EFFECT OF α-TOCOPHEROL AND ITS DERIVATIVES ON ATPase ACTIVITY AND OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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UDC 615.356:577.161.3/.015.42:/612.351.11+612.351.1:/612.398.145.1:612.262

KEY WORDS: α-tocopherol; mitochondria; oxidative phosphorylation; ATPase.

Evidence has been obtained recently of the important role of free-radical lipid peroxidation (LPO) in various biological membranes in the genesis and development of certain pathological processes [1-4]. In this connection, particular importance is attached to the search for a use of new inhibitors of free-radical reactions in biomembranes. Besides natural antioxidants, an important place also is occupied by the synthesis of new compounds possessing these properties.

The aim of this investigation was to study the effect of α-tocopherol (TP) and certain of its derivatives on the ATPase activity of liver mitochondria.

EXPERIMENTHAL METHOD

Mitochondria were isolated from the liver of noninbred rats by the method in [7]. ATPase activity and oxidative phosphorylation were recorded as changes in the H^+ concentration during the reaction [5]. The protein concentration in the mitochondrial suspension was determined by the biuret test in the presence of sodium deoxycholate [6]. The following reagents were used: TP was from "Serva," West Germany; EDTA, KH_2PO_4, and 2,4-dinitrophenol were from "Merck," West Germany; sucrose was from "Fluka," West Germany; the remaining reagents were from "Reakhim," USSR, and were of the chemically pure grade. TP analogs (Fig. 1) were generously provided by Corresponding Member of the Academy of Sciences of the USSR R. P. Evstigneeva (M. V. Lomonosov Moscow Institute of Fine Chemical Technology).

EXPERIMENTAL RESULTS

Depending on their effect on mitochondrial ATPase activity the compounds used can be divided into two groups. Group 1 contains TP and its esters, tocopheryl acetate and tocopheryl stearate, which have no effect on mitochondrial ATPase activity. Group 2 includes compounds which stimulate mitochondrial ATPase activity, including TP derivatives with a shortened phytol chain (C_1, C_3, and C_1-acetate). A typical kinetic curve illustrating the action of TP

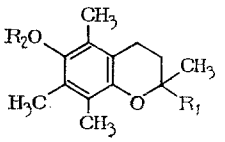

Fig. 1. Structural formulas of analogs of TP, 2,5,7,8-tetramethyl-6-oxy-2-alkyl-chromane (C). C_1 $R_1 = CH_3$, $R_2 = H$; C_1-acetate $R_1 = CH_3$, $R_2 = OC = CH_3$; C_3 $R_1 = CH_2 = CH_2 = CH_3$, $R_2 = H$; TP $R_1 = CH_2 = (CH_2 = CH_2 = CH(=CH_3) = CH_2)_3 = CH_3$, $R_2 = H$; α-Tocopherol acetate $R_2 = OC = CH_3$; α-Tocopherol stearate $R_2 = OC = (CH_2)_{16}CH_3$.

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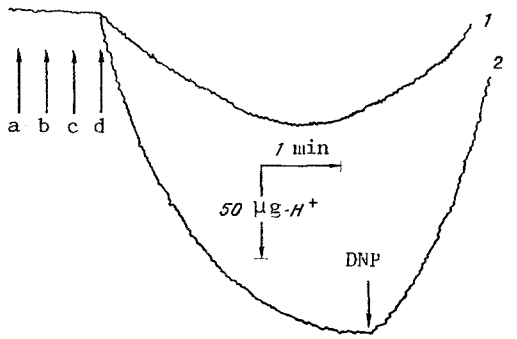

Fig. 2

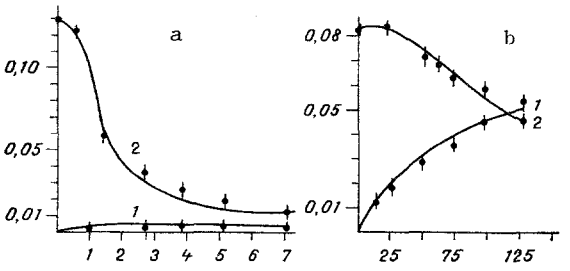

Fig. 3

Fig. 2. Effects of TP (1) and C_3 (2) on oxidative phosphorylation and ATPase activity of rat liver mitochondria. pH-metric recording in medium: 0.01 M KCl, 100 μM EDTA, 0.25 M sucrose, 2.5 mM KH_2PO_4 (pH 7.5, 25°C), final volume 4 ml. Arrows indicate addition of: a) rotenone (5 μM), b) succinate (10 mM), c) mitochondria (3.1 mg protein), d) ADP (200 μM); DNP (80 μM).

Fig. 3. Dependence of action of TP (a) and C_3 (b) on ATPase activity in intact liver mitochondria on concentration. Composition of incubation medium (4 ml): 0.2 M sucrose, 0.01 M KCl, 100 μM EDTA, 2 mM ATP, 5 mM Tris-HCl (pH 7.5, 25°C), 3.4 mg mitochondrial protein, 1) in absence of DNP, 2) in presence of 80 μM DNP. Abscissa, concentration (in M × 10^{-4}). Ordinate, rate of acidification (in μg H^+/min/mg protein).

derivatives with a shortened phytol chain on mitochondrial ATP-synthetase and ATP-hydrolase activity is illustrated in Fig. 2. In the presence of TP, added ADP undergoes phosphorylation, and the phosphorylation reaction can be reversed by the addition of 2,4-dinitrophenol (DNP; Fig. 2, 2). Addition of ADP to mitochondria after preliminary incorporation of C_3 into the mitochondrial membranes induces spontaneous (without the addition of NDP) reversal of the ATP-synthetase reaction (Fig. 2, 1), i.e., an uncoupling action of the short-chain TP derivative is recorded. Comparison of the effectiveness of action of C_3 and DNP showed that C_3 is a much weaker uncoupler. If ATPase activity in the presence of 80 μM DNP is taken as 100%, concentrations of C_3 up to and including 150 μM did not stimulate ATPase to the same degree: over the whole range of concentrations from 30 to 150 μM the effect of C_3 was from 15 to 60% of the stimulating activity of DNP on ATPase (Fib. 3b).

Similarly another short-chain TP derivative (C_1) also stimulated ATPase of intact mitochondria. Considering that C_1-acetate, a derivative without the 6-oxy-group in the chromane ring, also possesses these stimulating properties, it can be concluded that the action of short-chain TP derivatives is not based on their protonophore effect. Long-chain TP derivatives do not stimulate ATPase in intact mitochondria, i.e., they have no uncoupling action.

Besides stimulating ATPase activity, short-chain TP derivatives also inhibit DNP-stimulated ATPase. Addition of C_3 to the reaction medium, for instance, induced a concentration-dependent decrease of activity of DNP-stimulated ATPase (Fig. 3b). It is an interesting fact that TP also had a similar inhibitory effect on DNP-stimulated ATPase (Fig. 3a), for although TP itself does not act on the ATPase activity of intact mitochondria within the concentration range from 100 to 800 μM, it induced concentration-dependent inhibition of DNP-stimulated ATPase. Since TP does not affect ATPase activity in mitochondria subjected to freezing and thawing, it can be concluded that it does not interact directly with the ATP-synthestase complex of the mitochondria. This is also shown by the fact that in the concentrations used (300-400 μM) TP did not affect phosphorylation in intact mitochondria (Fig. 2). If the TP concentration was increased above 400 μM, it caused inhibition, probably on account of interaction with transport systems located in the membrane. Other long-chain TP derivatives — tocopheryl acetate and tocopheryl stearate — also had a similar action.

It can be concluded from these results as a whole that short-chain TP derivatives have a damaging action of the mitochondrial membrane, causing uncoupling of oxidative phosphorylation. TP itself, and also its long-chain derivatives, have no such perturbing properties.

The authors are grateful to the Committee for Science of the Council of Ministers of Bulgaria for financial support for the research.

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PHOSPHOLIPID REPAIR OF LIVER MEMBRANES IN RATS POISONED WITH CARBON TETRACHLORIDE

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UDC 616.36-091.8-099-0039.-02:615.451.234:547.953

KEY WORDS: carbon tetrachloride; cytochrome P-450; liposomes; phospholipids; liver microsomes.

Liposomes are being used increasingly more frequently in experimental and clinical medicine as microcapsules for administration of drugs, x-ray contrast compounds, enzyme preparations, and other biologically active substances.

This paper describes an attempt to use liposomes for the administration of phospholipid material in vivo for the repair of membranes of the endoplasmic reticulum (ER) of the liver, damaged by carbon tetrachloride (CCl_4). The criterion of repair was restoration of the phospholipid composition and enzyme activity of ER membranes.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 100-150 g. CCl_4 was injected by the intragastric route in a dose of 2.5 ml/kg in the form of a 50% solution in mineral oil.

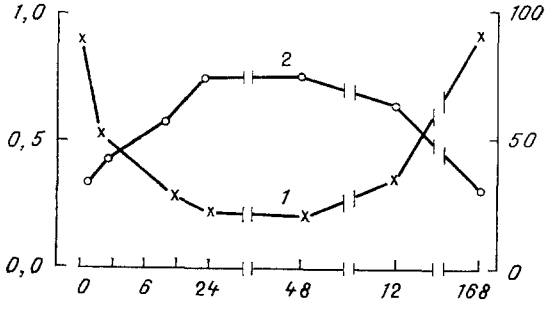

Fig. 1. Changes in cytochrome P-450 content and degree of its inactivation by the action of CCl_4. Abscissa, time after administration of CCl_4 (in h); ordinate: on left — cytochrome P-450 concentration (in mmoles/mg protein), on right — coefficient of inactivation (in %). 1) Cytochrome P-450 concentration; 2) coefficient of inactivation.

Department of Biochemistry, Medico-Biological Faculty, M. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 301-303, September, 1987. Original article submitted October 2, 1986.

 0007-4888/87/0009-1226$12.50