A TISSUE-SPECIFIC UNCOUPLER OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

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The effect of an endogenous uncoupler from rat liver on oxidative phosphorylation of mitochondria in the liver, kidney, heart, lung, and brain was investigated. Its action was shown to be tissue-specific. The selectivity of the action of the uncoupling factor on the mitochondrial membrane is manifested best at pH values from 6.3 to 6.9, close to those in the cytoplasm of the liver. Tissue specificity is independent of the initial state of the mitochondria within wide limits and it can abolished only by thermal injury to oxidative phosphorylation. Activity of the tissue-specific uncoupler is not reduced by sedimentation of ribosomes and microsomes. The addition of bovine albumin to the incubation medium does not affect the degree of tissue-specific uncoupling. The role of the uncoupler in the regulation of proliferation within the tissue is postulated.

KEY WORDS: *tissue-specific uncoupler of oxidative phosphorylation*; *tissue control of proliferation.*

The writers previously showed the existence in rat liver of a factor which, when added to mitochondria (MC) from the same tissue, increased their O_2 uptake in the active metabolic state (ΔO_{act}) and somewhat reduced the respiratory control (RC) after Chance [2, 3]. The observed stimulation of respiration was maintained in the presence of oligomycin, evidence of the uncoupling of mitochondrial oxidative phosphorylation [3]. A similar uncoupler also was found in the kidney [2]. Both factors acted much more strongly on MC from the same tissue than on heterologous MC. This indicated the tissue specificity of the observed effect.

The aim of this investigation was to test the hypothesis of tissue specificity of the action of the uncoupling factor from the liver experimentally and to study some of its properties.

EXPERIMENTAL METHOD

To obtain the uncoupling factor and MC male Wistar rats weighing 200-250 g were used. The uncoupling factor was activated by combined incubation of the water-soluble fraction of the nuclei (WFN) and the mitochondria-free cytoplasmic fraction (MFCF) from liver tissue in the proportion of 1:1 at 37°C and pH 7.5 for 15-20 min [3]. The fractions of ribosomes and microsomes were sedimented by ultracentrifugation of the mixture of WFN and MFCF at 105,000g for 1 h [7]. MC were obtained from the liver, kidney, heart, brain, and lung of a rat by Schneider's method [9] with modifications. Differences in the values of ΔO_{act} did not exceed 10-15%. The effect of the uncoupling factor on oxidative phosphorylation was assessed by polarographic measurement of respiration of MC (2 mg MC protein in 1 ml) in medium containing 5 mM succinate, in the active metabolic state and after its completion. The measurements were made in a constant-temperature covered cell with revolving open platinum electrodes at 32°C. The fraction containing the uncoupling factor was added to the cell before MC. The incubation medium, allowing for the addition of this fraction in the proportion of 1:1, contained 183 mM sucrose, 35 mM KCl, 3 mM KH_2PO_4, 3 mM EDTA, and 10 mM Tris buffer, pH 6.7. For the work with MC from brain and lung, bovine albumin (Koch-Light Laboratories Ltd.) was added to the incubation medium in a concentration of 5 mg/ml.

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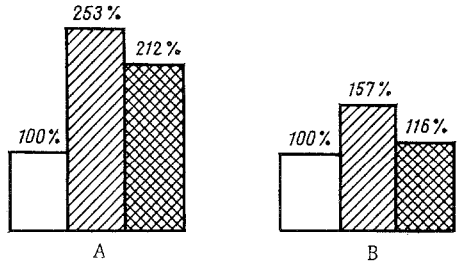

Fig. 1. Effect of sedimentation of ribosomes and microsomes on uncoupling of oxidative phosphorylation in MC of liver and kidney by endogenous factor from the liver. A) Values of ΔO_{act} for MC of liver; B) the same for MC of kidney. Unshaded columns represent intact MC; obliquely shaded columns addition of preincubated WFN and MFCF; cross-hatched columns, the same, after sedimentation of ribosomes and microsomes. pH of incubation medium, 7.2.

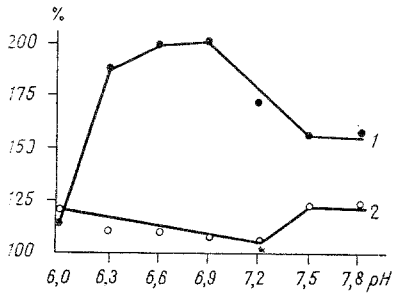

Fig. 2. Effect of pH of incubation medium of MC on degree of tissue-specific uncoupling of oxidative phosphorylation. Abscissa, pH values; ordinate, ΔO_{act} (in percent of their values in intact MC): 1) action of uncoupling factor from liver on homologous MC; 2) its action on MC of kidney.

TABLE 1. Effect of Uncoupling Factor from Liver on MC of Rat Liver, Kidney, Heart, Brain, and Lung

Tissue of origin of MC	ΔO_{act}	Phosphorylation time	RC
Liver	223±8	213±10	75±4
Kidney	112±5	83±4	123±6
Liver	240±10	302±15	87±6
Heart	92±5	62±3	133±3
Liver	189±4	144±7	92±4
Brain	95±2	89±5	141±6
Liver	191±7	167±9	83±5
Lung	105±4	61±4	144±7

Legend. Results expressed as percentages of values for intact MC.

EXPERIMENTAL RESULTS

In the preliminary experiments a mixture of WFN and MFCF containing ribosomes and microsomes, preincubated together, was added to MC. The cytosol used also possessed tissue-specific activity. For that reason, sedimentation of the fractions of ribosomes and microsomes was desirable not only to study the intracellular localization of the tissue-specific uncoupling factor, but also to differentiate its action as far as possible from tissue-nonspecific uncoupling. Results are shown in Fig. 1. After sedimentation of the above-mentioned fractions only the tissue-specific activity of the uncoupling factor remained. In the next experiments the microsomes and ribosomes were sedimented. As Fig. 2 shows, at pH 7.5-7.8 there was only a comparatively small difference in the strength of action of the uncoupling factor from the liver on MC from the liver and kidney. When the pH was lowered this difference increased, not only because of an increased effect of the uncoupler from the liver on oxidative phosphorylation of homologous MC, but also on account of weakening of its action on MC of the kidney. The optimal pH range for manifestation of the phenomenon of tissue-specific uncoupling of oxidative phosphorylation was 6.3-6.9. At pH 6.0 the differences in the action of the uncoupling factor on MC of both tissues disappeared completely on account of weakening of uncoupling in MC of the liver.

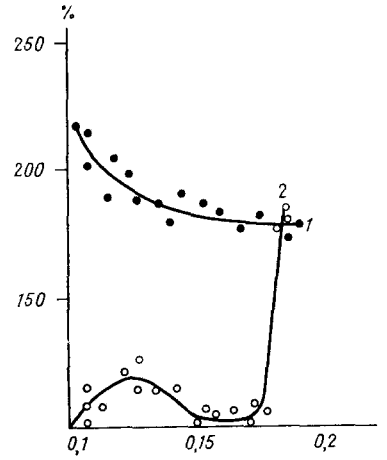

Fig. 3. Effect of thermal injury to MC on tissue-specific uncoupling: 1) MC from liver; 2) from kidney. Abscissa, ΔO_{act} of MC of liver and kidney, preincubated at 37°C (in μatoms O_2); ordinate, the same, after addition of uncoupling factor from liver to MC (in percent of values of ΔO_{act} for thermally injured MC).

The next task was to study the effect of uncoupling factor from the liver on MC of various rat tissues (Table 1). The action of this factor from liver on MC of heart, brain, and lung was similar to its action on MC of kidney and differed sharply from its action on oxidative phosphorylation of homologous MC. On the addition of the uncoupler to liver MC an increase in ΔO_{act}, an increase in the phosphorylation time, and a decrease in RC were observed. This uncoupling factor did not increase ΔO_{act} in MC of all other tissues, it reduced the phosphorylation time, and increased RC. The effect of the uncoupling factor from the liver on brain and lung MC, incidentally, cannot be explained by the presence of albumin in the incubation medium, for special experiments showed that bovine albumin, in a concentration of 5-10 mg/ml, does not affect tissue-specific uncoupling of oxidative phosphorylation.

Since no loss of tissue specificity could be observed in the action of the uncoupling factor from liver and kidney MC when kept in the cold, in the course of this investigation the initial state of MC was impaired by preincubation in isolation medium at 37°C for 3-20 min (Fig. 3). Exposure of the MC to heat led to an increase in ΔO_{act}. Weakening of the effect of the hepatic uncoupling factor on liver MC was observed under these circumstances. Preincubation of kidney MC led initially to a very small increase in uncoupling under the influence of liver uncoupling factor. Later, when ΔO_{act} of the preincubated MC had increased by 50% compared with the intact MC, the selectivity of action of the liver uncoupling factor increased again a little. During subsequent exposure of MC to heat, the specificity of action of the uncoupling factor was completely lost. No correlation was found between the values of RC and the phosphorylation time for the preincubated MC or the changes in these indices and ΔO_{act} under the influence of the uncoupler.

The results are evidence of the tissue specificity of uncoupling factor from the liver. The selectivity of its action on the mitochondrial membrane is best manifested at pH values close to those of the cytoplasm of the liver [5]. The tissue specificity of the endogenous uncoupling factor cannot be explained by differences in the initial state of the intact MC, for it can be abolished only by thermal injury to oxidative phosphorylation.

The question of the possible function of the tissue-specific uncoupler in living tissue arises. A role in regulation of proliferation within the tissue can be postulated for it [4]. In the light of modern views on the organization of this regulation, the effector inhibiting mitotic activity as the cell population density increases has to satisfy several demands [6, 10]. It must possess tissue specificity; its concentration in the tissue must rise proportionally to mitotic activity; accumulation of the effector in the cells must prevent their subsequent passage through mitosis. The uncoupler studied in the present experiments satisfies these demands. As this paper shows, it possesses tissue specificity. Its activation requires interaction between WFN and the cytoplasm; consequently, an increase in its concentration must be expected in the prophase of mitosis of the cell, when lysis of the nuclear membrane is complete. In view of this, the concentration of the uncoupling factor in the tissue should rise proportionally to its mitotic activity. Uncoupling of oxidative phosphorylation in MC is known to cause the ATP level in the cell to fall, with a corresponding rise in the concentration of AMP, a powerful inhibitor of the initial stage of synthesis of purine nucleotides [11]. The uncoupling of oxidative phosphorylation can therefore lead to inhibition of DNA synthesis and, consequently, to a reduction in the mitotic activity of

the tissues. The hypothesis of activation of the uncoupling factor in the course of mitosis is confirmed by stimulation of respiration of sea urchin and amphibian eggs, which has been found to reach a maximum in the prophase of mitosis [8, 12], and also by information on the fall in the ATP concentration in the regenerating rat liver at the time of maximal mitotic activity [1].

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DETECTION OF RESERPINE-LIKE SUBSTANCES IN THE MYOCARDIUM OF ANIMALS AND MAN

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Substances similar in some of their physicochemical properties to reserpine were found in the myocardium of animals and man. The tissues of the myocardium were shown to synthesize these substances from formate and tryptophan.

KEY WORDS: *myocardium; biosynthesis of reserpine-like substances.*

Alkaloids are a group of organic compounds, as a rule of plant origin, with considerable and varied biological activity. At the beginning of the 1970s the first reports appeared of the discovery of alkaloids in animal tissues [1-3] and the possibility of their biosynthesis in the tissues of mammals by condensation of L-dopa and two of its mono-O-methyl esters with formaldehyde and acetaldehyde [2].

These investigations led the present writers to look for the presence of alkaloid-like compounds in the tissues of animals and man and, in particular, in the myocardium.

EXPERIMENTAL METHOD

The substances for study were isolated from the myocardium of the right and left ventricles of man, ox, and Wistar rats. The human heart was used not later than 12 h after death;

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