EFFECT OF SODIUM DESOXYCHOLATE AND TRYPSIN ON THE MITOCHONDRIA OF THE GRAY AND WHITE SUBSTANCE OF SOME STRUCTURES OF THE RABBIT BRAIN

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The attention of investigators has recently turned to the study of the structure and properties of the mitochondrial membrane. This structure, which is semipermeable, is very important in the regulation of the exchange of material between the cytoplasm and the mitochondria. In the mitochondrial membrane are concentrated the basic oxidative enzymes. During the process of oxidative phosphorylation the high energy compound ATP is synthesized, that basic energy source for cell activity.

According to electron microscopic data, the mitochondria of nerve tissue possess a double external membrane and a number of internal membranes, or cristae, between which the matrix-substance, with a consistency similar to cytoplasm, is located [22].

The majority of investigators suggest that the external mitochondrial membrane consists of two dense protein layers separated by a less dense bimolecular lipid layer [20, 8, 19, 17, 18, 23].

However, observations made with the high resolving power of the electron microscope permit revelation of a more complex structure of the mitochondrial membrane [6, 13, 18, 24]. There are indications that mitochondria of different origins possess certain differences in membrane structure [6, 9, 16, 15].

It is known that different brain structures have different levels of oxidative activity [4, 5, 7, 11, 12]. It may be suggested that these peculiarities in certain degree are conditioned by the peculiarities of mitochondrial membrane structure and properties.

One method for studying the properties of the mitochondrial membrane is the selective disruption of its components (proteins and lipids). In this way the permeability of the envelope, and thus the volume of the mitochondria, is altered. The degree and kinetics of the swelling (or shrinking) of the mitochondria may, evidently, characterize the nature of the protein-lipid composition of the membrane of these organelles [9, 16, 15].

The present work was devoted to a comparative study of the dynamics of swollen mitochondria from different rabbit brain structures as affected by trypsin and sodium desoxycholate to obtain data which characterize the peculiarities of the protein and lipid components of these mitochondrial membranes.

METHODS

We studied mitochondria isolated from the cerebral cortex, the grey matter from certain brainstem structures (the nucleus of the optic tubercle, the lateral and medial geniculate bodies, the corpora quadrigemina), the white matter which lines the cerebral cortex (cerebral white substance) and the white matter of the rabbit spinal cord. The brains of 17 animals were used.

Mitochondria were isolated according to the method of Fonyo and Somogyi [12]. Cooled brain tissue was homogenized in four volumes of 0.25 M sucrose in 0.01 M Tris buffer, pH 7.4 at zero deg. The homogenate (10%)

was filtered through two layers of cheesecloth and was centrifuged for ten minutes in a TsLP-1 at 1000 rpm. The supernatant was centrifuged for 15 min at 11,300 rpm. The top, friable layer of the precipitate was carefully washed off. The dense part of the precipitate was resuspended in the isolation medium and was centrifuged for ten min at 13,600 rpm. The mitochondrial precipitate obtained was subjected to further study.

The degree of purity of the mitochondrial suspension was controlled by phase contrast microscopy and staining with acid fuchsin by the method of Altman [21]. Mitochondria in the suspensions being studied were counted under phase contrast in a Goryaev chamber.

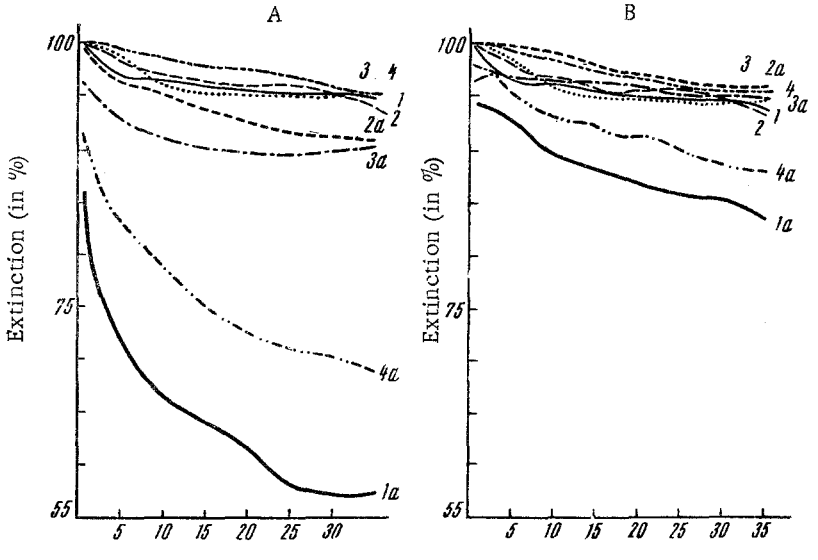
Change in mitochondrial volume was measured on an SF-4 spectrophotometer at 520 millimicrons, t = 22-23°, according to the method of Cleland [10], which permits recording of the degree and kinetics of the volume change in the mitochondria by the changes in optical density of the suspension. The mitochondria were incubated in 0.25 M sucrose in 0.02 M Tris buffer at pH 7.4. The final volume of the suspension was 3.2 ml, E_{520} = 0.350-0.360. Change in optical density was recorded each 3-5 min for 35 min. The results were expressed in per cent, taking the absorption at 10-30 seconds after the mitochondria were mixed with the incubation medium as 100.

Trypsin was used in a concentration of one mg/ml, sodium desoxycholate at 2.5×10^{-3} M. The degree of mitochondrial swelling in the presence of trypsin or desoxycholate was equal to the difference between the values of control absorption (spontaneous swelling) and the absorption of the mitochondrial suspension after 30 min of incubation with the reagents. The protein content of the mitochondria was measured according to the method of Lowry [14] with Folin's reagent [3].

RESULTS

In 3.2 ml of the suspension of cortical mitochondria which we studied (E_{520} = 350-360) there was 0.32 mg of protein. In the same volume of mitochondrial suspension from the white matter the protein content was considerably lower (0.17 mg). Samples of cortical mitochondria contained approximately 1.4 times more organelles than samples from the white matter. Each mitochondria from the cortex contained an average of 0.32 picograms (10^{-12} g) of protein, and mitochondria from the white matter contained 0.24 picograms.

In the figure are presented data concerning the mitochondrial swelling in mitochondria from the above-mentioned structures of the rabbit brain. The curves are constructed from the mean values (from 5-8 experiments). It follows from analysis of the curves that spontaneous swelling of the mitochondria from all the brain structures studied was of very minor degree and was of similar magnitude (4-6%).



Spontaneous swelling (1-4) and swelling in the presence of sodium desoxycholate (A, 2.5×10^{-3} M) or trypsin (1 mg/ml) (1a-4a) of mitochondria from rabbit brain.

In the presence of sodium desoxycholate and of trypsin the mitochondria exhibited different degrees of swelling. The greatest degree of swelling after the action of both substances was shown by mitochondria from the cerebral hemisphere, the least by mitochondria from the cerebral white matter and spinal cord white matter.

For mitochondria from the cortex, brainstem, white matter of the cerebrum and spinal cord the degree of swelling after application of trypsin was respectively, 9.3, 6.7, 2.3, 0.8%; after application of sodium desoxycholate—38, 25.4, 3.2, 4.8%.

Mitochondria from the white matter were practically resistant to the action of trypsin and swelled only to a very small degree as the result of sodium desoxycholate. After 30 min of incubation the degree of swelling of these mitochondria was almost the same as that of mitochondria from the spinal cord white matter.

It should be noted that the difference in degree of swelling between mitochondria of the brain structures being studied was much more marked when sodium desoxycholate was used than with trypsin.

Our attention now turns to certain similarities in the kinetics of the swelling of mitochondria from the cortex and gray matter of the brain stem during incubation of eight to 30 min.

The material obtained permits the hypothesis that there are essential differences in degree and kinetics of mitochondrial swelling in mitochondria from different brain structures and these differences are not related to the numerical inequality of mitochondria in samples studied. This assumption is confirmed by data from experiments with cat brain mitochondria, where it has been established that the degree of mitochondrial swelling is inversely proportional to the concentration of mitochondria in the incubated sample. In the present work the mitochondrial suspension of rabbit brain cortex contained almost 1.4 times more mitochondria than the suspension of white matter, but had a much greater degree of swelling. This suggests that the membranes of mitochondria from different brain structures differ in this property. It is possible that this is related to the different specific content of protein and lipids in the mitochondrial membranes. In such a case the specific content of proteins and lipids increases in the following order: cerebral cortex → brainstem gray matter → spinal cord white matter → cerebral cortex white matter.

On the other hand, the mitochondrial envelope in different brain structures may be distinguished not by the quantitative but by the qualitative composition of proteins and lipids and by the spatial arrangement of the corresponding complexes. In this connection it may be hypothesized that the proteins and lipids of the cortical mitochondrial membranes are more accessible to the action of trypsin and sodium desoxycholate than the membranes of the remaining brain structures we have studied.

The greater difference in degree of mitochondrial swelling in the brain structures under study as the result of sodium desoxycholate in comparison to trypsin treatment may indicate that the external mitochondrial membranes are significantly different in lipid content rather than in protein components.

The data obtained in this study, evidently, indicate a certain similarity in the composition or arrangement of proteins and lipids in the membranes of mitochondria from the grey matter (cortex and brainstem) and difference in these features of the latter from mitochondria from the white matter (spinal and cerebral) of the rabbit brain.

Thus, the results of the present study suggest that mitochondria isolated from different rabbit brain structures differ in functional respects, in structure and in metabolic activity and are characterized by different properties of the protein-lipid components in their membranes.

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