QUANTITATIVE ANALYSIS OF HETEROGENEITY OF RAT BRAIN MITOCHONDRIA IN ISOLATED FRACTIONS AND in situ

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The mitochondrial system of various objects in situ is in the form of a branching thread [12, 14]. Biochemical studies have shown that in the nerve cell this system has a similar organization [6]. The writers have postulated that if mitochondria (MCH) in nerve tissue are thread-like, the index of heterogeneity (IH) in the MCH fraction ought to change depending on tissue homogenization conditions. Solutions of different composition can be used as the factor modifying the parameters of brain homogenization. IH can thus be used as a criterion for assessing the structural organization of the mitochondrial system in the nerve cell.

The object of this investigation was to study IH of mitochondria in relation to the conditions of isolation of mitochondrial fractions and to compare it with IH of mitochondria in the cell, with particular reference to rat brain nerve cells.

EXPERIMENTAL METHOD

MCH were isolated in solutions (Table 1) by the method in [2]. The technique of electron microscopy was described in the same place.

The morphometric method of determination of the purity of MCH using electron micrographs is based on known principles [1, 13]. Its details are as follows: A Plexiglas plate, on which squares of equal size are drawn, is placed on a photograph of the MCH fraction. The side of each of the squares is equal to the diameter of the smallest MCH on the photograph. The number of squares (in per cent) corresponding to all MCH, divided by the number of squares occupied by all structures, gives the index of purity of the mitochondrial fraction.

TABLE 1. Indices of Heterogeneity of MCH in Isolated Fractions and inside the Cell and also of Portions of the Thread-like Structure in Sections of the Cell Model (M ± m)

Object	No. of exps.	MCH, %		Ratio of small MCH/large MCH
		small	large	
Model of arrangement of one thread-like structure inside ball	6	68±6,2	32±4,0	2,1±0,24
Nerve cell	20	66±2,6	34±1,2	1,9±0,08
Mitochondrial fractions obtained in solutions of:				
0.28 M sucrose	3	28±2,6	72±5,0	0,39±0,04
0.28 M sucrose + 0.1 mM EDTA	3	17±1,8	83±8,2	0,20±0,03
0.3 M mannitol with 0.1 mM EDTA	3	53±6,0	47±4,8	1,13±0,12
Krebs–Ringer solution with 5.5 mM glucose	3	32±3,1	68±4,4	0,47±0,04
Krebs–Ringer solution without glucose	3	26±2,5	74±6,5	0,35±0,04

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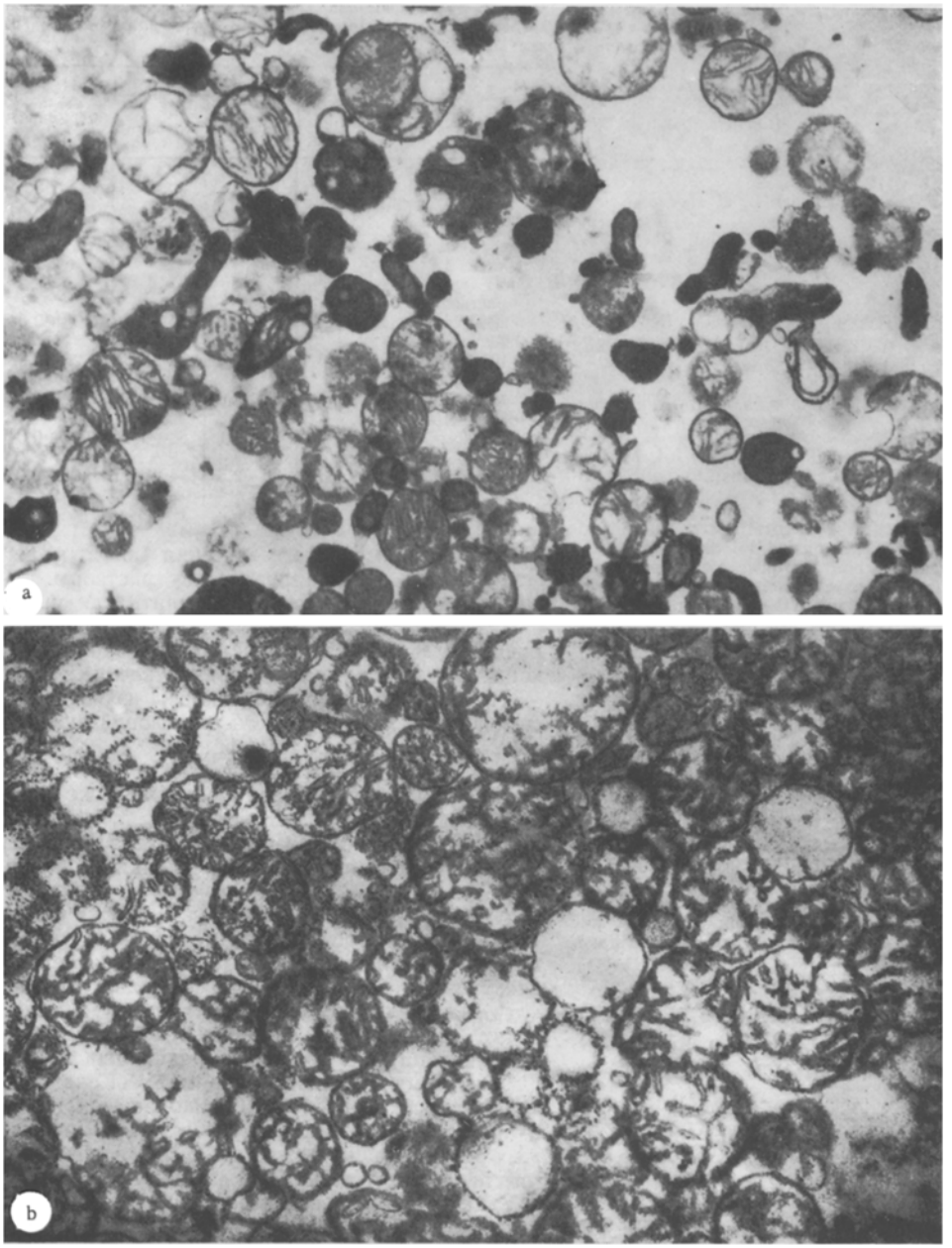

Fig. 1. Electron micrographs of rat brain MCH fractions. a) MCH isolated in sucrose solution (osmium tetroxide), 20,000×; b) MCH isolated in Krebs–Ringer solution with glucose, 30,000×.

The following parameters were used as IH: a) the ratio of the numbers of small MCH to the number of large MCH; b) the distribution of the ratio between width and length of MCH. Small MCH were those whose area was 1-3 squares.

A length of plastic cord, wound at random inside a hollow ball filled with phosphate cement, was used as the model of arrangement of the thread-like structure in the cell. The volume of cord in the ball, like the volume of MCH in the neuron, was about 7%. Sections through the model were analyzed.

EXPERIMENTAL RESULTS

The purity of the MCH fractions in solutions of sucrose, sucrose with EDTA, mannitol with EDTA, and Krebs–Ringer solution with and without glucose was 59 ± 3.7, 60 ± 5.4, 47 ± 3.8, 89 ± 6.1, and 78 ± 7.2% respectively.

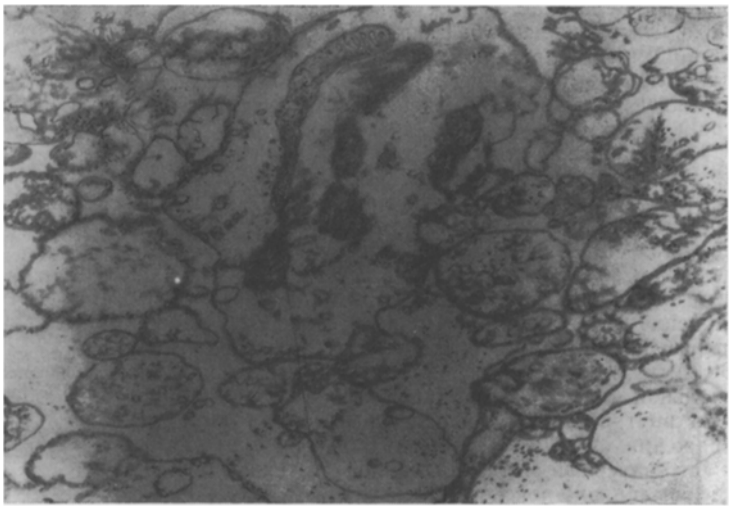

Fig. 2. Electron micrograph of nuclear residue of rat brain homogenate with large and thread-like MCH; 20,000×.

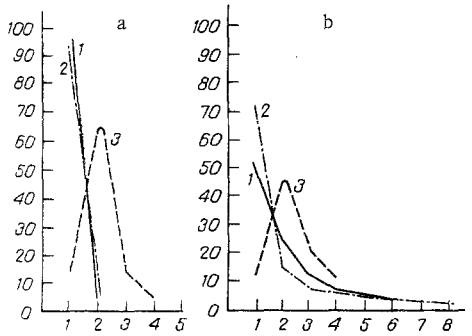

Fig. 3. Graphs of distribution of relative values of width (a) and length (b) of segments of thread-like structure in model (1), of MCH in section through cell (2), and in MCH fractions (3). Abscissa, ratio of width of MCH to width of smallest MCH (a); ratio of length of MCH to length of smallest MCH (b); ordinate, number of MCH (in %).

Electron micrographs of MCH isolated in the first (0.28 M sucrose) and fourth solutions (Krebs–Ringer solution with glucose) are illustrated in Fig. 1; photographs of MCH isolated in the second, third, and fifth solutions were published previously [6, 11]. Morphometric estimates of homogeneity of the MCH fractions were practically the same as purity calculated on the basis of a theoretical coefficient of 100% purity of brain MCH [5, 6]. The functional characteristics of these MCH fractions have been described in the literature [2, 5, 6-10].

The maximal yield of MCH did not exceed 20-30% [6]. Grinding brain tissue in a homogenizer of Dounce type leads to destruction of more than 80% of the cells. The comparatively small yield of MCH cannot therefore be explained by inadequate disintegration of the cells. The supernatant obtained after sedimentation of MCH from the postnuclear homogenate contained up to 3-5% of the total quantity of MCH in the homogenate, and the nuclear residue contained 70-80% [6]. It was suggested on the basis of these findings that the mitochondrial system in the nerve cell is a thread, large fragments of which are deposited in the nuclear residue during centrifugation. Electron-microscopic investigation of the nuclear residue revealed the presence of long MCH (fragments) in 5-6 or 25-30 fields (Fig. 2).

IH of the MCH fraction differed from each other and also significantly from IH in situ (Table 1). In the fractions there were more of the large MCH, in situ more of the small MCH. The number of small MCH in

the fractions was in fact less because some of the large organelles (about 10%) were only "scalped" in the plane of the section and in the photograph they appeared as small MCH. Consequently, heterogeneity of the MCH preparation was significantly lower than in the cell.

The graphs in Fig. 3 show that virtually all MCH in the cell were of the same width but of different lengths, like the segments of the cord seen in a section through the model. The parameters of distribution of relative width and length of MCH in the fraction differed in principle from the graphs of the model in situ. This shows that MCH in the fraction were spherical and discrete. It was impossible for there to be any small structures in the model, because one thread-like "organelle" had been taken. The practically identical IH values in the model and in the cell (Table 1, Fig. 3) suggested that the mitochondrial system in the cell has a thread-like structure.

Analysis of more than 50 electron micrographs of sections through nerve cells published by other workers, and in which at least several dozen MCH were seen, showed that long MCH were present in almost 100% of cases. Long structures were seen with the same frequency in the model. Large, oval, and long MCH were found in the cell [15]; the small MCH were round and oval, the large were elongated. Where the process of a nerve cell could be seen in cross section the MCH was round, where it was cut in longitudinal section it was long.

It can thus be concluded that the mitochondrial system in the nerve cell has the appearance of a thread or of several long thread-like organelles. The mitochondrial thread is perhaps one of the forms which appears at a certain stage in the life of the cell. Skulachev [3] put forward the idea that the reticular structure of MCH in muscle fibers facilitates conduction of signals for contraction within the fibers. Probably the same organization of the mitochondrial system in nerve cells determines intracellular conduction of nervous excitation.

Data from the literature cited in this paper were supplied by the "Biomed" Information Retrieval System [4], which can give answers to about 10^{12} types of questions concerning information on tissue respiration, the mechanism of oxidative phosphorylation, synthesis, metabolism, and the structure of substances, enzymes, receptors, the histology and cytology of the brain, liver, heart, and other systems of the body, their functions under normal and pathological conditions, and several model states (interrelations at levels from molecular to the whole organism, treatment, ontogeny, phylogeny, biorhythms, nutrition, inflammation, trauma, infection, immunity, transplantation, and the action of chemical and physical factors, including nonionizing and ionizing radiation, etc.).

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LITERATURE CITED

1. A. A. Glagolev, Geometric Methods of Quantitative Analysis of Rocks [in Russian], Moscow–Leningrad (1933).
2. L. F. Panchenko, A. A. Shpakov, A. M. Dudchenko, et al., Tsitologiya, 15, 1481 (1973).
3. V. P. Skulachev, in: Biochemistry of Mitochondria [in Russian], Moscow (1976), p. 8.
4. A. A. Shpakov, Nauch.-Tekh. Inform. (VINITI), Ser. I, No. 5, 14 (1978).
5. A. A. Shpakov, Biofizik, 23, 85 (1978).
6. A. A. Shpakov, A. M. Dudchenko, and V. F. Dudchenko, Tsitologiya, 18, 312 (1976).
7. A. A. Shpakov and A. V. Kosarev, Biofizika, 21, 1046 (1976).
8. A. A. Shpakov and A. V. Kosarev, Biofizika, 22, 164 (1977).
9. A. A. Shpakov and A. V. Kosarev, Biofizika, 22, 663 (1977).
10. A. A. Shpakov, A. V. Kosarev, and O. G. Zymaleva, Biofizika, 22, 829 (1977).
11. A. A. Shpakov, L. F. Panchenko, A. M. Dudchenko, et al., Tsitologiya, 16, 1124 (1974).
12. H.-P. Hoffman and C. J. Avers, Science, 181, 749 (1973).
13. A. V. Loud, J. Cell Biol., 15, 481 (1962).
14. E. Mugnaini, J. Cell, Biol., 23, 173 (1964).
15. E. B. Sandborn, Cells and Tissues by Light and Electron Microscopy, New York (1970).