CHANGES IN INCORPORATION OF LABELED AMINO ACIDS INTO INDIVIDUAL PROTEIN FRACTIONS OF RAT BRAIN TISSUE AFTER INTRAUTERINE HYPOXIA

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Acute intrauterine hypoxia leads to a disturbance of growth and differentiation in the CNS. This posthypoxic period is characterized by a succession of changes involving different aspects of brain tissue metabolism, including protein metabolism [2]. Moderate intrauterine hypoxia has been shown to cause activation of ^{14}C-lysine incorporation into total proteins of the cerebral cortex, and also into proteins of the fraction of mitochondria, synaptosomes, and nuclei in 10-day-old-rats. Normalization of protein synthesis takes place later, and by the 46th day of life incorporation of precursors into proteins of the nuclear fraction is reduced [6]. Inhibition of total protein synthesis also has been observed in the cerebral cortex of 3-month-old rats after severe intrauterine hypoxia [3]. The molecular mechanisms of the secondary worsening of the state of protein metabolism of brain tissue have still received only little study.

The aim of the present investigation was to study the effect of intrauterine hypoxia on incorporation of labeled precursors into individual protein fractions of rat brain tissue.

EXPERIMENTAL METHOD

Hypoxia was produced in rats on the 18th-19th day of intrauterine development by controlled compression of the umbilical vessels of the fetus without affecting its vessels from the uterus. In each experiment four fetuses were exposed simultaneously to hypoxia while the remainder served as the control [1]. The rats were studied at the ages of 7, 15, and 30 days of extrauterine life. The intensity of protein synthesis was estimated by measuring incorporation of a mixture of ^{14}C-amino acids (chlorella hydrolysate, from "Chemapol," Czechoslovakia) into proteins of the cerebral cortex and cerebellum 30 min after intraperitoneal injection of the precursors in a dose of 1 μCi/g. Each sample was treated with the tissue solubilizer NCS (37°C, 48 h) and kept in darkness at 15°C for 24 h. Radioactivity was determined in toluene-Triton scintillator on a Mark-3S liquid scintillation counter and expressed in cpm/mg/min of protein, or as the relative specific activity (RSA), namely the ratio of radioactivity of the homogenate to radioactivity of the protein. The protein concentration was determined by the microbiuret method [4].

Total brain proteins of 30-day-old rats were fractionated by an original method. After successive extraction of brain proteins with 0.1% Triton X-100 and 1% sodium dodecylsulfate (SDS) and treatment with 2-mercaptoethanol at 100°C for 2 min, the samples were fractionated on a column (1.6 × 45 cm), filled with Sephadex G-200 and equilibrated beforehand with 0.1 M sodium-phosphate buffer with 0.1% SDS, pH 7.4. The eluate was collected in volumes of 0.5 ml, treated like the total protein samples, and their radioactivity was counted. The following molecular weight markers were used: cytochrome c (12.3 kD), myoglobin (17.8 kD), egg albumin (45 kD), and bovine serum albumin (67 kD). The protein concentration was determined from the optical density of the solution at 280 nm. By means of the suggested method it is possible to carry out simultaneous chromatographic fractionation of membrane and nonmembrane brain proteins and to record the radioactivity of the individual isotope-labeled protein fractions reliably.

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TABLE 1. Intensity of Incorporation of Mixture of ^{14}C-Amino Acids into Proteins of Cerebral Cortex and Cerebellum of Control Rats (n = 19-22) and Rats Exposed to Hypoxia (n = 12), of Different Ages (M ± m)

Parameter	Age of animals, days		
	7	15	30
	Cerebral cortex		
Incorporation of amino acids, cpm/mg protein/min			
Control	145,7±14,6	70,4±21,1	55,1±3,4
Hypoxia	155,6±16,5	66,0±19,4	52,1±1,9
RSA, %:			
Control	46,1±2,6	43,0±3,2	36,4±2,8
Hypoxia	44,5±2,5	38,3±2,1*	38,4±2,0
	Cerebellem		
Incorporation of amino acids, cpm/mg potein/min			
Control	203,7±54,1	113,8±16,2	44,1±4,1
Hypoxia	174,9±30,5	129,2±28,7	45,6±3,3
RSA, per cent			
Control	42,8±2,2	37,8±2,0	36,0±3,0
Hypoxia	38,7±3,0	36,7±3,5	40,9±3,1

Legend. *p < 0.05.

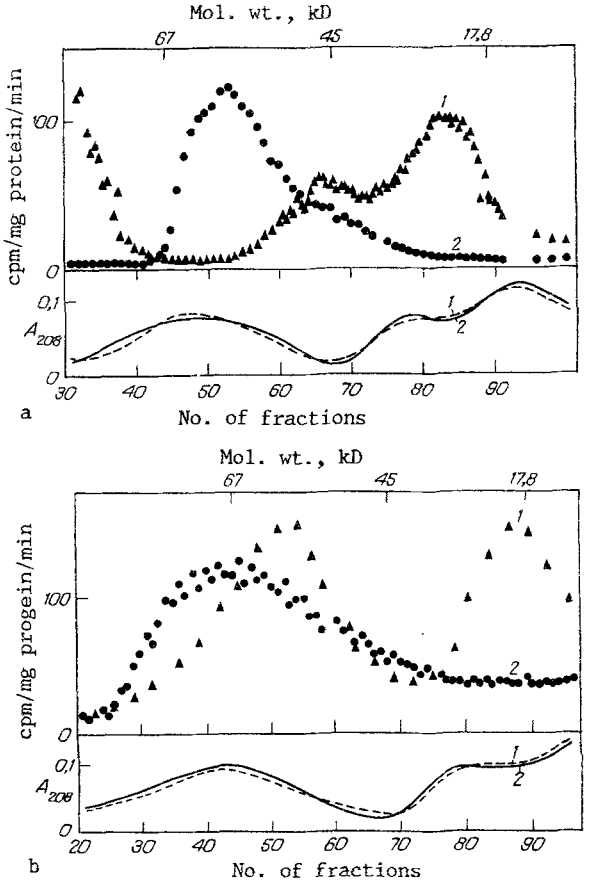

Fig. 1. Incorporation of mixture of ^{14}C-amino acids into proteins of cerebral cortex (a) and cerebellum (b) and elution profile of these proteins in control (1) and after hypoxia (2).

EXPERIMENTAL RESULTS

Incorporation of a mixture of ^{14}C-amino acids into brain proteins of control rats and rats exposed to hypoxia, of different ages, is illustrated in Table 1. The intensity of incorporation of the precursors into total proteins of the cerebral hemispheres and cerebellum of the control rats fell from the 7th to the 30th days of life (by 62.2 and 78.4% respectively). The RSA, reflecting the efficiency of protein synthesis, showed little change with age and averaged 40.8% for both brain regions tested. This parameter makes it

possible to take account of the difference in availability of the precursors for protein synthesis and changes in permeability of the blood-brain barrier.

During the period of the investigation no significant differences were found between the control and experimental animals except a small decrease in RSA in the cerebral cortex at the age of 15 days. The level of total incorporation of labeled amino acids was equalized in this case by an increase in the supply of precursors entering through the blood-brain barrier.

Thus the systems responsible for maintaining total protein synthesis at the normal level in rats exposed to hypoxia during the first month of life remain effective.

Elution profiles of proteins of the rat cerebral cortex and cerebellum and also incorporation of ^{14}C-amino acids, calculated per milligram protein of each fraction, are shown in Fig. 1. With a level of resolution allowing chromatographic fractionation, differences cannot be found in the elution profile of proteins in all brain regions between experimental and control animals. Meanwhile the distribution of the radioactive label among different fractions in rats exposed to hypoxia differed significantly from the control. The low-molecular-weight peak (30 kD), characteristic of the control rats, was found in the experimental animals at the background level both in the cerebral cortex and in the cerebellum. In the higher-molecular-weight region, peak incorporation was shifted considerable relative to the control: in the cerebellum from 52 to 67 kD and in the cerebral cortex instead of two peaks with mol. wt. of 40 and 80 kD, only one maximum was observed, with mol. wt. of 53 kD.

Several workers have shown disparity between the pattern of protein fractionation and the intensity of incorporation of precursors into particular protein fractions in the case of brain tissue. It was not into fractions which contained a larger quantity of protein that more intensive incorporation took place [5]. The reasons for this disparity were not discussed. It can be tentatively suggested that during an exposure of 30-40 min the radioactive precursors are incorporated mainly into proteins with a rapid turnover, which does not necessarily include the majority of brain proteins. Changes in incorporation of amino acids into different brain polypeptides after intrauterine hypoxia may have many different causes: a disturbance of synthesis of regulatory proteins (30 kD), a change in the average rate of synthesis of high-turnover proteins, acceleration or deceleration of maturation processes in the tissue, so that a pattern of incorporation characteristic of a later or an earlier stage of normal development can be observed. Furthermore, a disturbance of coordination of ontogenetic formation of structures and the formation of the metabolic pathways of the brain may take place. Further studies of the age dynamics of synthesis of individual protein fractions are needed to shed light on this problem.

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