The role of lipid peroxidation in the induction of cation transport in rat liver mitochondria

The antioxidant effect of oligomycin and dicyclohexylcarbodiimide

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Lipid peroxidation in mitochondria induced by Fe^{2+} in the presence of ascorbate or by cumene hydroperoxide in the presence of phosphate results in a drop of the membrane potential and in K^+ efflux. The inhibitors of ATP-synthetase (oligomycin and dicyclohexylcarbodiimide (DCCD)) are capable of preventing lipid peroxidation, stabilizing the membrane potential and inhibiting potassium efflux. The same effects are observed in the presence of ionol or α-tocopherol. In contrast to antioxidant protection the effects of oligomycin and DCCD are reversed by the uncoupler (FCCP). The functional link between non-enzymatic lipid peroxidation, proton conduction through F_o component of ATP-synthetase and induced cation transport is suggested.

Mitochondria *Lipid peroxidation* *Cation transport* *ATP-synthetase* *Membrane potential* *Cumene hydroperoxide*

1. INTRODUCTION

The study of lipid peroxidation in rat liver mitochondria has shown that oxidation reactions may be catalysed by ferrous ions [1–4], ascorbate [5,6], Fe^{2+} with ascorbate [7] (this induction being of non-enzymic nature) and organic cumene and *tert*-butyl hydroperoxides [8–10]. The development of lipid oxidation processes correlates with changes in the integrity of the mitochondrial membranes, causes their irreversible swelling, disruption and the efflux of cations from the mitochondria [1–6].

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Abbreviations: DCCD, dicyclohexylcarbodiimide; FCCP, carbonylcyanide-*p*-fluoromethoxyphenylhydrazone; CuOOH, cumene hydroperoxide; EDTA, ethylenediamine tetraacetic acid; $\Delta\Psi$, electric membrane potential

It was generally accepted [1–6] that lipid peroxidation processes disturb mitochondrial functions. According to this concept, the enzymes superoxide dismutase [11], catalase [12], as well as glutathione-peroxidase and glutathione-reductase [13] protect mitochondria against the damaging effect of lipid peroxidation processes.

However, lipid peroxidation may have a physiological importance [14].

The purpose of this work is to elucidate the relationship of non-enzymatic lipid oxidation reactions with the system of induction of cation transport, on one hand, and functioning of the ATP-synthetase enzymic mitochondrial complex on the other.

We show that the process of induction of peroxidation reactions in mitochondrial membranes with Fe^{2+} in the presence of ascorbic acid or with cumene hydroperoxide in the presence of phosphate brings about the induction of K^+

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transport, the increase in mitochondrial respiration rate in state 4 and the drop in membrane potential.

It is shown that the induction of lipid peroxidation is an essential stage in the process of induction of K^+ transport. The ATP-synthetase inhibitors oligomycin and dicyclohexylcarbodiimide control the induction of ion transport in rat liver mitochondria by suppressing lipid peroxidation.

2. METHODS

Rat liver mitochondria were isolated by the differential centrifugation in a medium containing 250 mM sucrose, 5 mM Tris–HCl, 250 μM EDTA (pH 7.8) [15]. The last washing of the mitochondria was performed in a medium without EDTA. Protein concentration in the mitochondrial suspension were determined by the biuret reaction, using bovine serum albumin as a standard [16].

Mitochondria (1–2 mg protein/ml) were incubated in a medium containing 200 mM sucrose, 30 mM Tris–HCl, 10 mM succinate, 5 mM H_3PO_4, 0.2 mM KCl, 2 μM tetraphenylphosphonium bromide, 2 μM rotenone (pH 7.5) at room temperature during 2 min whereupon either cumene hydroperoxide (200 μM) or 5 μM $FeSO_4$ and 0.5 mM ascorbic acid (time '0' min) were added. During measurement of accumulation of malonic dialdehyde the uncoupler [carbonylcyanide-*p*-fluoromethoxyphenylhydrazone (FCCP 0.5 μM)] was added simultaneously with inductors of peroxidation process (both after 2 min incubation).

Mitochondrial respiration rate was measured by the polarographic method using a Clark electrode. K^+ in the incubation medium were recorded with the potassium-selective electrode 'Orion Research'. The transmembrane potential was monitored with a selective electrode by the distribution of tetraphenylphosphonium between the mitochondrial matrix and the incubation medium. The electrode was made in our laboratory according to [17].

The level of peroxidation processes was determined from the accumulation of malonic dialdehyde which forms a coloured complex with thiobarbituric acid. Samples of 0.2 ml were taken at specified time (as indicated in fig.1,2) from the measuring cell of 4 ml and were fixed in 1.5 ml 20% acetic acid (pH 3.5). The amount of malonic dialdehyde was determined in supernatant after centrifugation of the sample at 5000 rev./min according to [18], using the molar extinction coefficient $\epsilon = 1.56 \times 10^5\ M^{-1}.cm^{-1}$.

3. RESULTS

Addition of Fe^{2+} in the presence of ascorbic acid to rat liver mitochondria led to a stimulation of mitochondrial respiration in state 4, a drop in the membrane potential (fig.1) and the induction of K^+ efflux from mitochondria (fig.3). Under these conditions we observed an enhancement of lipid peroxidation, as shown by the accumulation of malonic dialdehyde in the course of the experiment (fig.1). The induction of lipid peroxidation with Fe^{2+} in the presence of ascorbic acid was suppressed by the inhibitors of free-radical processes [ionol (fig.1) and α-tocopherol]. This was accompanied by the stabilization of the membrane potential (fig.1) and the inhibition of the efflux of K^+ (fig.3).

We observed the suppression of lipid peroxida-

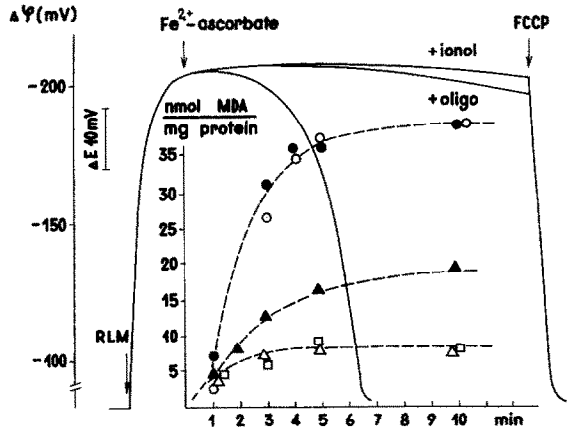

Fig.1. The magnitude of the membrane potential and the accumulation of malonic dialdehyde during lipid peroxidation in rat liver mitochondria induced by Fe^{2+} in the presence of ascorbic acid. Additions: (●) $FeSO_4$, ascorbate; (▲) $FeSO_4$, ascorbate, oligomycin; (○) $FeSO_4$, ascorbate, oligomycin, FCCP; (△) $FeSO_4$, ascorbate, ionol; (□) $FeSO_4$, ascorbate, ionol, FCCP. For the composition of the incubation medium see section 2. Reagents: $FeSO_4$, 5 μM; ascorbic acid, 0.5 mM; oligomycin, 2 μg/mg protein; ionol, 10 μM; FCCP, 0.5 μM.

tion by a specific ATP-synthetase inhibitor [oligomycin (fig.1)] as well as by dicyclohexylcarbodiimide (DCCD) under the conditions of Fe^{2+}–ascorbate-induced lipid peroxidation.

In response to addition of cumene hydroperoxide in the presence of phosphate, as well as addition of Fe^{2+} in the presence of ascorbic acid, we observed a stimulation of mitochondrial respiration in state 4, a drop in the membrane potential (fig.2) and the efflux of K^+ (fig.3).

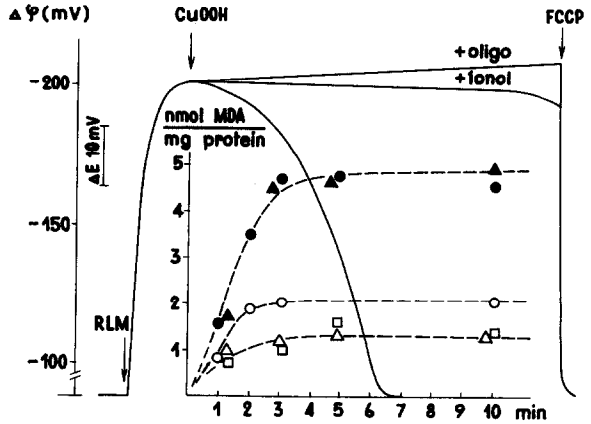

Fig.2. The magnitude of the membrane potential and the accumulation of malonic dialdehyde during lipid peroxidation in rat liver mitochondria induced by cumene hydroperoxide in the presence of phosphate. Additions: (●) CuOOH; (○) CuOOH, oligomycin; (▲) CuOOH, oligomycin, FCCP; (△) CuOOH, ionol; (□) CuOOH, ionol, FCCP. Reagents: CuOOH, 200 μM; oligomycin, 2 μg/ml protein; ionol, 10 μM; FCCP, 0.5 μM.

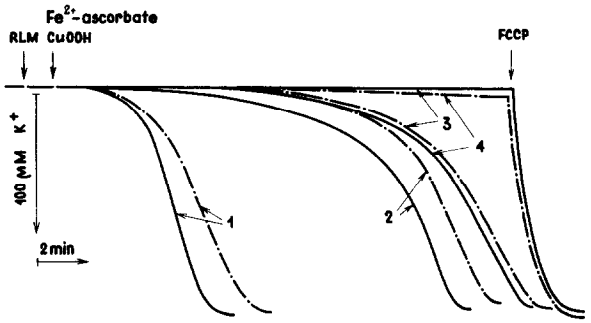

Fig.3. The effect of ionol, oligomycin and DCCD on the release of K^+ from mitochondria during lipid peroxidation induced by Fe^{2+} in the presence of ascorbic acid (——) or by cumene hydroperoxide in the presence of phosphate (-----): control (1); in the presence of oligomycin (2); with ionol (3); with DCCD (4). For reagent concentrations see fig.1,2.

Under these conditions the lipid peroxidation in mitochondria was accelerated concomitant to the decrease in the membrane potential (fig.2). These processes were also effectively suppressed both by ionol (fig.2) and α-tocopherol, as well as by ATP-synthetase inhibitors [oligomycin and DCCD (fig.2,3)].

The discovered suppression of oxidation reactions by the ATP-synthetase inhibitors [oligomycin and DCCD (fig.1,2)] was completely reversed by the uncoupler of oxidative phosphorylation (FCCP). The effect of ionol and α-tocopherol on lipid peroxidation was not reversed by FCCP.

4. DISCUSSION

The induction of lipid peroxidation with ferrous ions in the presence of ascorbic acid or with cumene hydroperoxide in the presence of phosphate brings about the release of K^+ and a drop in the membrane potential. The inhibitors of peroxide processes, ionol and α-tocopherol, completely suppress the release of K^+ from mitochondria and a drop in the membrane potential, by inhibiting the lipid peroxidation processes. These data indicate that the lipid peroxidation process is a significant stage of ion transport induction in mitochondria [7,19,20].

Here, we have discovered a new phenomenon: the suppression by the ATP-synthetase inhibitors oligomycin and DCCD of the potassium ion transport in mitochondria induced by lipid peroxidation (fig.3). It is shown that the inhibitors suppress the very process of lipid peroxidation both in the case of induction of lipid peroxidation with Fe^{2+} in the presence of ascorbic acid (fig.1) and in the case of induction of lipid peroxidation with cumene hydroperoxide in the presence of phosphate (fig.2).

A comparison between the effect of ionol and α-tocopherol and the antioxidant action of oligomycin and DCCD has revealed a qualitative difference in the effect of these lipid peroxidation inhibitors under experimental conditions. The effect of oligomycin and DCCD, but not that of ionol and α-tocopherol is observed only in the energized mitochondria and is reversed by the uncoupler of oxidative phosphorylation [FCCP (fig.1,2)].

The inhibitory action of oligomycin and DCCD

indicates the existence of close relationship between non-enzymatic peroxidation reactions and the functioning of the ATP synthetase mitochondrial complex. It seems possible to explain this interrelationship by assuming a positive feedback between the peroxidation reaction rate in mitochondria and the operation of the proton channel in ATP-synthetase.

The fact that the rate of peroxidation reactions in mitochondrial membranes is controlled by the proton leakage through the ATP-synthetase proton channel contradicts with the accepted opinion that lipid peroxidation processes can only damage mitochondrial membranes.

It may be assumed that lipid peroxidation processes directly contribute to regulation of the system of cation transport and the ATP-synthetase mitochondrial complex.

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