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Effect of Carnosine on Self-Organization of Mitochondrial Assemblies in Rat Liver Homogenate

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Abstract—The effect of carnosine on self-organization of mitochondrial assemblies was studied in rat liver homogenate of quiescent and excited animals. It was shown in separate electron microscopy experiments with serial slices that under our conditions of preparation of homogenate, blocks of native mitochondrial-reticular network in the cell, assemblies of mitochondria, are kept. Carnosine was shown to prevent dissociation of assemblies during storage. Its effect is maximal for more dissociated assemblies from excited animals with decreased ability for self-organization. Prevention of disassembly of organelles by carnosine can serve as one of the mechanisms of carnosine-induced diminishing of muscle fatigue under prolonged work.

Key words: mitochondria, carnosine, morphometry, self-organization

Mitochondria in their native state in intact cells exist in the form of filamentous structures or assemblies bound through the endoplasmic reticulum. This branched structure is called the mitochondrial reticular network [1-4]. Interaction of endoplasmic reticulum with mitochondria is necessary for their biogenesis, including transport of proteins and lipids [5-7] and Ca^{2+} [8-11] into mitochondria. The preservation of structural interaction between mitochondria and reticulum provides milder activation of Ca^{2+}-dependent mitochondrial enzymes than on the addition of this ion to isolated single organelles, as well as makes possible biosynthetic processes during activity. On the other hand, succinate oxidation, the most powerful energy process in mitochondria, serves as a factor providing formation of native reticulum in the form of parallel tubules [12].

Maintaining the ability of living systems for restoration in the course of activity is a crucial condition for physiological work without fatigue. It can be suggested that phenomenon of prevention of muscle fatigue under work, discovered by S. E. Severin [13], is based on the ability of carnosine to support the structural organization of the mitochondrial network in the cell.

Rat liver homogenate was taken for initial investigations considering that mechanisms of cytoskeleton support in different tissues have much in common and probably include contractile proteins, as well as the protective effect of carnosine is manifested not only in muscle, but also in other tissues [14]. Earlier we reported a convenient method for investigation of self-organization of mitochondrial assemblies in rat liver homogenates [15, 16]. Our previous investigations showed that structural properties are more sensitive tests of functional state of mitochondria in the living organism than conventional biochemical measurements [15-18]. This is apparently because video-microscopic investigations are carried out under less influence of conditions damaging native assemblies of mitochondria, such as tissue dilution, incubation media containing sucrose, and incubation with stirring.

The investigation of the effect of carnosine on the ability of mitochondria for self-organization was carried out based on the developed approaches.

MATERIALS AND METHODS

Male Wistar rats weighing 200-240 g bred in a vivarium of the Institute of Theoretical and Experimental Biophysics were used throughout the work. Experiments were carried out in a group of 11 rats, including those in the maximally possible quiescent state and in a state of mild excitation. Excitation was induced by transfer of animals from the warm room in the vivarium to cooler (10°C) for 14 h or by administration of different doses of adrenaline. Adrenaline (25 or 100 μg/100 g body weight)

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was administrated intraperitoneally 30 min before decapitation.

To keep the quiescent state control animals, they were not injected with saline.

Preparation of assembled mitochondria in homogenate. After decapitation the liver was quickly excised, put in homogenization medium (125 mM KCl, 10 mM HEPES, pH 7.4), weighed, and transferred into a cold room. Tissue was crushed through a cooled steel press with holes 1-mm in diameter. Minced tissue was homogenized with addition of 1 ml of medium per 1 g tissue in a loose-fitting Teflon–glass homogenizer at 200 rpm, 3-4 strokes. Homogenate was filtered through a double layer of kapron. The homogenate was ready for measurements in 10 min.

Measurements of area of mitochondrial assemblies. Control and experimental samples (1 ml) of homogenate were kept in thermostatted cells at 12°C. Carnosine solution was added to the experimental cell to final concentration 50 μM. The first experimental sample was analyzed 2 min after preparation of homogenate with subsequent measurements each 12 min for 1 h.

For microscopic measurements 30 μl of homogenate was added in 1 ml of homogenization medium, stirred with standard speed for 5 min, transferred into a Neibauer chamber, and observed using dark field techniques.

A MINTRON CCD MTV-1 802 CB video camera (Candela, Russia) combined with an MBI-11 microscope (Lomo, Russia) was used for observations. The velocity of video frames is about 3 sequences per 1 sec for maximal resolution. The video camera was attached to a Pentium 133 computer with DE-12B2 video card coupled device for image entry from Candela.

The level of scanning was selected based on automatic determination of background density and gray level mode (256 total). Level of scanning was: mode + 45. Each point on the morphometric graphs is the mean of scanning 9 fields of vision containing total number 800-2000 objects. The data were computed by quantitative morphometric analysis using the Image Tool (version 2) program (The University of Texas Health Science Center, San Antonio, USA).

Statistical treatment was made by the Student *t*-test.

Chemicals. The used reagents were: HEPES (Sigma, USA); KCl (Reakhim, Russia), highly pure; carnosine (Rostech, St. Petersburg, Russia).

RESULTS AND DISCUSSION

We have shown earlier that the initial area of assemblies is related to the state of the animal: in control animals mean area is greater than in excited animals or in animal models of pathology [15-18]. During storage of preparations self-organization of blocks of assemblies with endoplasmic reticulum in larger structures occurs. Assemblies of mitochondria contact each other and form joined structures because we use concentrated homogenate with respect to protein (tissue/solution ratio 1 : 1, w/v). Slow dissociation of structures begins with prolongation of storage because of destructive processes in the homogenate.

One of the typical dependences of mean area changes on duration of storage is given in Fig. 1 for control animal.

Index of assembling, Δp, was introduced as an additional quantitative evaluation of self-organization during storage. It is calculated as the ratio of minimal value of mean area within 50-60 min storage (Fig. 1, point *A*) to its maximal value in this experiment (Fig. 1, point *B*) and is expressed in percent. Values of $\Delta p > 100\%$ indicate that self-organization overcomes dissociation, while $\Delta p < 100\%$ indicates dissociation of assemblies. According to our data, processes of dissociation prevail in tissues under excitation, stress, in animal models of diseases, and in human diseases. Under these conditions, support and strengthening of the mitochondrial-reticular network can be a crucial condition for preservation of the function of the cell.

Analysis of kinetics of assembly area changes during storage showed that the effect of carnosine is mostly expressed at the second stage of storage, at 50-60 min, when processes of dissociation prevail (a typical example is presented in Fig. 2). The effect of carnosine was calculated in percents of the control sample.

The influence of carnosine was investigated under different physiological states, in quiescent and excited animals, which are characterized by different kinetics of self-organization of assemblies. These results are presented in the table.

Large mitochondrial assemblies (50-60 μm^2) are typical for control animals. In them, stable maintaining of area or even enlargement by 20-70% was observed. This

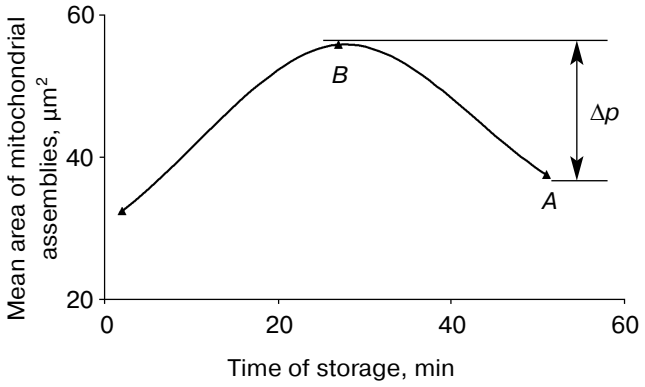

Fig. 1. Changes in mean area of assemblies during storage.

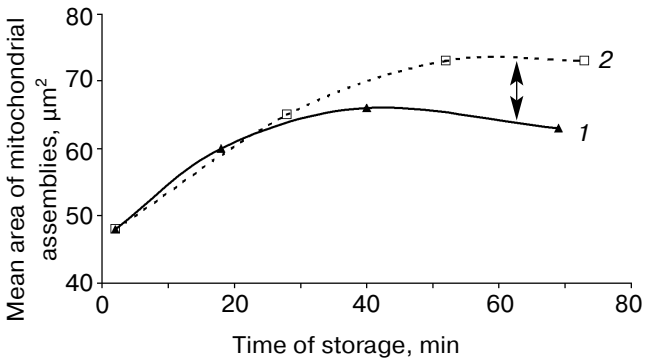

Fig. 2. Effect of carnosine (50 μM) (*2*) on changes in area of assemblies during storage (*1*) (control).

indicates good self-organization and stability of membrane structures. In such preparations carnosine addition did not induce increase in area of assemblies.

The considerably smaller area is typical of assemblies of excited animals as compared with quiescent (about twofold). It is also seen that their self-organization is considerably weakened—dissipation of assemblies occurred during storage. In such preparations, pronounced increase in self-organization under the influence of carnosine was observed. It was twofold in some cases. Pair comparison of mean area of assemblies in control and experimental samples showed that in the presence of carnosine this was nearly twofold larger, $p < 0.01$.

Thus, the effect of carnosine depends on the state of the preparation. Correlation analysis showed that changes in mean area of assemblies under the influence of carnosine is in inverse dependence ($R = -0.66$) on index of assembling (Δp) with high probability: the effect is maximal for impaired structures of excited animals and is practically absent in preparations with good self-organization in control animals.

The presented data show that the effect of carnosine is the strengthening of self-organization. This is mostly pronounced in 50 min of storage, when dissipation of assemblies begins. Dissipation of assemblies is probably the result of destructive processes due to impairment of supporting energy processes or induced by long storage in media with high oxygen pressure.

The reported effect of carnosine, strengthening of self-organization and slowing down of dissociation of assemblies, can be due to two of its properties—cationic nature and antioxidant effect [14, 19]. It was reported that cations Na^+ and K^+ facilitate self-organization of mitochondria in assemblies and filamentous structures [20]; we confirmed this for polycations [21]. However, Na^+ and K^+ increase formation of assemblies at higher concentrations, 5-50 mM, than carnosine. Its concentration used in our work was 50 μM. It should be also considered that some portion of the carnosine could be destroyed by carnosinase. Therefore, the effect of carnosine is considerably more pronounced than that of monovalent cations. The participation of antioxidant properties of carnosine in realization of the reported effect seems to be less probable because in the homogenate, prepared by our method, native antioxidant defense of the cell is well preserved and peroxide oxidation of lipids is one order of magnitude lower than in standard preparation of mitochondria [15].

According to modern views mitochondria can function as an electric cable in cells, namely in the form of filamentous structures [22]. This function is very important for maintaining workability of muscles during their prolong function and for slowing down fatigue. Strengthening of self-organization of mitochondria in assemblies by carnosine, slowing down their dissociation under stress influences, can be supposed to serve as one of the mechanisms of the phenomenon of muscle fatigue prevention under prolonged work.

Therefore, the phenomenon of muscle fatigue prevention under prolonged work by carnosine, discovered by S. E. Severin, can be based also on the ability of carnosine to support self-organization of mitochondria and

Self-organization of mitochondrial assemblies in rat liver homogenate and carnosine effect (50 μM) under different conditions (quiescent and excited animals)

Conditions	Area of assemblies after 50-60 min storage, μm^2	Index of mitochondrial assembling during storage in control samples, Δp, %	Changes in area of assemblies in experimental samples (50 μM carnosine), % of control sample
Control animals ($n = 4$)	57.8 ± 11.3	126.3 ± 35.6	99.3 ± 13.3
Excited animals ($n = 7$)	33.1 ± 8.3*	79.3 ± 22.2*	149.7 ± 36.8**

* Data are valid with respect to control, $p < 0.05$.
** Data are valid with respect to control, $p < 0.01$.

reticulum in network, providing preservation of more stable state of organelles and decreasing their dissipation under load.

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