

ELECTROPHORETIC INVESTIGATION OF BRAIN
PROTEINS OF MICE WITH CONGENITAL MICROPHTHALMIAM. G. Uzbekov
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831.31-008.939.6-074

Soluble and membrane proteins of the cerebral cortex of normal mice (line C57BL/6J) and mice with microphthalmia (line OR-OR) were investigated by disk electrophoresis in polyacrylamide gel at pH 8.9. The membrane proteins were solubilized with 1% Triton X-100 and 0.1% sodium dodecylsulfate solutions. Significant differences were found in the composition of the proteins extracted with sodium dodecylsulfate in the normal and congenitally blind mice. Thirteen separate fractions were isolated from the proteins of these cortical extracts of normal mice, but only 11 protein fractions were found in the same brain extracts from the mice with microphthalmia. No differences in principle were found in the character of distribution or the content of the protein fractions in soluble proteins from the cortex of the normal and congenitally blind mice. It is concluded from the results that the genetically determined exclusion of photic stimulation in these animals leads to marked structural and biochemical changes in the brain.

KEY WORDS: microphthalmia; proteins of the cerebral cortex; electrophoresis in polyacrylamide gel.

The study of the changes taking place in the brain of animals as a result of congenital exclusion of the functions of brain systems provides an approach to the elucidation of the principles governing the morphochemical differentiation of the CNS. To investigate some aspects of this problem, the brain of congenitally blind mice in which microphthalmia develops at about the 12th day of intrauterine development [1], was investigated [1]. Considerable disturbances of the metabolism of various compounds in the ganglionic cells of the retina were found in such mice, together with simultaneous inhibition of the electrical activity of these cells [5, 7]. The rate of the slow component of the axoplasmic current in the optic nerve is also reduced [5]. Histochemical investigations on the visual cortex of mice with microphthalmia have shown a decrease in the total protein reserves in the bodies of the neurons [2] and a decrease in the intensity of the reaction for nucleoproteins in their nuclei [6].

The object of this investigation was to make an electrophoretic study of the cerebral cortical proteins of mice with microphthalmia.

EXPERIMENTAL

Adult microphthalmic mice of line OR-OR (ocular retardation) and mice with normal sight of line C57BL/6J were used. In each experiment the brain of a normal and a mutant mouse was investigated simultaneously. A weighed sample of cerebral cortex was homogenized in 10 volumes of 0.02 M phosphate buffer, pH 6.3. The homogenates were centrifuged for 1 h at 100,000 g in the VAC-601 ultracentrifuge (East Germany) and the supernatant (saline extract) was collected. The residue was again extracted with 1.5 ml % Triton X-100 solution in the same phosphate buffer and the resulting suspension was centrifuged for 1 h at 100,000 g; the supernatant was discarded. The residue that remained was extracted with 1.5 ml of 0.1% sodium dodecylsulfate solution (SDS) in phosphate buffer, centrifuged for 1 h at 100,000 g, and the supernatant (the SDS extract) was collected.

Laboratory of Biohistochemistry, Brain Institute, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR B. N. Klovskii.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 78, No. 12, pp. 31-34, December, 1974. Original article submitted February 19, 1974.

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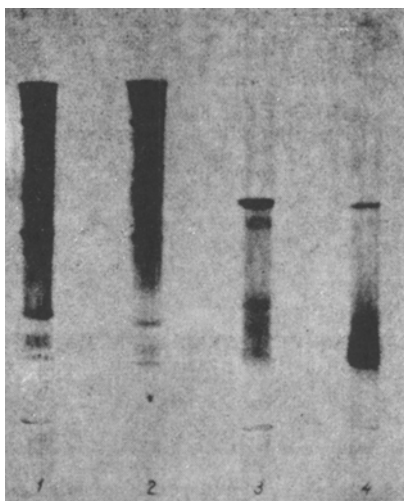


Fig. 1. Electrophoresis of proteins in saline and SDS extracts of the cerebral cortex of normal and congenitally blind mice: 1, 2) saline extracts of normal and blind mice, respectively; 3, 4) SDS extracts of normal and blind mice, respectively.

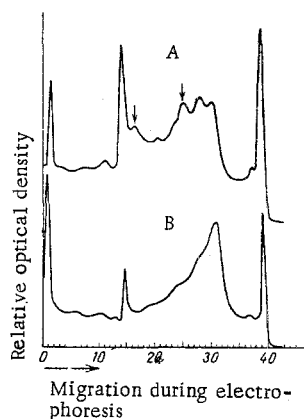


Fig. 2. Densitograms of gels after electrophoresis of protein in SDS extracts of normal (A) and congenitally blind (B) mice. Arrows indicate protein fractions not present in SDS extracts of the cortex of the blind mice.

Disk electrophoresis of proteins of the saline extract was carried out in 7.5% gel at pH 8.9 with reagents and apparatus manufactured by Reanal (Hungary). The length of the separating gel was 50 mm and that of the concentrating gel 15 mm. The solution of the protein (190–210 μ g) containing 25–30% sucrose was applied to each gel and a current of 4 mA applied to each tube. Electrophoresis was carried out for 3 h at 20°C. The gels were then stained with 1% Amido Black 10B in 7% acetic acid. Fractionation of the proteins of the SDS extract was carried out in the same way, but 0.1% SDS was included in the composition of the separating and concentrating gels and the electrode buffer. The densitometric investigation of the stained gels was carried out with a Carl Zeiss (East Germany) instrument.

The protein content in the extracts was determined by the method of Lowry et al. [8].

RESULTS AND DISCUSSION

Figure 1 shows photographs of the stained gels obtained after electrophoresis of saline extracts (gels 1, 2) and SDS extracts (gels 3, 4) of the cortex of normal and congenitally blind mice. Visual and densitometric examination revealed no difference in principle

in the character of the distribution or quantity of protein fractions in the saline extracts from the brain of normal and mutant mice. Meanwhile the intensity of staining of some protein components was reduced in the saline extracts of the brain of the blind mice; this evidently reflects a change in the relative proportions of the fractions of soluble proteins in the control and experimental animals.

The normal and blind mice differed significantly in the composition of proteins extracted by the anionic detergent SDS, i.e., proteins comprising mainly the structural proteins of the membranes. It will be clear from Fig. 2 that densitometry of the stained gel obtained after electrophoresis of the SDS extracts of the normal mouse brain revealed 13 separate fractions, whereas only 11 protein fractions were found in the SDS extract of the brain of the mice with microphthalmia. The two fractions missing from the SDS brain extract of the congenitally blind mice are indicated in Fig. 2 (A) by arrows.

In mice with congenital microphthalmia qualitative changes are thus found in the composition of the insoluble brain proteins. These findings are in agreement with results obtained by Pigareva et al. [2].

Using cytochemical methods these workers found a decrease in the protein content in the bodies and processes of neurons in layers IV and V of the visual cortex of mice with microphthalmia. It is also known that three fractions present in the retina of normal animals are not found on disk electrophoresis among proteins soluble in SDS in the retina of adult congenitally blind mice [4]. The specific protein opsin has been identified among the proteins of these three fractions. These results suggest that proteins specific with respect to visual impulses exist at the cortical level also, in the membranous structures of its visual area.

The results of the present investigation in conjunction with data in the literature [2, 4, 5] suggest that the composition and, probably, the metabolism of the cell membrane proteins not only in the retina, but also in the cortex, have certain characteristic features in mice with microphthalmia. This is shown also by data on interaction between the synaptosomes of the brain of congenitally blind mice and serotonin and tryptamine obtained by a method developed by the present writers [3]. According to these results the brain synaptosomes of mice with microphthalmia bind the above-mentioned indolamines almost twice as actively as the brain synaptosomes of mice with normal sight.

It can be concluded from the analysis of these experimental results and data in the literature that genetically determined exclusion of visual impulses in animals leads to considerable structural and biochemical changes in the visual system and, probably, in the brain as a whole. In mice with normal sight, impulses reaching the central visual system by some means or other evidently bring about the complete effectuation of the genetic information lodged in the cells, especially as regards the quantity and composition of the proteins. This hypothesis is confirmed by observations showing that the retinas of normal and congenitally blind mice do not differ in the composition of their protein fractions during the first three weeks of birth [4], i.e., up to a certain period their development in this respect is identical. However, in the adult state, distinct differences are found between these structures. It is difficult at present to say whether these differences in the protein composition in the cerebral cortex of adult mice with microphthalmia are associated entirely with the incomplete age-biochemical differentiation of the cells, or whether under these conditions the development and metabolism of the cells also exhibit certain pathological features.

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