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The effect of butylated hydroxytoluene, an inhibitor of lipid peroxidation, on the calcium-induced uncoupling of rat liver mitochondria

Sergei A. Novgorodov*, Vladimir G. Gogvadze, Boris I. Medvedev and Valeryi P. Zinchenko

*A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899 and Institute of Biological Physics, USSR Academy of Sciences, Pushchino, Moscow Region 142292, USSR

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The accumulation of Ca^{2+} by rat liver mitochondria in the presence of P_i results in spontaneous activation of respiration, accompanied by progressive loss of the accumulated cation. The lipid peroxidation inhibitor, butylated hydroxytoluene, completely prevents and reverses the loss of accumulated Ca^{2+} and restores respiration to the state 4 level, but exerts no effect on the rate of Ca^{2+} accumulation and respiration in the presence of the uncoupler. The strong inhibition by butylated hydroxytoluene of ruthenium red-insensitive Ca^{2+} efflux has also been observed. No correlation between the BHT-sensitive Ca^{2+} loss and the formation of malonic dialdehyde in mitochondria has been found. The data obtained suggest that the Ca^{2+}-induced uncoupling of mitochondria is mainly due to the appearance of electrogenic ion fluxes that are controlled by the initial steps of lipid peroxidation.

Ca^{2+} accumulation; Lipid peroxidation; Antioxidant; Mitochondria; Ion flux

1. INTRODUCTION

It is well established that mitochondria from different tissues are capable of respiration- or ATP-dependent massive accumulation of Ca^{2+} (reviews [1,2]). An intriguing property of the mitochondrial Ca^{2+}-transporting system in vitro is that the accumulation of Ca^{2+} inside mitochondria in the presence of P_i gives rise to time-dependent uncoupling, i.e. irreversible activation of respiration and efflux of Ca^{2+} and other cations from mitochondria. Several factors are known to increase the ability of mitochondria to accumulate Ca^{2+} in the presence of P_i, for example oligomycin, adenine nucleotides, Mg^{2+}, alkalinization of the reaction media and treatment of mitochondria with SH reagents [1-4]. The mechanisms by which the above factors increase the ability of mitochondria to accumulate Ca^{2+} remain obscure. Here, we show that the Ca^{2+}-induced uncoupling can be completely prevented and reversed by an inhibitor of lipid peroxidation. The results obtained suggest that the accumulation of Ca^{2+} in the mitochondrial matrix induces electrogenic ion fluxes, controlled by the very initial steps of lipid peroxidation. A preliminary report has been presented [5].

Correspondence address: S.A. Novgorodov, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Abbreviations: BHT, butylated hydroxytoluene; MDA, malonic dialdehyde; $\Delta\psi$, transmembrane electrical potential; TPP^+, tetraphenylphosphonium

2. MATERIALS AND METHODS

Rat liver mitochondria were prepared by the conventional procedure [6] in 0.25 M sucrose containing 5 mM Hepes (pH 7.4) and 0.25 mM K^+ EDTA. EDTA was omitted from the final washing solution and sedimented mitochondria were suspended in the same solution at 100 mg protein/ml. The standard incubation medium contained 0.25 M sucrose, 10 mM potassium succinate, 10 mM H_3PO_4, 2 μM rotenone, 2 μM TPP^+ chloride and 10 mM Hepes (pH 7.4). The mitochondrial transmembrane potential was estimated from the distribution of TPP^+ between

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the mitochondrial matrix and surrounding medium using a TPP^+-sensitive electrode [7]. The changes in Ca^{2+} and O_2 concentrations in the incubation medium were recorded using a Ca^{2+}-selective electrode (Radiometer F2002-Ca^{2+}, Denmark) and a Clark-type electrode, respectively. Lipid peroxidation was estimated by the accumulation of MDA using the thiobarbituric acid test in the presence of the iron chelator diethylenetriaminepentaacetate [8]. Protein concentration was determined by the biuret method using bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

Fig.1B shows the time course of oxygen consumption by the suspension of mitochondria. Addition of a limited amount of Ca^{2+} transiently stimulated respiration, accompanying the accumulation of cations, and after some time, a spontaneous increase in the rate of respiration occurred (curve 1). The presence of BHT in the incubation medium (curve 2) totally prevented spontaneous acceleration of oxygen consumption but had no effect on the rate of respiration during the accumulation of Ca^{2+}. Addition of BHT after the respiration rate had attained a maximum also restored it to the level of state 4 (curve 3).

In both cases, the effect of BHT was completely reversed by the protonophore uncoupler, CCCP, indicating the lack of a direct action of BHT on the respiratory chain. A powerful inducer of lipid peroxidation, Fe^{2+} (in the presence of ascorbate) [9], considerably decreased the ability of mitochondria to maintain coupled respiration after Ca^{2+} addition (curve 4). BHT restored the ability of mitochondria to maintain controlled respiration after the addition of Ca^{2+} in the presence of Fe^{2+} and ascorbate (curve 5). The ability of BHT to suppress Ca^{2+}/phosphate-induced electrogenic ion fluxes is manifested distinctly in its effect on $\Delta\psi$ (fig.1A).

Fig.1C shows the distribution of Ca^{2+} between mitochondria and the incubation medium unter the conditions described for fig.1B. From comparison of fig.1B,C, it is obvious that the spontaneous increase in respiration rate is accompanied by the loss of Ca^{2+} from mitochondria (curves 1,4). BHT increases the ability of mitochondria to retain Ca^{2+}, in both the presence and absence of Fe^{2+} and ascorbate (fig.1C, curves 2,5) and gives rise to reaccumulation of Ca^{2+} after the onset of its efflux (curve 3).

The results obtained show that the accumulation of Ca^{2+} in the presence of P_i in the mitochondrial matrix activates processes that, with respect to some parameters, are similar to those initiated by the lipid peroxidation inducer Fe^{2+} and ascorbate.

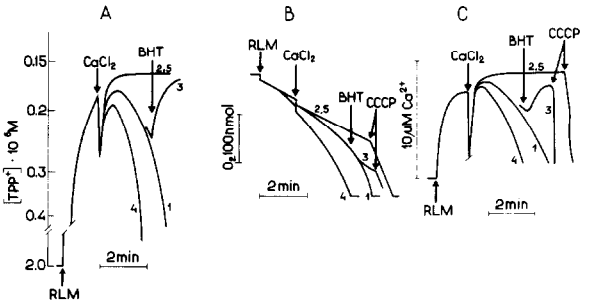

Fig.1. Effects of Ca^{2+}, BHT, and Fe^{2+}-ascorbate on transmembrane potential (A), rate of respiration (B) and Ca^{2+} distribution (C) in mitochondria (RLM). RLM, 1 mg/ml. (1) 50 μM $CaCl_2$ (2) 50 μM BHT in the medium + 50 μM $CaCl_2$, (3) 50μM $CaCl_2$ + 50 μm BHT, (4) 15 μM $FeSO_4$ and 250 μM ascorbate in the medium + 50 μM $CaCl_2$, (5) 50 μM BHT, 15 μM $FeSO_4$ and 250 μM ascorbate in the medium + 50 μM $CaCl_2$.

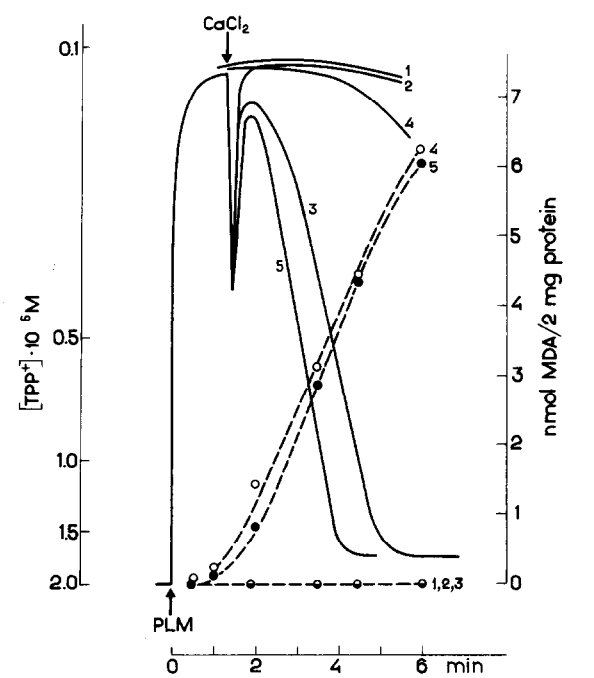

Fig.2. Effects of Ca^{2+}, BHT, and Fe^{2+}-ascorbate on the transmembrane potential (——) and accumulation of lipid peroxidation products (– – –)in mitochondria (RLM). RLM, 2 mg/ml. (1) Control, (2) 50 μM BHT in the medium + 100 μM $CaCl_2$, (3) control + 100 μM $CaCl_2$; (4) 15 μM $FeSO_4$ and 250 μM ascorbate in the medium, (5) 15 μM $FeSO_4$ and 250 μM ascorbate in the medium + 100 μM $CaCl_2$.

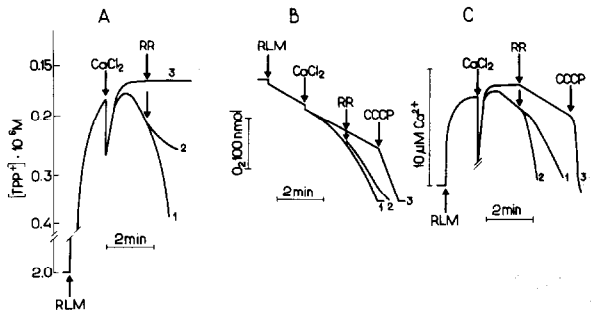

Fig.3. Effects of Ca^{2+} and ruthenium red (RR) on transmembrane potential (A), rate of respiration (B) and distribution of Ca^{2+} in mitochondria (RLM). RLM, 1 mg/ml. (1) 50 μM $CaCl_2$, (2) 50 μM $CaCl_2$ + 3 μM RR, (3) 50 μM BHT in the medium + 50 μM $CaCl_2$ and 3 μM RR.

Activation of lipid peroxidation by various inducers increases the ion permeability of the inner mitochondrial membrane [9,10]. This is not surprising, since it is well known that lipid peroxidation products increase the ion permeability of mitochondrial and artificial membranes [9,11,12].

However, as demonstrated in fig.2, dissipation of $\Delta\psi$ in Ca^{2+}/phosphate medium in the absence (curve 1), as opposed to the presence (curve 4) of Fe^{2+} and ascorbate, is not accompanied by accumulation of the end product of lipid peroxidation, MDA, whereas stabilization of $\Delta\psi$ in the presence of BHT is pronounced. Mitochondrial Ca^{2+}/phosphate-induced uncoupling is frequently attributed to induction of ruthenium red-insensitive, nonelectrogenic Ca^{2+} efflux and dissipation of $\Delta\psi$ as a result of Ca^{2+} recycling across the mitochondrial membrane [1,2,4]. Indeed, addition of ruthenium red after the onset of Ca^{2+} efflux induced significant acceleration of Ca^{2+} release (fig.3C, curve 2), which is considerably suppressed by BHT (fig.3C, curve 3). Ruthenium red addition, however, is not accompanied by restoration of $\Delta\psi$ (fig.3A, curve 2) or the initial rate of respiration (fig.3B, curve 2), as occurs on addition of BHT (fig.1). Therefore, the contribution of Ca^{2+} cycling to the electrogenic fluxes arising in the system is only slight.

The present results show that the initial stages of the peroxidation process are a factor controlling Ca^{2+}-induced uncoupling of mitochondria and ruthenium red-insensitive Ca^{2+} efflux.

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