ACTION OF A CYTOTOXIN FROM THE VENOM OF A CENTRAL ASIATIC COBRA ON RAT LIVER MITOCHONDRIAL FUNCTION

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The action of various concentrations of cytotoxin (C), free from contamination with phospholipase A, on respiration and oxidative phosphorylation of rat liver mitochondria and their "ghosts" and also on the permeability of their internal membranes for various ions was studied. Low concentrations of C (10-25 μM) do not affect functional parameters of intact mitochondria but increase the permeability of the internal membranes for monovalent ions sharply. The uncoupling action of C on mitochondrial "ghosts" is evidently due to the formation of nonspecific "channels" of conductance in the membrane with low selectivity for size and charge of the ion.

KEY WORDS: cytotoxin; mitochondria; oxidative phosphorylation; permeability; rat liver.

A system of structural toxins consisting of phospholipase A and membrane-active polypeptides which, by their joint action, cause destruction of the lipid skeleton of membranes, has been found in the venom of snakes of the family Elapidae [6, 9]. Membrane-active polypeptides, known in the literature as cytotoxins, and phospholipase A have now been isolated in the pure form from venoms of the Elapidae [1, 2, 5, 9, 11]. The cytotoxins have been shown to induce passive permeability of biological and artificial phospholipid membranes for monovalent cations [12]. The writers showed previously [3] that disturbance of the structure and functional parameters of isolated mitochondria is produced by the phospholipase A of the venom of the Central Asiatic cobra and a fraction of the cytotoxin containing only trace amounts of phospholipase A as an impurity.

In the present investigation the action of the pure cytotoxin, uncontaminated by phospholipase A, on mitochondria was studied.

EXPERIMENTAL METHOD

Cytotoxin was obtained from the venom of the Central Asiatic cobra Naja naja oxiana E. in the pure form by the method described previously [5]. The cytotoxin is a basic polypeptide with hydrophobic properties; its molecular weight is 6500 and its molecule consists of 60 amino acid residues, the order of which has been completely established [10].

Rat liver mitochondria were isolated by differential centrifugation [3] and mitochondrial "ghosts" were obtained [12] by the osmotic shock method in medium containing 2 mM Tris-HCl (ph 7.4). The functional characteristics of the mitochondria and their "ghosts" were as follows: velocity of respiration, after Chance, in the active state (V_3) and in the resting state (V_4), the ADP/O coefficient, and the respiratory control (RC) were investigated polarographically in medium [8] containing 100 mM sucrose, 75 mM KCl, 10 mM succinate, 2.5 mM Na_2HPO_4, and 10 mM Tris-HCl (pH 7.45).

The state of permeability of the internal membranes of the unenergized mitochondria was judged from their swelling, recorded as a change in the optical density of the suspension [7]. Optical density was measured at 520 nm on the SF-4A spectrophotometer or LMF-69 photometer, the output of which was connected to an ON-102 automatic writer. The permeability of the mitochondrial "ghosts" for potassium, sodium, Tris, magnesium, calcium, strontium, and barium ions was investigated in isoosmotic solutions of the nitrates of these cations, buffered with 10 mM Tris-nitrate (pH 7.5). Permeability for H^+ was measured in an isoosmotic solution of ammonium nitrate [7], and for Cl^- in $CaCl_2$ solution. To rule out energy-dependent cation accumu-

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TABLE 1. Effect of Cytotoxin on Some Parameters of Function of Mitochondria and Mitochondrial "Ghosts" of Rat Liver

Concentration of cytotoxin, μM	Intact mitochondria				Mitochondrial "ghosts"			
	V_3	V_4	RC	ADP/O	V_3	V_4	RC	ADP/O
0	90	30	3,0	1,9	48	32	1,5	2,0
1	90	30	3,0	1,9	60	46	1,3	1,8
5					54	44	1,2	1,6
10	90	30	3,0	1,9	67	67	1,0	—
50					67	67	1,0	—
100	90	32	2,8	1,9	50	50	1,0	—

Legend. Velocity of respiration expressed in ng-atoms O_2/min/mg protein.

TABLE 2. Action of Cytotoxin (C) on Permeability of Inner Membranes of Mitochondrial "Ghosts" for Different Ions

Ion tested	Experimental conditions	ΔE_{520}/min (×100)	Activity, %
Ca^{++}	Control	5,	100
	C, 10 μM	11,0	220
	C, 25 μM	17,0	340
Mg^{++}	Control	0,4	100
	C, 10 μM	0,4	100
	C, 25 μM	0,4	100
Sr^{++}	Control	8,0	100
	C, 10 μM	11,0	140
	C, 25 μM	13,0	160
Ba^{++}	Control	1,4	100
	C, 10 μM	1,5	110
	C, 25 μM	1,8	130
H^+	Control	2,0	100
	C, 10 μM	4,0	200
	C, 25 μM	8,0	400
	TTFB*	30,0	1500
Na^+	Control	1,5	100
	C, 10 μM	3,0	200
	C, 25 μM	11,0	730
	Gramicidin D, 1.33 μg/ml	128,0	8500
K^+	Control	0,3	100
	C, 10 μM	4.0	1300
	C, 25 μM	11,0	3700
	Valinomycin, 0.6 μM	23,0	7700
Tris	Control	1,0	100
	C, 10 μM	8,0	800
	C, 25 μM	11,0	1100
Cl^-	Control	3,0	100
	C, 10 μM	14,0	460
	C, 25 μM	18,0	600

Legend. 1. In the case of H^+, Na^+, and K^+ effects of the corresponding ionophores are given for comparison. 2. Here and in Table 2, ΔE_{520} denotes change in optical density of mitochondria at 520 nm with time during swelling of mitochondria. [TTFB – unidentified Russian acronym; possibly should be TTPB – Consultants Bureau.]

lation, antimycin A and rotenone (0.33μg/ml of each) were added to the medium. Energy-dependent swelling of the mitochondrial "ghosts" was thus limited only by the rate of transmembrane transport of the ions studied, for all the media contained penetrating counterions.

EXPERIMENTAL RESULTS

The results of investigation of the action of the cytotoxin on intact mitochrondria show that cytotoxin free from contamination with phospholipase A does not disturb the functional parameters of intact mitochondria (Table 1). The action of the whole venom of N. naja oxiana and of the preparation known as cytoxin, which was

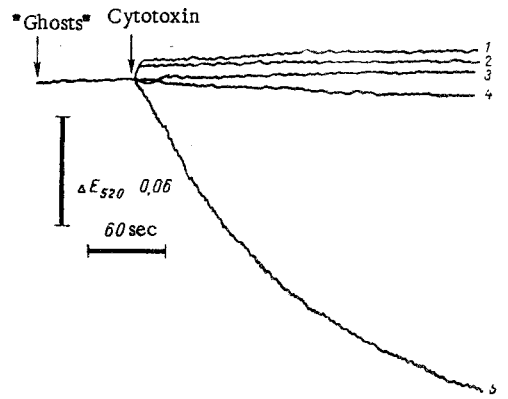

Fig. 1. Action of various concentrations of cytotoxin on kinetics of energy-dependent swelling of mitochondrial "ghosts" in solutions of 270 mM sucrose and 250 mM sucrose containing 10 mM Tris-HCl. Incubation media: curves 1-4) 270 mM sucrose, 5) 250 mM sucrose + 10 mM Tris-HCl, pH 7.5. Besides the above-mentioned components the medium also contained antimycin A and rotenone. 1) 5-10 μM cytotoxin; 2) 25 μM cytotoxin; 3) control; 4) 50 μM cytotoxin.

TABLE 3. Effect of Mg^{2+} and La^{3+} Ions on Rate of Swelling of Mitochondrial "Ghosts" Induced by Cytotoxin in Medium with Sodium Nitrate

Experimental conditions	ΔE_{546} nm (×100)	Inhibition, %
Cytotoxin, 10 μM (control)	1,9	—
Mg^{2+} ions		
5 mM	1,7	11
10 mM	1,5	22
15 mM	0,8	58
20 mM	0,3	84
La^{3+} ions		
0.5 μM	1,9	—
1,0 μM	1,5	22
10.0 μM	1,1	62

used previously [3], on the energy metabolism of the mitochondria is evidently due to the effect of phospholipase A. However, the cytoxins were shown to have membrane activity and, in particular, to increase passive permeability of plasma membranes, leading to swelling of the cell [13]. The absence of effect of cytotoxin on intact mitochondria could be accounted for by the different phospholipid composition of the mitochondrial membranes, preventing interaction with cytotoxin, or by the presence of an outer membrane preventing the cytotoxin from binding with the inner membrane of the mitochondria. The second hypothesis was tested on mitochondrial "ghosts," deprived of their outer membrane, but which preserved their respiratory and phosphorylating activity. Investigations showed that cytotoxin, in a concentration as low as 1 μM, caused uncoupling of oxidative phosphorylation, manifested by a decrease in the RC and ADP/O coeffcients and stimulation of respiration of the "ghost" mitochondria in state V_4. The effect of the cytotoxin increased with an increase in its concentration, and in the presence of 10 μM cytotoxin RC of the mitochondrial "ghosts" was 1 (Table 1).

Disturbance of the energy coupling of the mitochondria was evidently due to modification of the permeability of the inner membranes by cytotoxin, and for that reason the effect of different concentrations of cytotoxin on passive permeability of mitochondrial "ghosts" for monovalent and bivalent ions was studied (Table 2). The results showed that cytotoxin sharply increased the permeability of the inner mitochondrial membranes for monovalent ions and increased permeability for bivalent ions by a lesser degree. The ions studied could be

arranged in the following order of velocity of transport through the inner membranes in the presence of 10 μM cytotoxin: $K^+ > Tris^+ > Cl^- > H^+ > Na^+ > Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$. The effect of cytotoxin on permeability of the inner mitochondrial membranes could be detected even in sucrose medium in the presence of low concentrations of electrolytes (10 mM Tris-HCl) (Fig. 1). It can accordingly be postulated that cytotoxin forms nonspecific conduction channels in the membrane with low selectivity for charge and size of the ion. However, these channels are impermeable for sucrose, although with an increase in the cytotoxin concentration to 50 μM only weak swelling was observed in sucrose medium free from electrolytes also. This may have been due to the formation of larger pores in the membrane by aggregates of cytotoxin molecules, which was possible only when high concentrations of the polypeptide were used.

Mg^{2+} ions, whose function is to stabilize the inner membranes of mitchondria [4], inhibited the effect of 10 μM cytotoxin; micromolar concentrations of La^{3+} ions, which also have a stabilizing effect on membranes, had a similar action (Table 3).

The results may indicate competition between cytotoxin and Mg^{2+} ions for binding sites on the membrane. Similar competition has been established for cytotoxin and Ca^{2+} ions on plasma membranes [6, 9].

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