the enzyme system for Ca transport in SR was stronger (Fig. 3). The presence of exogenous HP of LA in this case also had no effect on the permeability of SR membranes for Ca ions (Fig. 3, Table 1).

It can be concluded from these results that among the primary molecular products of lipid peroxidation, namely hydroperoxides of free fatty acids and hydroperoxides of phospholipids, only the latter are effective modifiers of permeability of SR membranes for Ca ions.

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TISSUE-SPECIFIC UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION OF MITOCHONDRIA FROM THE RAT HEART, KIDNEY, THYMUS, AND LUNG

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The effect of microsome-free cytoplasmic fractions from the rat heart, kidney, thymus, and lung on oxidative phosphorylation (OP) of mitochondria (MCH) from these tissues and also of MCH from liver and brain was investigated. Crossed experiments showed the existence of tissue-specific uncouplers of OP in these fractions, similar in their properties to that found previously in rat liver. The possible role of these regulators in the initiation of enzymic degradation of MCH through activation of phospholipase A during the outflow of calcium ions from MCH is discussed. Activation of DNase I associated with the mitochondrial membrane is postulated under these conditions.

KEY WORDS: tissue-specific uncouplers; calcium; phospholipase A; DNase I.

The writer showed previously that rat liver tissue contains a factor whose action on homologous mitochondria (MCH) has been identified as uncoupling [2]. On the addition of the fraction containing this factor to MCH from the kidney, heart, brain, and lung its action on oxidative phosphorylation (OP) was found to be tissue-specific [3]. Tissue-specificity was expressed as a selective increase in the uptake of oxygen by MCH in state 3 (ΔO_{act}) and an increase in the duration of phosphorylation (t_p) in homologous MCH. The addition of bovine albumin, which inhibits the uncoupling action of free fatty acids on OP, did not affect the amplitude of tissue-specific uncoupling.

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TABLE 1. Effect of Uncoupling Factors from Rat Heart, Kidney, Thymus, and Lung on OP of MCH from These Tissues and also of MCH from Brain Liver, M ± m

Tissue from which uncoupler obtained	Series of experiments	Tissue from which MCH obtained	ΔO_{act}, mμ atoms		ΔO_{act}, %	t_p, sec		t_p, %
			C	UF		C	UF	
Heart	I	Heart	94±3	173±12	184	8±2,0	18±3,2	227
		Kidney	98±4	107±8	109	12±2,2	13±1,5	106
		Liver	105±6	119±6	114	23±3,0	25±4,2	110
	II	Heart	96±2	185±10	192	9±2,0	25±3,6	272
		Lung	110±4	110±5	100	28±2,6	30±2,8	106
		Brain	108±3	117±3	109	26±3,0	22±2,5	85
Kidney	I	Kidney	100±4	345±15	345	14±2,0	32±3,4	226
		Liver	103±5	129±3	126	21±3,0	13±2,2	60
	II	Kidney	102±3	253±13	248	12±1,0	25±1,8	210
		Heart	98±4	103±4	105	8±2,0	7±1,2	86
	III	Kidney	96±6	258±8	268	11±1,6	26±1,5	240
		Lung	107±4	134±5	125	30±4,0	37±2,8	125
	IV	Kidney	105±2	216±5	206	12±2,2	34±2,6	280
		Brain	112±6	141±3	126	25±2,0	21±1,8	85
Thymus	I	Thymus	104±3	210±9	200	32±3,0	52±5	162
		Kidney	102±4	110±5	109	12±1,6	7±1,2	60
		Liver	105±6	120±7	114	22±2,2	22±2,4	100
		Heart	97±3	99±8	102	9±1,8	10±1,5	105
		Lung	109±5	124±10	113	27±3,2	29±3,4	107
		Brain	106±4	113±6	107	29±3,6	26±2,6	94
Lung	I	Lung	110±3	217±7	197	31±3,2	65±6,2	209
		Heart	95±4	96±4	101	7±1,0	8±1,2	112
		Kidney	99±6	101±6	102	11±1,6	13±1,6	115
		Liver	106±3	117±4	110	25±3,4	27±2,6	108
		Brain	109±5	112±5	103	27±2,4	26±2,2	95

Note. 1) n = p× P≤0.05. Data given in absolute values and as percentages of their values in intact MCH. 2) C – control (intact MCH); UF) the same parameter for MCH in presence of uncoupling factor.

The results of the experiments described below demonstrated the existence of such regulators of OP in all the rat tissues listed in the title.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. To obtain endogenous uncouplers the corresponding tissue was homogenized in a solution of 280 mM sucrose with 10 mM Tris buffer, pH 8.5, in the proportion of 1 : 7. The nuclear fraction (600g, 10 min) mitochondrial fraction (6000g, 15 min), and fraction of total lysosomes of the endoplasmic reticulum (105,000g, 60 min) were sedimented successively from the resulting homogenate [4]. Activity of the tissue-specific uncoupler was determined in microsome-free cytoplasm (MFC) obtained in this manner. MCH were obtained by Schneider's method in the modification in [8]. The effect of MFC on OP of MCH was as assessed by polarographic measurements of their respiration in medium containing 5 mM succinate in state 3 and after the end. Measurements were made in a closed constant-temperature cell with a volume of 1 ml, with a rotating open platinum electrode at 26°C. In the experiments with thymus MCH the temperature in the cell was 32°C. MFC were added to the cell before addition of MCH in a dose equivalent to 2 mg protein. 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 experiments with brain, thymus, and lung MCH and also in experiments in which factors from thymus and lung were added to MCH, highly purified bovine albumin (from Koch-Light) was added to the incubation medium in a concentration of 10 mg/ml.

EXPERIMENTAL RESULTS

To detect tissue-specific uncoupling activity in MFC from the heart, the action of this fraction on the homonymous tissue was compared with its effect on OP of MCH from rat kidney, liver, lung, and brain (Table 1). The results show that MFC from the heart, although almost doubling ΔO_{act} of MCH from homologous tissue, had practically no effect on this parameter of MCH from heterologous tissues. Similar selectivity of action of this fraction also was found in relation to the values of t_p.

The results of the experiments to study the effect of MFC from the kidney on MCH from the liver, kidney, heart, lung, and brain also demonstrated the existence of an uncoupling factor in rat kidney tissue with the

property of selectively increasing ΔO_{act} and t_p for MCH from the homonymous tissue (Table 1). As Table 1 shows, the effect of this fraction on OP of MCH was indistinguishable from the action of MFC from the heart.

The next experiments were carried out with MFC from rat thymus (Table 1). This fraction also was found to have the tissue-specific property of increasing the oxygen uptake by MCH from this tissue in state 3 and increasing the phosphorylation time of added ADP.

The effect of MFC from the lung on OP of MCH from homologous and heterologous tissues also was investigated (Table 1). The results showed that MFC from this tissue has a tissue-specific effect on ΔO_{act}, and also on the value of t_p for MCH from the homonymous tissue.

Comparison of the character of action of uncouplers from rat kidney, heart, thymus, and lung on MCH from homonymous and heterologous tissues with the properties of the tissue-specific uncoupler from the liver showed that their effect on ΔO_{act} and t_p of MCH is similar. Changes in the values of Chance's respiratory control under the influence of the endogenous regulators listed above in homologous MCH coincided with those obtained during a study of uncouplers from the liver [3]. Meanwhile the respiratory control in heterologous MCH was changed only a very little in the presence of all the uncouplers tested, and these changes were not consistent in character. No increase in the respiratory control, characteristic of addition of uncoupler from the liver to MCH from heterologous tissues, was observed in these experiments.

To sum up the data described above, it can be concluded that rat heart, kidney, thymus, and lung tissues contain tissue-specific uncouplers with properties similar to that of the uncoupler from the liver which the writer found previously [2,3]. The wide distribution of tissue-specific uncouplers suggests the existence of similar regulators in the other tissues of the rat.

The character of the action of tissue-specific uncouplers on OP of MCH suggests that in the presence of a sufficiently deep disturbance of mitochondrial function these regulators, like their synthetic analogs [7], can reduce the calcium capacity of MCH and so promote the passive outflow of calcium ions from the intramitochondrial specimen. Under these conditions phospholipase A, which degrades the mitochondrial membrane [9], is known to be activated. Under certain conditions it can thus be expected that tissue-specific uncouplers can participate in the initiation of enzymic degradation of MCH from the homonymous tissue. The writer's previous experiments showed that tissue-specific uncoupler from the liver is activated by interaction between the water-soluble fraction of the nuclei and the cytoplasm. For this reason an increase in the concentration of the above-mentioned regulator in the cell must be expected in the course of its mitotic division, which is accompanied by total degradation of the nuclear membrane. The stimulation of respiration [10] and fall in the ATP level [1] observed under these circumstances are in good agreement with this hypothesis. It is interesting to note that mitotic cell division is also accompanied by a decrease in the total mass of MCH on account of their degradation [5]. It is also suggested that degradation of the mitochondrial membrane during the discharge of calcium under the influence of an endogenous uncoupler can lead to activation of DNase I, associated with this membrane [6].

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