DYNAMICS OF Ca^{++} TRANSPORT IN RAT LIVER MITOCHONDRIA IN ANOXIA

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The antibiotic tetracycline was used in the experiments as a fluorescent probe for Ca^{++} ions in rat liver mitochondria. Incubation of isolated mitochondria under anaerobic conditions at 20°C led to rapid (30 min) loss of ability of the mitochondria to accumulate Ca^{++}. Disturbances of the Ca^{++}-accumulating power of the mitochondria develop much more slowly (over 2 h) and irregularly during survival of the liver: the maximal values are found after survival for 5-10 and 60 min.

KEY WORDS: anoxia; rat liver mitochondria; calcium accumulation; tetracycline as a fluorescent probe.

An important problem in modern pathology is the study of the mechanisms of early disturbances taking place in the cell in anoxia. The role of Ca^{++} ions in the structural and functional changes in biological objects is particularly interesting. In various pathological states disturbances of energy-dependent processes, including the active transport and "packing" of Ca^{++} ions, are observed [11].

The antibiotic tetracycline has been used as a fluorescent probe for Ca^{++} ions [3, 4]. The writers showed previously that actively accumulated Ca^{++} gives a considerable and slowly developing increase in tetracycline fluorescence [8]. If, however, Ca^{++} ions are passively adsorbed on the surface of organelles, e.g., on membranes of mitochondria inactivated by boiling, a very slight and step-like increase in the fluorescence of this antibiotic is observed. De-energization of the mitochondria, leading to the liberation of Ca^{++} accumulated previously, is accompanied by a decrease in tetracycline fluorescence [8].

The object of this investigation was to study changes in active Ca^{++} transport in anoxia in isolated mitochondria and tissue sections.

EXPERIMENTAL METHOD

Mitochondria were isolated by Schneider's method [12] and washed twice. The incubation medium for the mitochondria contained 0.25 M sucrose, 10 mM tris-HCl, pH 7.4, and 10 mM succinate. In experiments to study the action of hypoxia on isolated mitochondria, anaerobiosis was produced by placing the thick suspension of mitochondria (35 mg protein/ml) in a closed test tube after 4-60 sec, as shown by polarographic determination of oxygen. The final tetracycline concentration in these experiments was 50 μM, and $CaCl_2$ was added in the required concentration in the course of the experiment.

Liver slices 4-5 mm thick were cut at different periods of survival of the organ and stained for 3 min in a solution of tetracycline in a concentration of 1500 μM. The sections were washed twice in the corresponding solutions.

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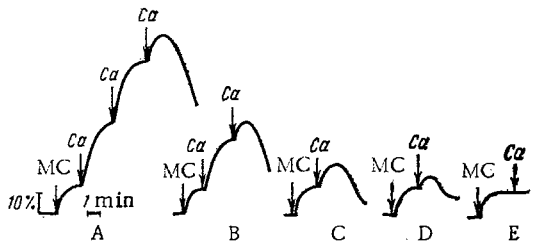

Fig. 1

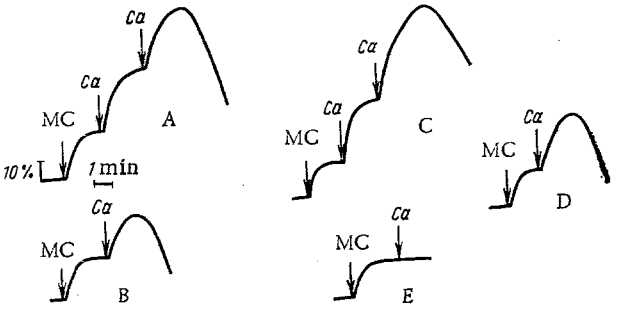

Fig. 2

Fig. 1. Changes in tetracycline fluorescence on addition of Ca^{++} to suspension of mitochondria incubated *in vitro*: A) 0 min of incubation; B) 5 min; C) 10 min; D) 15 min; E) 30 min. Arrows denote times of additions: MC) mitochondria in concentration of 8 mg protein/ml; Ca) 200 μmoles of $CaCl_2$. Here and in Fig. 2: abscissa, time (in min); ordinate, intensity of fluorescence (in %).

Fig. 2. Changes in tetracycline fluorescence on addition of Ca^{++} to suspension of mitochondria isolated from rats decapitated at different times: A) 0 min of survival of liver; B) 30 min; C) 60 min; D) 90 min; E) 120 min. MC) Mitochondria in a concentration of 4.2 mg protein/ml; Ca) 100 μmoles $CaCl_2$.

Fluorescence of tetracycline was excited by light with a wavelength of 380 nm and the intensity of fluorescence was measured at a wavelength of 540 nm.

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments of series I the dynamics of changes in the fluorescence of tetracycline in response to addition of Ca^{++} to the mitochondrial suspension was investigated after various periods of anaerobic incubation at 20°C. To estimate the level of coupling of the energy processes in the mitochondria at the different stages of incubation the respiratory control (RC) was determined. The results of one typical experiment are given in Fig. 1. Clearly the addition of 200 μmoles Ca^{++} to the original suspension (RC 4.8) caused a slow increase in tetracycline fluorescence, evidence of active accumulation of the cation. Repeated addition of 200 μmoles Ca^{++} gave similar changes in this parameter. Further addition of Ca^{++} led to a decrease in the intensity of fluorescence, indicating the liberation of Ca^{++} from the damaged mitochondria [8]. Liberation of Ca^{++} was observed from mitochondria incubated for 5 min (RC 3.5) in response to the second addition. Anaerobic incubation for 10 and 15 min led to a decrease in RC to 1.8 and 1.6, respectively, and to a marked decrease in the Ca^{++}-accumulating power. After incubation for 30 min the energy-dependent functions studied were totally lost: RC was 1 and there was virtually no increase in the intensity of tetracycline fluorescence.

In the experiments of series II the dynamics of the fluorescent responses of mitochondria isolated from the liver of rats at different times after decapitation of the animal to addition of Ca^{++} were studied. It will be clear from Fig. 2 that the addition of Ca^{++} ions in a concentration of 100 μmoles to mitochondria isolated 30 min after decapitation caused a very slight increase in tetracycline fluorescence followed by a decrease. This indicates that, at that stage of survival of the organ, the ability of the mitochondria to accumulate Ca^{++} was reduced. However, in mitochondria obtained 1 h after decapitation of the animal, ability to accumulate Ca^{++} ions was restored, as shown by the marked increase in the intensity of tetracycline fluorescence after addition of Ca^{++}. At later periods of survival of the liver a gradual decrease in the Ca^{++}-accumulating function of the mitochondria was observed until its total loss after 2 h.

In the experiments of series III fluorescent responses of liver slices taken at different times after death and stained with tetracycline were investigated. Since tetracycline is known to stain only mitochondria in tissues [5] and since the intensity of its fluorescence is directly related to the Ca^{++} content in the mitochondria [8], changes in fluorescence of the tissue slices during incubation reflect the state of active Ca^{++} transport by the mitochondria rather than the Ca^{++} content in the cell. It follows from Fig. 3 that the order and magnitude of the changes in the intensity of fluorescence of the stained

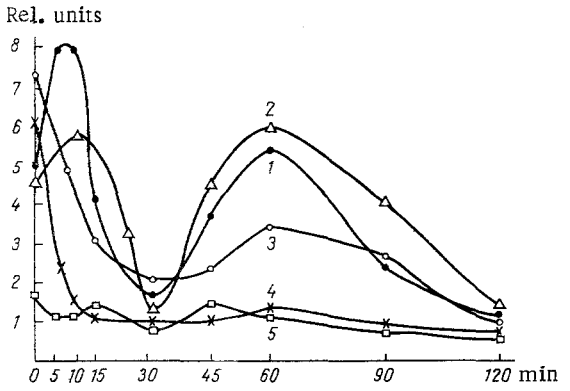

Fig. 3. Changes in fluorescence of liver slices stained with tetracycline during survival. Medium for staining: 1) 0.25 M sucrose; 2) distilled water; 3) Hanks's solution; 4) calcium-free Hanks's solution; 5) 0.95 M NaCl. Abscissa, time (in min); ordinate, intensity of fluorescence (in relative units).

tissue depend on the times of survival and on the composition of the medium in which the material was stained. The character of the fluorescence curve of liver slices stained with tetracycline in sucrose, water, and Hanks's solution suggests that changes in the Ca^{++}-accumulating power of the liver mitochondria during survival takes place in two phases of time: an increase after 5 and 60 min and a decrease after 30 and 120 min. These results, it must be pointed out, agree satisfactorily with those of the study of Ca^{++}-dependent fluorescent responses of mitochondria isolated from the liver after the same periods of survival (Fig. 2). If Hanks's solution not containing Ca^{++} ions was used as the solvent for tetracycline, the high initial level of tetracycline fluorescence in the slices fell steadily and became stabilized after 10-15 min of postmortem incubation. The most probable explanation is that the decrease in tetracycline fluorescence in this case was due to the presence of uncompensated phosphate, which is a stronger competitor for Ca^{++} than tetracycline [6, 7].

If the liver slices were stained in tetracycline in 0.95 M NaCl the intensity of fluorescence of the antibiotic was very low and remained practically unchanged with time, because of the predominant formation of a Na–tetracycline complex, with a low intensity of fluorescence [3, 7].

The most probable cause of the disturbance of functional integrity of the mitochondria in anoxia may be activation of phosphorylase A_2 by Ca^{++} ions entering the cytoplasm. This could also explain the fact that isolated mitochondria, kept under anaerobic conditions *in vitro*, lose their functions much sooner than mitochondria isolated from the liver after various periods under anaerobic conditions. Anoxia of the whole organ is evidently not accompanied by the rapid degeneration of the mitochondria and by liberation of Ca^{++} ions, by contrast with experiments *in vitro*, for the protective mechanisms of the cells or, at least, anaerobic glycolysis may be mobilized. This could lead to the slower development of anoxic damage to the mitochondria. The cause of the increase in the Ca^{++}-accumulating power of the mitochondria after survival of the liver for 1 h is less clear. It must, however, be noted that these results are in close agreement with those of the study of the morphological and biochemical characteristics of the liver and heart tissues during survival [2, 9, 11, 13].

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