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THE EFFECT OF UNCOUPLERS ON MITOCHONDRIA, RESPIRATORY ENZYME COMPLEXES AND ARTIFICIAL PHOSPHOLIPID MEMBRANES

V. P. SKULACHEV, A. A. SHARAF, L. S. YAGUJZINSKY and A. A. JASAITIS
Department of Bioenergetics, Laboratory of Bioorganic Chemistry, Department of Animal Biochemistry, Moscow State University, USSR

and

E. A. LIBERMAN and V. P. TOPALI
Institute of Biophysics, Academy of Science, USSR

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1. INTRODUCTION

One of the experimental approaches to the problem of the mechanism of oxidative phosphorylation is the study of various effects of the uncoupling agents. Substances of this type are capable of uncoupling the process of electron transfer from phosphorylation. Among inhibitors which disturb energy transformation in mitochondria, uncouplers act the closest to the site of energy transformation in the respiratory chain.

We have studied the mechanism of the uncoupling action using intact mitochondria, reconstructed respiratory chains, artificial phospholipid membranes, and solutions of phospholipid in heptane.

2. METHODS

Mitochondria were separated from liver homogenate prepared in a glass vessel with a teflon pestle. The medium for homogenization contained 0.25 M sucrose and 0.001 M EDTA. Nuclei and cell membranes were precipitated by centrifugation at 600 *g*, and mitochondria at 9.000 *g*. The reaction mixture contained 0.25 M sucrose, 0.02 M KCl, 0.01 M KH_2PO_4, 0.005 M $MgCl_2$, 0.03 M tris buffer and 0.002 M EDTA. *p*H of the mixture was 7.5. Mitochondria were added in quantities of about 2mg of protein/ml. Separation of mitochondria was performed at 0°C, and incubation carried out at room temperature. The enzyme complex II+III (succinate - cytochrome c oxidoreductase) was prepared by a modification of the method of Hatefi et al. (1961), and the complex IV (cytochrome oxidase) by the method of Fowler et al. (1962). The oxygen consumption was measured polarographically, reduction of NAD^+ - fluorimetrically and reduction of cytochromes - with the Chance differential spectrophotometer. Solution of brain phospholipids in heptane (10mg/ml) was used for obtaining bimolecular artificial phospholipid membranes. In some experiments, instead of brain phospholipids, lecithin or synthetic distearic cephalin was used. The membrane was formed in a hole of a small teflon vessel with double wall (diameter of the hole was 1-1.5 mm). The space between the walls was filled with phospholipid solution. The original resistance of the membranes was about 10^9 Ω/cm^2. Measurements of the electric conductivity of the membrane were performed by the method described before (Babakov et al., 1966).

3. RESULTS

The following uncouplers were studied; *p*-triflouromethoxy-carbonylcyanidephenylhydrazone (FCCP), tetrachloro-triflouromethylbenzimidazole (TFB), *p*-2,4-dinitrophenol (DNP), and salicylic acid. The effects of