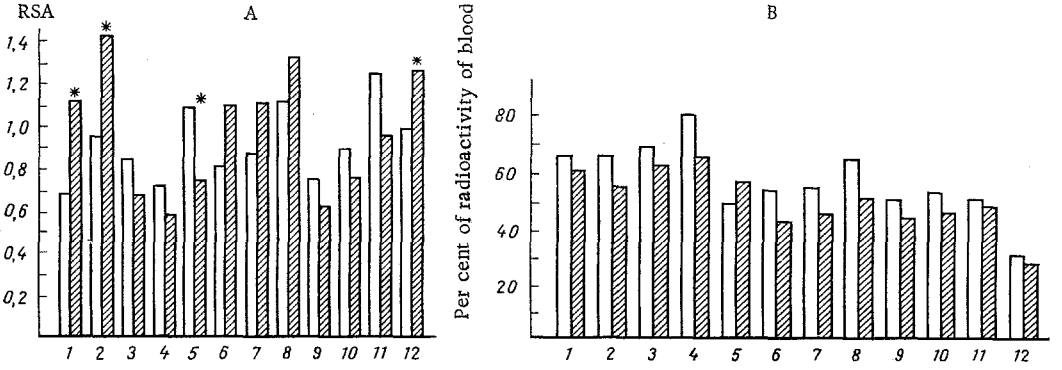

Fig. 2. Incorporation of leucine-^{14}C into rat brain proteins (A) and transport of leucine through BBB into brain tissue (B). 1) Visual cortex; 2) sensomotor cortex; 3) temporal cortex; 4) olfactory lobes; 5) hippocampus; 6) basal ganglia; 7) cerebellum; 8) corpora quadrigemina; 9) thalamus; 10) hypothalamus; 11) medulla; 12) spinal cord. Remainder of legend as in Fig. 1.

alkaline digests was determined by Lowry's method. The intensity of incorporation of labeled amino acids into brain proteins was expressed as specific activity (in counts/100 sec/10 mg protein) and relative specific activity (RSA, the ratio between the radioactivities of the protein and TCA extract). To characterize the state of the BBB the radioactivity of the brain tissue was expressed as a ratio of the activity of the blood and also of the injected radioactivity per unit body weight. Statistical analysis of the results was carried out with the aid of the nonparametric Wilcoxon–Mann–Whitney criterion. Altogether, 40 noninbred rats were used in the experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

Changes in protein synthesis were observed in the animals exposed to hypoxia in the antenatal period and they differed in different parts of the brain. In the animals in the experiments with methionine-^{35}S incorporation of the amino acid into proteins was reduced compared with the control in the sensomotor cortex and hippocampus and also, but to a lesser degree, in the basal ganglia and corpora quadrigemina. Meanwhile renewal of proteins was increased in the hypothalamus, cerebellum, medulla, and spinal cord (Fig. 1A). In concurrent investigations of the penetration of labeled methionine into tissue homogenates from various parts of the brain it was found that the changes in radioactivity in the brain tissue of the experimental animals did not

correspond to changes in the incorporation of label into proteins. A decrease in radioactivity was found in the tissues of the hypothalamus, medulla, and spinal cord, i.e., in structures in which protein renewal was intensified. Increased radioactivity was observed in the hippocampus and basal ganglia, characterized by depression of protein synthesis (Fig. 1B).

In the experiments with leucine-^{14}C a significant decrease was found in the renewal of hippocampal proteins and there was a tendency for protein renewal to be diminished in the temporal cortex, thalamus, hypothalamus, and medulla. At the same time, in several structures (visual and sensomotor cortex, spinal cord) significant activation was observed, whereas in the basal ganglia, cerebellum, and corpora quadrigemina there was a tendency toward activation of protein synthesis (Fig. 2A). Investigation of the penetration of labeled leucine into the tissues of various parts of the brain showed a tendency toward an increase in the accumulation of label in the hippocampus and to a decrease in its accumulation in most brain structures in which activation of protein synthesis was observed. Just as in the experiments with methionine-^{35}S, no correlation was thus found between the changes in protein synthesis and the accumulation of labeled amino acids in the brain structures.

The results of these investigations indicate significant changes in protein renewal in the various brain structures and in the transport of labeled amino acids through the BBB into tissue homogenates in animals exposed to acute hypoxia in the antenatal period. The fact will be noted that the incorporation of labeled leucine and methionine into hippocampal proteins and also into individual cortical structures was significantly reduced in the experimental animals, whereas protein synthesis in certain other parts of the brain was activated. The hippocampus is known to play a special role in the mechanisms of memory [2, 3, 14]. Special investigations in the writers' laboratory [5] have shown disturbances of fixation of temporary connections and of their conversion into long-term memory in rats exposed to acute hypoxia in the antenatal period.

Definite correlations were thus found in the experimental animals between the disturbance of fixation of temporary connections and the depression of protein synthesis in certain brain structures and, in particular, in the hippocampus.

Substantial changes in function of the BBB are found in animals exposed to acute hypoxia in the antenatal period. Disturbances of the accumulation of labeled amino acids in the tissues of different parts of the brain as a rule did not correspond to the changes in their incorporation into proteins of those same structures. According to modern views on the BBB, the main factor determining its function is the intensity of tissue metabolism in the CNS [9]. The results of the present investigations show that a characteristic feature of the experimental animals was that the function of the BBB did not correspond to the level of protein metabolism in the various parts of the CNS. Possibly in consequence of this, the requirements of amino acids necessary for brain function was not satisfied in certain structures.

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