Effects of Antibiotics Nogalamycin, Cirolemycin and Tubercidin on Endogeneous Respiration of Tumor Cells and Oxidative Phosphorylation of Mammalian Mitochondria

In our previous paper, effects of the above-mentioned antibiotics on glycolysis, biosynthesis of protein, nucleic acids and transplantability of Ehrlich ascites carcinoma (EAC) cells have been studied[1]. Little is known, however, about the effects of nogalamycin, cirolemycin and tubercidin on mammalian mitochondrial functions and/or on respiration of tumor cells. This paper deals with the effects of these antibiotics on endogeneous respiration of tumor cells as well as on mitochondrial oxidative phosphorylation.

Materials and methods. ICR albino mice inoculated with Ehrlich-Lettré ascites cells were provided by Dr. E. Patterson of the Cancer Institute, Fox Chase, Pa., USA. ELD/Ehrlich-Lettré hyperdiploid/ascites tumor cells were harvested 6–8 days after inoculation, washed in a saline phosphate medium[2] and suspended in the same medium as described in earlier papers[3,4].

Intact pigeon heart and rat liver mitochondria were isolated in a medium containing 0.225 *M* mannitol, 0.075 *M* sucrose, 0.2 m*M* EDTA and 2 m*M* morpholinopropane sulphonate (MOPS), pH 7.2 according to the method of CHANCE and HAGIHARA[5]. If not otherwise indicated, the mitochondria were suspended in the same medium. The protein concentration of the final suspension was determined by the biuret method with bovine serum albumin as standard[6].

Rates of oxygen uptake were measured polarographically with a Clark-type oxygen electrode as described previously[4]. All mitochondrial preparations were checked for structural integrity using the criterion of respiratory control[7]. Antibiotics nogalamycin, cirolemycin (U-12,241) and tubercidin were kindly supplied by Dr. HANKA, Research Laboratories The Upjohn Co., Kalamazoo, Mich., USA, and were dissolved in dimethyl sulphoxide (DMSO). The same volume was added to the control samples to verify that the presence of DMSO in experiments had no effect (final concentration of DMSO 1%)[8]. All other reagents were obtained from Sigma Chemical Co. The substrates were prepared in stock solutions at pH 7.4.

Results and discussion. Figure 1 shows the effects of antibiotics nogalamycin, cirolemycin and tubercidin on endogeneous respiration of Ehrlich-Lettré cells. From the antibiotics tested, only nogalamycin inhibited endogeneous respiration of Ehrlich-Lettré cells. Maximal inhibition of respiration (30%) was observed at concentration of 100 μg/ml. Figures 2, 3 and 4 show the effects of different antibiotics on respiration of pigeon heart mitochondria (PHM) as well as rat liver mitochondria (RLM) in state 4 with succinate or glutamate plus malate as

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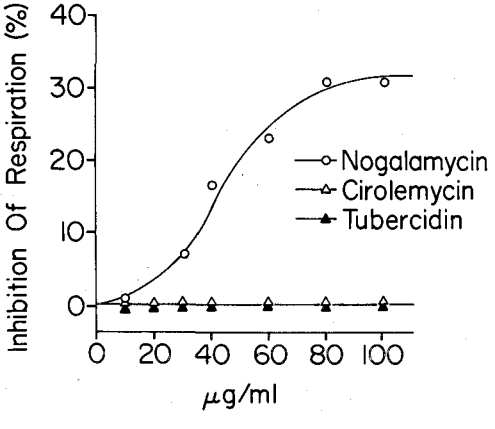

Fig. 1. Effects of nogalamycin, cirolemycin and tubercidin on endogeneous respiration of Ehrlich-Lettré tumor cells. 0.2 ml of cell suspension containing 15 mg dry weight cells, were added to 2.0 ml of isotonic saline-phosphate medium pH 7.4 (154 m*M* NaCl, 6.2 m*M* KCl and 11 m*M* sodium phosphate buffer). Oxygen uptake was measured at 30 °C. Rate of oxygen consumption for control was 135 nmoles per min.

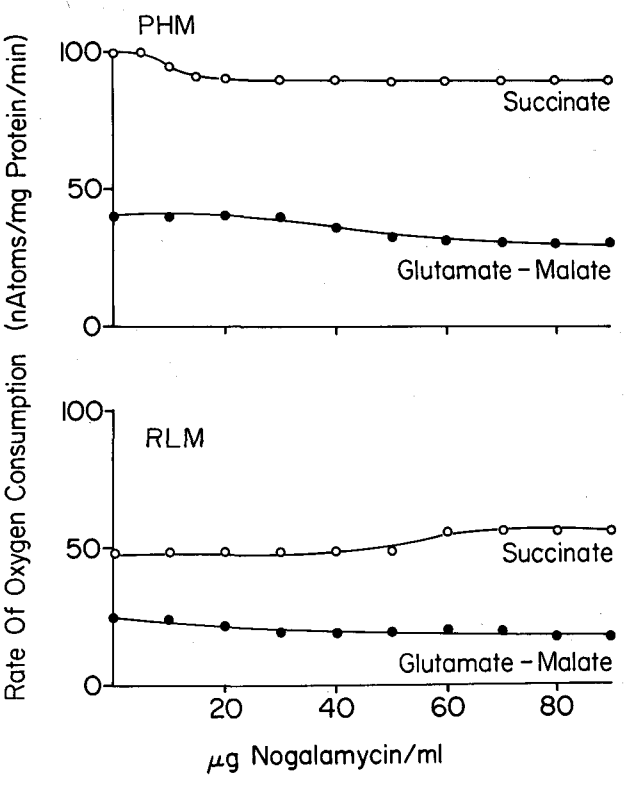

Fig. 2. Effect of nogalamycin on the rate of oxygen consumption of intact pigeon heart mitochondria (PHM) and rat liver mitochondria (RLM). The mitochondrial respiration was assayed in a medium containing 0.225 *M* mannitol, 0.075 *M* sucrose, 10 m*M* K_2HPO_4. 0.2 m*M* EDTA and 10 m*M* MOPS, pH 7.2. The mitochondria were suspended at 0.6 mg protein/ml PHM or 1.1 mg protein/ml RLM. The substrates were 10 m*M* succinate in the presence of 3 μ*M* rotenone or 5 m*M* glutamate plus 5 m*M* malate. The reaction temperature was 25 °C.

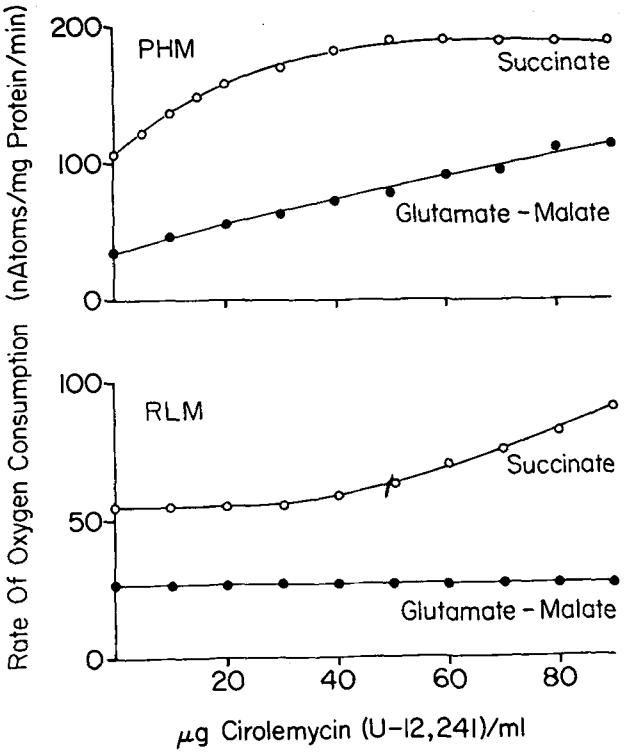

Fig. 3. Effect of cirolemycin on the rate of oxygen consumption of intact pigeon heart mitochondria (PHM) and rat liver mitochondria (RLM). The other conditions were the same as for Figure 2.

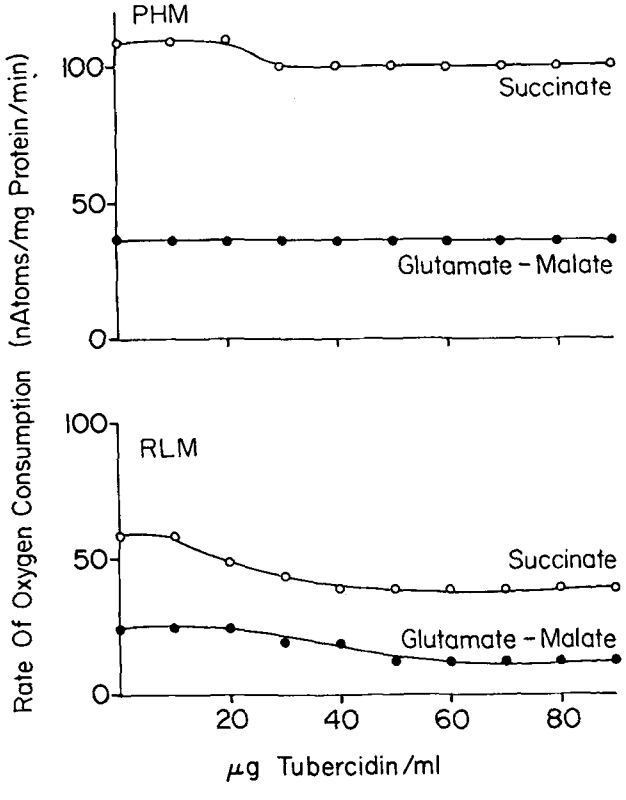

Fig. 4. Effect of tubercidin on the rate of oxygen consumption of intact pigeon heart mitochondria (PHM) and rat liver mitochondria (RLM). The other conditions were the same as for Figure 2.

The effect of nogalamycin, cirolemycin and tubercidin on the respiration of pigeon heart mitochondria

Antibiotic	Rate of oxygen consumption (natoms/mg/min)			
	Control		25 μg/ml of Antibiotic	
	State 4	State 3	State 4	State 3
Nogalamycin	89.5	365.0	97.0	365.0[a]
	34.5	248.0	34.5	248.0[b]
Cirolemycin	93.5	350.0	117.0	372.0
	40.4	282.0	40.4	262.0
Tubercidin (50 μg/ml)	89.5	372.0	96.7	358.0
	34.4	262.0	27.6	241.0

[a] Succinate, 150 μM ADP. [b] Glutamate-malate, 200 μM ADP.

substrates. Nogalamycin had no effect on respiration of both mitochondria, either in the presence of succinate or glutamate plus malate in state 4 respiration. Cirolemycin stimulated respiration in state 4 mainly in pigeon heart mitochondria. In both PHM and RLM, respiration was more stimulated if succinate was substrate. To stimulate respiration in state 4 of rat liver mitochondria, higher concentrations of cirolemycin were needed (with succinate as substrate). In the case of RLM oxidizing glutamate plus malate, there was no stimulation of respiration. Tubercidin inhibited respiration a little in rat liver mitochondria with both succinate as well as glutamate plus malate as substrates. The effects of three antibiotics on oxidative phosphorylation (state 3) and state 4 respiration are shown in the Table. It is evident from the results presented in the Table that antibiotics, nogalamycin and cirolemycin in concentrations of 25 μg/ml had no significant effect on state 3 respiration of pigeon heart mitochondria. Antibiotic tubercidin at 50 μg/ml inhibited a little state 3 respiration (262 in control to 241 natoms of oxygen/mg protein/min). In general, the same concentrations of antibiotics cause a greater stimulation of respiration in pigeon heart than in rat liver mitochondria. This may be explained by the higher concentration of cytochrome a per mg protein in pigeon heart mitochondria[9]. Results included in this paper indicate that antibiotics tested, even in relatively low concentrations, interfere with respiratory functions of eucaryotic cells (nogalamycin) and with isolated mitochondria as well (tubercidin). This may also be interesting from the point of view of the elucidation of the secondary effects of the antibiotics under discussion.

Zusammenfassung. Von den Antibiotika Nogalamycin, Cirolemycin und Tubercidin inhibiert nur Nogalamycin die endogene Atmung von Ehrlich-Lettré - Zellen. Tubercidin interferiert in relativ niedrigen Konzentrationen mit der Atmung isolierter Mitochondrien.

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