EFFECT OF THE CENTRAL CHOLINOLYTIC CYCLOSIL ON RAT LIVER MITOCHONDRIAL FUNCTION

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During incubation of rat liver mitochondria in the presence of the central cholinolytic cyclosil (0.04 mg/ml) the effectiveness of formation of high-energy compounds is increased. The rate of O_2 utilization remained unchanged. Some tendency was observed for the inward K^+ transport into the mitochondria to be increased in the presence of cyclosil. The results point to an increased degree of energization of the mitochondria in the presence of cytosil.

KEY WORDS: central cholinolytics; cyclosil; liver mitochondria; oxidative phosphorylation; K^+ transport.

Central cholinolytics are widely used in clinical practice at the present time. However, when drugs of this group are used with the object of achieving a central cholinergic blocking effect, frequently insufficient account is taken of the character of their effect on peripheral organs and tissues. When peripheral action of central cholinolytics has been recorded, the conclusions have been based as a rule on changes in the functional composition of a particular physiological system as a whole. The problem of the direct action of the compounds of this group on cellular and subcellular structures of peripheral tissues still remains unsolved.

The high pharmacological activity of the central cholinolytics suggests that their action is based on definite metabolic shifts. In this connection the analysis of function of the energy metabolism systems and ion transport, which is coupled with them, is particularly interesting.

The object of this investigation was to study the action of the central cholinolytic cyclosil (hydrochloride of the dimethylamino ester of phenylcyclopentylglycolic acid) on oxidative phosphorylation and K^+ transport in rat liver mitochondria in vitro. This compound also has a marked peripheral effect.

Data in the literature on the action of central cholinolytics on oxidative phosphorylation are few in number and contradictory in nature [1, 4, 12].

EXPERIMENTAL METHOD

Experiments were carried out on 50 rats of both sexes weighing 260-320 g. By differential centrifugation [14] mitochondria were isolated from liver homogenates in medium containing 0.25-0.3 M sucrose and 10 mM Tris-HCl, pH 7.4. The uptake of oxygen by the mitochondria was recorded by a polarographic method [9] on the LP-7 polarograph (Czechoslovakia) with closed platinum electrode of the Clark type [10]. The composition of the incubation medium was: KCl 150 mM, Tris-HCl 5 mM, KH_2pO_4 5 mM (pH 7.4). The protein content was determined by the biuret method [11]. The experiments were carried out by the following scheme: incubation medium (1 ml) was poured into the polarographic cell, to it were added the oxidation substrate (10 mM succinate) and 0.05 ml of a suspension of mitochondria in the isolation medium, containing 2.9-3.4 mg protein/ml; the initial rate of oxygen uptake (V_0) was then measured. ADP was then added to the samples in a dose of 100 μmoles/ml in two successive portions and the rate of oxygen uptake was measured in Chance's state 3 (V_3), when respiration was activated by the addition of phosphate acceptor to the cell, and in state 4 (V_4), which arises after the end of phosphorylation and is characterized by the slowing of respiration. All measurements were made at 28°C. After the addition of 40 μmoles 2,4-dinitrophenol (DNP) to the sample the rate of O_2 uptake was then measured in the presence of the uncoupler (V_{DNP}). Cyclosil was added to the

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TABLE 1. Oxidative Phosphorylation and Ion Transport in Rat Liver Mitochondria during Incubation with Cyclosil (0.04 mg/ml), M ± m

Group of animals	Δt_1	Δt_2	RC_L	RC_C	Uptake of K^+ ions, μg-eq/mg protein
Control	1,00±0,01	0,94±0,02	1,93±0,16	1,93±0,11	0,034±0,005
Experimental	0,84±0,001	0,77±0,03	2,33±0,10	2,41±0,03	0,0046±0,013
P	<0,1	<0,1	<0,1	<0,1	<0,5

Legend. RC_L) Respiratory control after Lardy and Wellman – ratio of rate of O_2 uptake in presence of ADP (V_3) to initial rate of O_2 uptake (V_0); RC_C) respiratory control after Chance and Williams – ratio of V_3 to rate of O_2 uptake when all ADP is phosphorylated to ATP (V_4); Δt_1) phosphorylation time of first addition of ADP; Δt_2) phosphorylation time of second addition of ADP. Number of experiments 12-15.

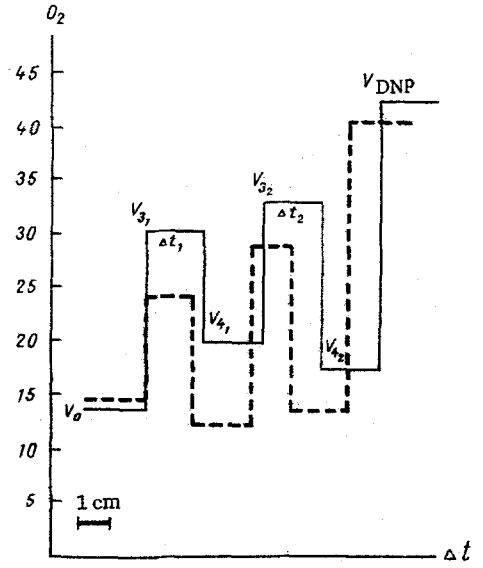

Fig. 1. Effect of cyclosil on oxidative phosphorylation in rat liver mitochondria. Abscissa, phosphorylation time (in min, 1 min = 2 cm); ordinate, rate of respiration (O_2 uptake) in nanoatoms O_2/mg protein/min. Continuous curve – control, interrupted curve – in presence of cyclosil. Indices 1 and 2 attached to values V_3 and V_4 denote first and second additions of ADP.

freshly isolated mitochondria in a final concentration of 0.04 mg/ml of the suspension of particles (corresponding to a dose of the cholinolytic of 10 mg/kg if uniformly distributed after parenteral injection) and the samples were incubated for 20 min.

The K^+ concentration was determined by means of a flame photometer (from Carl Zeiss, East Germany). The mitochondria were suspended in 0.25 M sucrose and placed in medium containing 11 mM K-phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 50 mM KCl, 0.25 M sucrose, 2 mM EDTA, 17 mM α-ketoglutarate, 0.83 mM NAD^+, and 4.7 mM ADP. In the control samples the mitochondria were sedimented by centrifugation immediately after addition to the incubation medium. The experimental samples were incubated at 20°C for 20 min. The residue of mitochondria was washed with medium without K^+, dried, and suspended in 10 ml 0.1 N HCl, and then left to stand overnight. The K^+ concentration in the mitochondria was determined from a calibration and the protein content by Lowry's method [15].

EXPERIMENTAL RESULTS

Preliminary addition of cyclosil in a concentration of 0.04 mg/ml did not affect the oxygen uptake of the mitochondria: the rates of oxidation of succinate without phosphate acceptor (V_0) were the same in the control and experiment. However, the presence of the cholinolytic increased the effectiveness of formation of high-energy compounds in mitochondria incubated with them compared with intact particles (Table 1). This is shown (Fig. 1) by the decrease in the velocities V_{4_1} and V_{4_2} (rates of O_2 uptake after the addition of ADP), ob-

served after the completion of the phosphorylation cycle, indicating improvement of the energy regulation of respiration because of the accumulation of ATP. The sample of mitochondria incubated with cyclosil was a more closely coupled, energized system (Table 1), as is confirmed by the decrease in the phosphorylation time and the increase in the values of the respiratory control. Repeated addition of ADP revealed more fully the "reserves of strength" of the energy regulation of the mitochondria. It will be clear from Fig. 1 that mitochondria incubated with cyclosil could complete a second phosphorylation cycle, just like the first, without any changes of any kind, which would have indicated damage to the mitochondria as a result of incubation with the cholinolytic.

The experiments to study the effect of cyclosil (0.04 mg/ml) on the active translocation of K^+ rat liver mitochondrial membranes showed that there is a tendency for K^+ accumulation in the mitochondria to be increased.

The results of these experiments suggest that during incubation of mitochondria with cyclosil direct interaction is observed between the cholinolytic and membranes. Evidence that this may be so is given by the results of experiments with the cholinolytics benactyzine and adiphenine [3]. According to observations by Volkova [2], central cholinolytics increase O_2 uptake in rat brain, liver, and kidney tissues.

However, most investigations [1, 4, 7, 8, 13] have shown that cholinolytics such as hydrallazine, aprophen,* difazin,† and caramiphen reduce O_2 uptake in different organs. There is evidence [12] that benactyzine does not disturb the oxidative phosphorylation cycle. The present experiments show that incubation of mitochondria with cyclosil does not lead to changes in O_2 uptake. Meanwhile the process of formation of high-energy compounds becomes more effective, and this in turn favors the transition of the mitochondria into a more closely coupled state. Mitochondria preincubated with cyclosil are a highly energized preparation, i.e., the energy regulation of electron transport by high-energy phosphates is intensified in these mitochondria [6].

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*2-diethylaminoethyl-2,2-diphenylpropionate hydrochloride.
†10-diethylaminoacetylphenothiazine.