TISSUE-SPECIFIC REGULATION OF OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA BY SOLUBLE PHASE OF LIVER CELLS

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KEY WORDS: mitochondria; uncouplers; organization of tissue metabolism; soluble phase of cells.

The soluble phase of cells (SFC) has various effects on the course of intramitochondrial processes. The study of the action of this fraction on oxidative phosphorylation (OP) in mitochondria is important for the understanding of mechanisms regulating the functions of these organelles in the cell. The writer showed previously that the SFC of rat liver [4, 5], and also of rat kidney, heart, lung, and thymus [3], contains tissue-specific regulators reducing the coupling of OP in mitochondria from homologous tissue.

It can be tentatively suggested that the action of these regulators on OP is supplemented by other regulatory influences of SFC on these organelles. That is why the study of the effect of a purified preparation of tissue-specific OP inhibitor on mitochondria does not necessarily give an exact idea of the mechanism of intracellular tissue-specific control of this process.

This paper describes an attempt to study some particular features of the action of the tissue-specific OP inhibitor in liver mitochondria present in the composition of the SFC.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250 g. Mitochondria were obtained from the liver and kidney by Schneider's method with modifications. The mitochondria were "aged" by incubation of a thick suspension of these organelles at 37°C. The method of obtaining tissue-specific uncoupler activity in SFC was described previously [1]. The effect of this fraction on OP was assessed by recording mitochondrial respiration polarographically in a medium with substrate, in the active metabolic state and after its completion [4]. Allowing for addition of the ingredients of SFC in the ratio of 1:1, the incubation medium contained 183 mM sucrose, 35 mM KCl, 3 mM KH_2PO_4, 3 mM EDTA, and 10 mM Tris buffer, pH 6.7. In experiments to study the effect of K^+ ions on the action of SFC on OP, the incubation medium and all other solutions used were free from this cation. Mitochondrial ATPase activity was measured by a pH-metric method based on proton liberation during enzymic hydrolysis of ATP [6]. The incubation medium contained 100 mM sucrose, 100 mM KCl, and 5 mM Tris buffer, pH 6.5.

EXPERIMENTAL RESULTS

The writer showed previously [2] that in the presence of SFC from rat liver, respiration in mitochondria from homologous tissue is stimulated in state "4'." Addition of 2,4-dinitrophenol (2,4-DNP) in a concentration of 0.5 mM, causing complete uncoupling of oxidative phosphorylation, after previous addition of SFC, was shown to inhibit tissue-specific stimulation of respiration induced by this fraction. In this case tissue-nonspecific acceleration of respiration by 1.1-1.2 times was observed compared with the rate of respiration of intact liver and kidney mitochondria in the presence of 2,4-DNP.

On addition of oligomycin, simultaneously with the SFC, to the incubation medium in a

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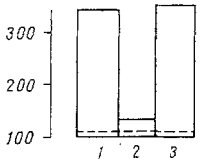

Fig. 1

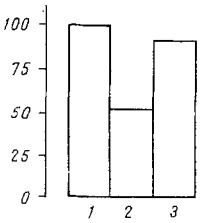

Fig. 2

Fig. 1. Effect of rotenone on tissue-specific uncoupling of OP in liver mitochondria in presence of SFC from same tissue. Ordinate, respiration rate of liver (columns) and kidney (broken line) mitochondria in percent of values for intact mitochondria (1, 2) and of their values in tests with rotenone (3). 1) Addition of liver SFC, 2) addition of rotenone (0.5 μg/mg mitochondrial protein), 3) addition of rotenone and SFC. Rotenone and SFC added to incubation medium of mitochondria before introduction of suspension of these organelles into cell.

Fig. 2. Effect of SFC on ATPase activity of liver and kidney mitochondria. 1) Initial ATPase activity of liver and kidney mitochondria, 2) liver ATPase activity in presence of liver SFC, 3) the same for kidney mitochondria. All data are percentages of their values for intact mitochondria.

concentration of 1 μg/mg mitochondrial protein, stimulation of respiration in liver mitochondria under the influence of SFC reached 160% compared with intact mitochondria. In kidney mitochondria the rate under these conditions reached 105%, demonstrating the tissue-specific character of stimulation of respiration in the presence of oligomycin.

The uncoupling action of some endogenous compounds is known to be due to passage of K^+ through the mitochondrial membrane. Accordingly, the effect of K^+ on the tissue-specific increase in mitochondrial respiration in the presence of SFC was studied. Addition of 38 mM exogenous K^+ was found to reduce ΔO_{act} in liver mitochondria by 25-30% compared with mitocondria incubated in medium not containing exogenous K^+. Replacement of KCl by choline chloride, providing an equivalent increase in the ionic strength of the incubation medium, led to the same decrease in the uncoupling effect of SFC.

It will be clear from Fig. 1 that addition of rotenone to intact mitochondria increased their ΔO_{act} by one-third. On simultaneous addition of rotenone and SFC, ΔO_{act} also was increased by one-third compared with its value in tests in which only SFC was added. Since addition of rotenone to intact mitochondria served as the control for this test, it was found that the uncoupling effect of SFC in the presence of rotenone was equal in value to the uncoupling action of this fraction without inhibitor. Rotenone did not affect the tissue-specificity of the action of SFC.

Measurement of the ATPase activity of liver and kidney mitochondria in the presence of liver SFC showed that the test fraction inhibited activity of this enzyme from liver mitochondria by half. Inhibition of ATPase in kidney mitochondria was much weaker (Fig. 2).

The effect of SFC on respiration and phosphorylation of mitochondria with disturbed OP also was studied (Fig. 3). A disturbance of OP was induced by "aging" the mitochondria in the course of their incubation. The uncoupling effect of SFC was found not to be intensified on weakening of the initial coupling of OP. The tissue specificity of uncoupling remained in this case. Addition of SFC to liver mitochondria with a profound disturbance of OP (inhibition of respiration in the presence of ADP) led to restoration of the parameters of coupling of OP. Unlike liver mitochondria, in kidney mitochondria the disturbed respiratory control, according to Chance, was not restored in the presence of liver SFC.

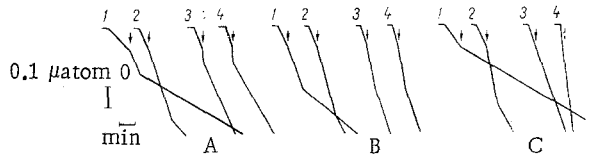

Fig. 3. Effect of liver SFC on liver and kidney mitochondria with different initial states of OP. A) Intact mitochondria; B) mitochondria after "aging" for 20 min; C) after "aging" for 40 min. 1, 3) Liver and kidney mitochondria without additions, 2) liver mitochondria with SFC, 3) kidney mitochondria with SFC.

The data given above are evidence of the complex character of tissue-specific regulation of mitochondrial OP by the soluble phase of liver cells. Inhibition of the uncoupling effect of this fraction by the addition of 2,4-DNP indicates the presence of a tissue-specific factor in liver SFC which disturbs OP in homologous mitochondria. The experiments with oligomycin showed that the tissue-specific stimulation of respiration observed under the influence of SFC is due to uncoupling of OP and not to "extraction" of high-energy compounds. The results of the experiments to study the effect of K^+ on the tissue-specific uncoupling effect of SFC suggest that this effect is evidently not connected with the passage of K^+ through the mitochondrial membrane. Weakening of the tissue-specific uncoupling effect in the presence of this cation can be attributed to an increase in the ionic strength of the incubation medium.

The results of the experiments with rotenone indicate that the tissue-specific uncoupling action of SFC is unconnected with selective disturbance of coupling at the rotenone point.

The tissue-specific inhibition of ATPase activity of the liver mitochondria is evidence of the ability of SFC to exert an opposite to uncoupling effect on OP. These results were confirmed in experiments in which the parameters of coupling were restored in mitochondria with a profound disturbance of OP when SFC was added to them. Consequently, SFC can limit coupling of OP tissue-specifically not only at the maximum, but also at the minimum. It does this by stabilizing OP at around average values of coupling by lowering it in highly coupled mitochondria and raising it in mitochondria with disturbed OP.

Tissue-specific regulators of the influence of liver SFC on liver mitochondrial OP thus determine the tissue organization of liver metabolism through selective "averaging" of the parameters of mitochondrial coupling in different liver cells, without however affecting OP of mitochondria from other tissues. The existence of such a mechanism can be explained by the demands of hormonal regulation of intracellular processes. It is well known that the efficacy of hormone action depends on the functional state of the target tissue. There is no doubt that a change in the degree of coupling of mitochondrial OP directly influences the state of the tissue. Consequently, tissue-specific regulation of the energy mechanisms of cells may be responsible for the identity of their response to the hormonal stimulus.

The writer previously argued in support of the role of tissue-specific uncouplers in the intrinsic regulation of tissue proliferation [2]. Such a regulator was later found by the writer in the adult rat heart, in which, as we know, mitotic activity is absent. Correlation also was demonstrated between the activity of the tissue-specific uncoupler in liver SFC and the duration of starvation of the animal [1]. Besides the results given in the present paper, these data are evidence of a role of tissue-specific mechanisms controlling mitochondrial OP in the intrinsic organization of metabolism of all tissues, including nonproliferating tissues. As regards the role of tissue-specific uncouplers in the regulation of tissue proliferation, it can be regarded as a case of manifestation of intrinsic control of tissue metabolism, brought about by these regulators when mitotic activity of the tissue is increased.

The possibility cannot be ruled out that this regulation of the coupling of OP is effected not by one, but by several regulators, whose action on this process is opposite in sign.

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STATE OF CARDIAC PYRIDINE NUCLEOTIDES AND FLAVOPROTEINS DURING PRESERVATION IN 0.5% FORMALIN SOLUTION

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KEY WORDS: formaldehyde; energy metabolism; myocardial cells.

Papers have recently been published on the use of formaldehyde as a preservative for organs [4]. However, comparatively little can be found in the literature on the mechanism of its action on tissues.

It was accordingly decided to study whether formaldehyde can interact with the mitochondrial respiratory chain and, if so, to determine the site of such interaction.

EXPERIMENTAL METHOD

Rats' hearts preserved by intravenous injection of 0.5% formalin solution were studied. Intact hearts were used as the control.

The energy state of the mitochondria in heart tissue was assessed by luminescence analysis [1, 2]. The fluorescence spectra of the hearts were recorded at 10, 120, and 180 min by means of an expedition microspectrofluorometer with FMÉL-1 probe system [2]. The objective was focused on the muscle cells to record radiation predominantly from the myocardium [2]. The wavelength of the exciting light was 365 nm. Radiation recorded under these circumstances in the region of 460 and 530 nm was due mainly to fluorescence of mitochondrial NADH and flavoproteins (FP) [1, 5]. The energy state of the mitochondria was assessed by determining the ratio between the intensities of luminescence of oxidized FP and NADH [2]:

$$\xi = \frac{I_{530} - {}^1/_2 I_{460}}{I_{460}}.$$

EXPERIMENTAL RESULTS

In the control the coefficient ξ did not exceed 0.1, and it remained at the same level for 2 h (Fig. 1). A very small increase was observed only after 3 h, to 0.14. The value of ξ in the experiment 10 min after preservation was 0.17, and after 3 h it rose to 0.52 (Fig. 1).

Measurement of fluorescence of NADH (Fig. 2) showed that the decrease in the intensity of luminescence immediately after preservation of the heart was 34%, but after 3 h it was 57%. The increase in luminescence of FP of the preserved heart relative to NADH was most marked after 3 h (Fig. 1).

To assess the possibility of the direct effect of formaldehyde on fluorescence of mitocondrial NADH, luminescence of 1 mM solution of NADH was measured after addition of 0.5 mM formaldehyde to it; no change in the intensity of luminescence was observed.

The increase in ξ immediately after preservation of the heart indicates an increase in the degree of oxidation of the respiratory chain of the mitochondria.. In the ischemized organ this could be observed as a result of a block to the progress of reducing equivalents

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