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PHOSPHOLIPASE A_2 ACTIVITY DETERMINES RATE OF MITOCHONDRIAL RESPIRATION IN HIBERNATING ANIMALS

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When animals sink into a state of hibernation the intensity of their oxidative metabolism decreases by 50-100 times within a few hours, and it increases just as quickly to the normal level on awakening [5]. In full agreement with the physiological state of the animals, isolated mitochondria (MCh) from the liver of hibernating susliks are characterized by a greatly reduced rate of respiration compared with the MCh of active animals [1]. No sufficiently convincing explanation of inhibition of mitochondrial respiration in hibernating animals has yet been given. One probable cause of the slowing of respiration may be a change in the physicochemical properties, microviscosity for example, of the membranes of MCh, leading to disturbances of the working of the membrane-bound enzymes. In turn, changes in the physicochemical properties of the membranes may be based on a change in mitochondrial phospholipase A_2 (PLA_2) activity [3].

To test this hypothesis, an investigation was carried out to study the role of PLA_2 in regulation of the rate of mitochondrial respiration in hibernating susliks.

EXPERIMENTAL METHOD

Susliks of the species Citellus undulatus were used in the experiments. MCh were isolated from the liver and incubated as described previously [1]. The rate of mitochondrial respiration was determined with the aid of a Clark's oxygen electrode in a thermostatically controlled cell with a volume of 2 ml at 27°C, with constant mixing. To assess the microviscosity of the membranes, polarization of fluorescence of 1,6-diphenylhexatriene was used [2]. The rate of ATP synthesis was determined enzymatically, by measuring accumulation of glucose-6-phosphate in the presence of 0.1 U hexokinase and 10 mM glucose, and recording fluorescence of NADPH [12]. The rate of substrate transport was estimated from swelling of MCh in 100 mM solutions of ammonium succinate, β-hydroxybutyrate, and glutamate [7]. Swelling of MCh was judged by the decrease in optical density of the mitochondrial suspension at 520 nm. The protein concentration was determined by Lowry's method.

EXPERIMENTAL RESULTS

The measurements showed that the microviscosity of the mitochondrial membranes in hibernating animals was higher than in active susliks: 0.252 ± 0.028 compared with 0.194 ± 0.017 P, respectively (n = 3). The increased microviscosity of the mitochondrial membranes

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TABLE 1. Rate of Mitochondrial Respiration (in ng-at O_2/min/mg protein) in Liver of Active (A) and Hibernating (B) Susliks Depending on Sucrose Concentration in Medium and Presence of BPB (M ± m)

Concentration of sucrose, mM	Rate of respiration					
	−ADP		+ADP		+ClCCP	
	A	B	A	B	A	B
250	35,0±4,8	19,2±4,0	145,0±10,8	46,6±5,8	216,6±14,7	57,5±4,1
60	51,4±8,3	25,7±3,8	111,1±8,4	91,3±7,2	208,3±18,3	160,6±9,8
250 + BPB	42,7±6,4	25,9±4,4	147,8±11,4	48,4±6,0	204,0±10,1	69,6±5,7
60 + BPB	38,8±5,1	23,7±3,0	138,9±10,4	50,0±5,6	210,2±12,1	72,4±6,1

Legend. Substrate 4 mM succinate. Additives: ADP 100 μM, ClCCP 1 μM, BPB 10 μM. ClCCP denotes carbonyl cyanide-m-chlorophenylhydrazone.

of hibernating susliks may possibly be one cause of the lowering of their oxidative activity. This suggestion is confirmed by the fact that activation by Ca^{++} ions of PLA_2, which has a "liquifying" action on the membranes as a result of hydrolysis of phospholipids [4], almost completely abolishes inhibition of mitochondrial respiration in hibernating animals [1]. Mitochondrial respiration of hibernating susliks also was significantly activated by a decrease in tonicity of the medium (Table 1). However, in this case, by contrast with activation of respiration by Ca^{++}, coupling of oxidation and phosphorylation is preserved to an adequate degree. Accordingly, with a fall in tonicity of the medium the rate of ATP synthesis in MCh of hibernating animals rose considerably: from 52.5 ± 5.0 to 75.3 ± 6.8 nmoles/min/mg protein (n = 4). Incidentally, MCh of active animals responded to lowering of the tonicity of the medium by a decrease in the rate of phosphorylating respiration and an increase in the rate of respiration in the absence of ADP. Incubation of MCh in a hypotonic medium caused them to swell, and this was accompanied by activation of lipolysis [6]. The PLA_2 inhibitor bromphenacyl bromide (BPB) [8] completely prevented activation of mitochondrial respiration in hibernating animals in hypotonic medium (Table 1). Consequently, abolition of inhibition of respiration in medium with low tonicity is the result of PLA_2 activation; BPB had hardly any effect on MCh of active animals or on MCh of hibernating susliks, incubated in isotonic medium. It must also be pointed out that BPB had virtually no effect on the actual process of swelling of MCh in hypotonic medium.

It is difficult at present to state how mitochondrial respiration of hibernating susliks is stimulated during activation by PLA_2, or how closely this is linked with a decrease in viscosity of the mitochondrial membranes. The difficulty is due to the fact that the mechanisms leading directly to the deep inhibition of mitochondrial respiration in hibernating animals are still unknown. One cause of inhibition of respiration may be inhibition of the transport of oxidation substrates across mitochondrial membranes. Our measurements showed that the rate of swelling of MCh of hibernating susliks in isotonic solutions of ammonium succinate, β-hydroxybutyrate, and glutamate is 2-2.5 times slower than that of MCh of active animals (Fig. 1). This is evidence of inhibition of substrate transport in MCh of hibernating susliks. Addition of 10-15 mmoles Ca^{++} per milligram protein to the cuvette considerably accelerated the swelling of MCh of hibernating susliks in ammonium succinate solution (Fig. 2) but had virtually no effect on the rate of swelling of MCh of active animals. The local anesthetic sovcaine (an analog of cinchocaine and a PLA_2 inhibitor) prevented the effect of Ca^{++}. Thus in these experiments activation of mitochondrial PLA_2 was accompanied by acceleration of substrate transport within MCh. These results are in good agreement with those obtained by other workers. It was recently shown that the therapeutic preparation piracetam, which activates PLA_2 [11], stimulates transport of ^{14}C-succinate into the mitochondrial matrix [9].

It can be concluded from these results that stimulation of mitochondrial respiration of hibernating susliks during activation of PLA_2 in the case of calcium loading, and also during swelling in a hypotonic medium may be connected with acceleration of transport of oxidation substrates through the membranes inside MCh. The possibility cannot be ruled out that this is due to lowering of the microviscosity of the lipid bilayer of the mitochondrial membranes.

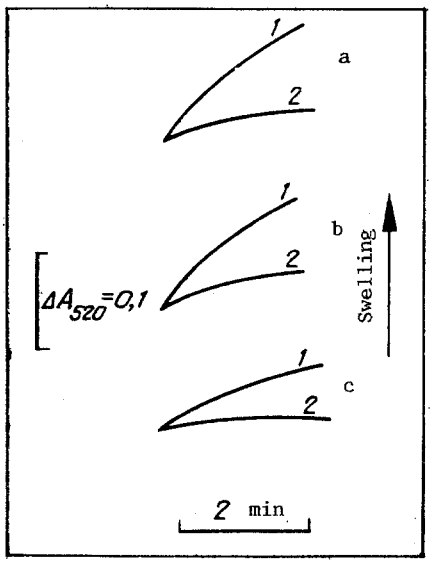

Fig. 1. Swelling of MCh in liver of hibernating and active susliks in 100 mM solutions of ammonium succinate (a), glutamate (b), and β-hydroxybutyrate (c). 1) MCh of active susliks; 2) MCh of hibernating susliks. All solutions contained 20 mM Tris, pH 7.4. In the case of succinate and glutamate, 3 mM KH_2PO_4 was added to the cuvette; to prevent changes in volume of the mitochondria due to metabolic processes, in all cases 1 μg antimycin A was added to the mitochondria.

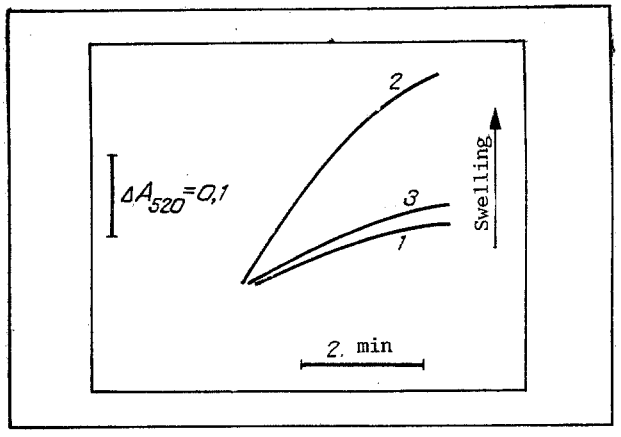

Fig. 2. Effect of Ca^{++} ions and cinchocaine on rate of swelling of liver MCh of hibernating susliks in 100 mM ammonium succinate solution. 1) Swelling of MCh without extra additives; 2) swelling of MCh after accumulation of 15 nmoles Ca^{++}/mg protein; 3) the same as 2, but in the presence of 300 μM cinchocaine.

The writers previously obtained evidence of a decrease in PLA_2 activity in hepatic MCh of hibernating susliks [1]. With these data in mind, and also the results given in this paper, it can be concluded that inhibition of phospholipase activity and associated changes in the state of the mitochondrial membranes of hibernating susliks are among the main causes of the inhibition of substrate transport and of oxidative metabolism as a whole when animals sink into a state of hibernation. On emergence from this state, activation of mitochondrial PLA_2 may bring about the rapid rewarming of the animals due to intensification of oxidative processes in MCh taking place with the liberation of a considerable quantity of heat [10].

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CROSSED RESISTANCE OF TUMOR CELLS WITH HIGH RESISTANCE TO COLCHICINE

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During chemotherapy with any antitumor preparation the tumor cells may acquire resistance not only to the same preparation, but also to several other chemical compounds differing in their structure and mechanism of action. This phenomenon has been called multiple drug resistance (MDR). Despite much progress in the study of MDR [1, 2], many aspects of this phenomenon remain unclear. A matter of undoubted interest for the understanding of the mechanisms of MDR is a study of crossed resistance (CR). The list of substances exhibiting CR is long and includes the Vinca alkaloids, colchicine, anthracycline antibiotics, and antinomycin D. Investigation of various cell lines with MDR has led to the following ideas: during the development of MDR the highest level of resistance is observed most frequently to a selective agent, and the level of CR to other preparations is as a rule lower. The degree of CR in different cell lines varies considerably, but it usually correlates directly with the level of resistance to the selected agent [1, 2]. However, this picture is not always observed. For instance, CR to Vinca alkaloids and to gramicidin C in some cell lines with MDR was found to be considerably higher than resistance to the selective preparation [3, 4]. In human cells resistant to podophyllotoxin, CR was observed to a wide range of preparations, but sensitivity to Vinca alkaloids, colchicine, and actinomycin D was completely preserved [5].

In the investigation described below, to study the causes and principles of variability of the character of CR in MDR, a series of cell lines with a very high level of resistance to colchicine was obtained. This model was used to study the development of CR to several preparations while the resistance to the selected agent was increased.

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