DISTANT-OPTICAL INTERACTION OF MITOCHONDRIA THROUGH QUARTZ

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Several investigations based on the effect of distant-optical interaction between various biological objects have recently been published [1, 2, 4, 5]. The presence of the effect in these investigations, just as in earlier ones [3, 8], was determined on the basis of stimulation of cell division and various morphological changes in the object. The choice of a well studied object, changes in which induced by distant interaction are recorded by an accessible and objective method, is of definite interest. In the writer's opinion, mitochondria are one such object.

The study of distant interaction between mitochondria, separated by a quartz partition, was the aim of the investigation described below. The effect of interaction was assessed on the basis of changes in the rate of oxygen consumption by the mitochondria, expressed in nanoatoms O_2/min/kg protein.

EXPERIMENTAL METHOD

Mitochondria were isolated from rat liver by differential centrifugation at −4°C. The tissue was homogenized in ice-cold isolation medium containing 0.25 M sucrose and 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 600g for 10 min and the supernatant at 14,000g for 10 min.

The mitochondria thus obtained were resuspended successively in isolation medium and in 0.25 M sucrose and centrifuged twice at 14,000g (10 min each time). The residue of mitochondria was suspended in 0.25 M sucrose and kept at 0°C. Protein was determined by the biuret method. The incubation medium contained 0.14 M sucrose, 0.015 M KCl, 5 mM KH_2PO_4, 2.5 mM $MgCl_2$; pH 7.4. The rate of oxygen consumption was determined polarographically by means of a closed Clark electrode in a quartz cell 1 ml in volume (Fig. 1), made from

TABLE 1. Difference Between Rate of Respiration of Control and Experimental Mitochondria

Substrate and state	Rate of respiration, in nanoatoms O_2/min/mg protein		Change, % of control	p
	control	experiment		
Endogenous respiration	4,6±0,3 (n=14)	3,6±0,2 (n=23)	76	<0,01
Substrate respiration of succinate (5 mM)	10,7±0,3 (n=20)	8,7±0,2 (n=30)	81,5	<0,01
ADP (0.2 μM)	33,2±1,6 (n=13)	30,0±1,7 (n=25)	90,4	>0,05
(V_4) "rest"	11,5±0,7 (n=14)	8,4±0,4 (n=25)	76,5	<0,01
ADP (0.2 μM)	32,8±1,4 (n=13)	26,7±1,5 (n=22)	81,4	0,01
(V_4) "rest"	8,7±0,6 (n=7)	5,8±0,3 (n=25)	66,7	<0,05

Legend. n) Number of experiments.

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Fig. 1

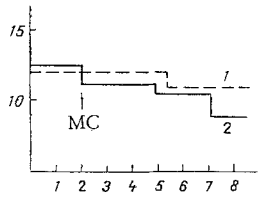

Fig. 2

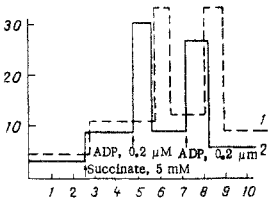

Fig. 3

Fig. 1. Scheme of arrangement of combined cell. A) Outer cell, B) inner quartz cell, C) Clark electrode.

Fig. 2. Diagram of oxygen consumption by mitochondria in inner quartz cell after addition of mitochondria (arrow) to outer cell. Legend (to Figs. 2 and 3): 1) control, 2) experiment. Abscissa, time (in min); ordinate, oxygen consumption (in nanoatoms O_2/min/mg protein).

Fig. 3. Diagram of oxygen consumption by mitochondria in inner quartz cell (mitochondria added previously to outer cell).

optically transparent quartz with a lower limit of transmission of 200 nm and with walls 1 mm thick. Throughout the experiment the quartz polarographic cell was lowered into an outer cell (volume 3 ml) made of transparent plastic, filled with incubation medium + 15 mM succinate. Addition of 10 mg mitochondria + 0.3 μM dinitrophenol to the outer cell was carried out beforehand, 1-2 min before addition of 3.5 mg mitochondria to the inner quartz cell, and before recording of the polarogram began, and again 2 min after addition of the mitochondria to the inner quartz cell.

Contact between mitochondria in the inner and outer cells was effected through a quartz wall 1 mm thick. In the control experiments, the outer cell contained incubation medium + 15 mM succinate without mitochondria and dinitrophenol (DNP). All experiments were carried out in uniform daylight and at a temperature of 18°C.

EXPERIMENTAL RESULTS

The rate of oxygen consumption by mitochondria in the inner quartz cell, when 5 mM succinate was used as the substrate, after activation of the mitochondria in the outer cell fell progressively with time on average by 3.1%/min, and after 7 min the decrease in oxygen consumption amounted to 27% of the level at the beginning of recording of the polarogram ($p < 0.01$ for 10 measurements). In control experiments a decrease in oxygen consumption also was observed during the same period of time. However, unlike in the experimental series, in the control the decrease did not begin until 5.5 min after the beginning of incubation and reached 10% compared with the level at the beginning of recording. The difference between the control and experiment thus amounted to 17% (Fig. 2).

In the case when mitochondria were introduced into the outer cell 1-2 min before they were added to the inner quartz cell the rate of oxygen consumption in the inner quartz cell also fell progressively throughout the period of recording the polarogram and its character was as follows: Endogeneous respiration was depressed by 24%, substrate respiration on 5 mM succinate was 18.5%, and the rate of oxygen consumption during phosphorylation fell by 9.6 and 18.6% respectively after the first and second additions of ADP. Respiration of the mitochondria during the "rest" after phosphorylation was reduced by 23.5 and 33.3% (Fig. 3). Averaged results of this series of experiments are given in Table 1.

The results thus indicate the presence of distant interaction between mitochondria separated by a quartz partition. Distant interaction is expressed as a significant decrease in oxygen consumption by mitochondria in optical contact with other mitochondria. The physical nature of the phenomena described above may be interpreted on the basis of the following facts. Mitochondria are known to possess spontaneous chemiluminescence in the visible region of the spectrum [12] and also to be highly sensitive to both visible [9] and UV radiation [10, 11]. It has been shown that UV radiation induces activation of oxidative phosphorylation and swelling and destruction of mitochondria. There is also much evidence in favor of a free-radical mechanism of oxidative phosphorylation [6, 7]. On the basis of the facts described above, the appearance of single quanta of UV radiation can be postulated during mitochondrial metabolism. Many investigations [1-3, 5, 8] have demonstrated the exceptionally active role of exposure of biological objects to the action of single ultraviolet photons (mitogenetic radiation). However, the final elucidation of the physical nature of these phenomena require further investigation.

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