The role of phospholipase A_2 in lipid peroxidation-induced fall of membrane potential of rat liver mitochondria

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Cumene hydroperoxide (230 μM)-induced fall of the membrane potential takes place only in Ca^{2+}-loaded mitochondria. Inhibitor of phospholipase A_2 *p*-bromphenacyl bromide prevents uncoupling of mitochondria, having no effect on the accumulation of lipid peroxidation products.

Mitochondria; Lipid peroxidation; Calcium; Phospholipase A_2

1. INTRODUCTION

It is well established that activation of lipid peroxidation regardless of the nature of prooxidant (Fe^{2+}-ascorbate, organic hydroperoxides) causes the time-dependent fall of $\Delta\phi$ and efflux of Ca^{2+} and other cations from mitochondria [1,2]. In the absence of prooxidant the same changes occur as a result of so called 'massive' Ca^{2+} loading in the presence of P_i, and are potentiated by activation of mitochondrial Ca^{2+}-dependent phospholipase A_2 and accumulation of free fatty acids and lysophospholipids in the mitochondrial membrane [3,4]. It should be mentioned that phospholipase A_2 activity also increases when mitochondria are peroxidized by iron and ascorbate [5]. So it is of interest to clarify the role of phospholipase A_2 in prooxidant induced deenergization of mitochondria. In the present paper we show that inhibition of phospholipase A_2 by *p*-bromphenacyl bromide prevents uncoupling of mitochondria, having no effect on the accumulation of lipid peroxidation products. The result obtained suggests that prooxidant-induced uncoupling of mitochondria is mediated by phospholipase A_2 activation.

2. MATERIALS AND METHODS

Rat liver mitochondria were prepared by the conventional procedure in 0.3 M sucrose containing 0.2 mM EGTA and 5 mM Tris-HCl, pH 7.5. EGTA was omitted from the final washing solution and sedimented mitochondria were suspended in the same solution at 60–70 mg protein/ml. The standard incubation medium contained 0.1 M sucrose, 0.1 M KCl, 2 mM KH_2PO_4, 2 μM rotenone, 2 μM TPP^+ chloride, 5 mM potassium succinate and 5 mM Tris-HCl. $\Delta\phi$ changes were evaluated by TPP^+ distribution between the incubation medium and the mitochondrial matrix with a TPP^+-selective electrode [6]. The changes in Ca^{2+} and O_2 concentrations in the incubation medium were recorded using a Ca^{2+}-selective electrode (Orion 93-90, USA) and Clark-type electrode, respectively. Lipid peroxidation was estimated by accumulation of MDA using the TBA-test [7]. Protein concentration was determined by Lowry's method [8]. All incubations were carried out at 24°C.

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Abbreviations: CuOOH, cumene hydroperoxide; TPP^+, tetraphenylphosphonium; MDA, malonic dialdehyde; TBA, 2-thiobarbituric acid; $\Delta\phi$, mitochondrial inner membrane potential; *p*-BrPhBr, *p*-bromphenacyl bromide; CCCP, carbonyl cyanide *m*-chlorphenylhydrazone

3. RESULTS AND DISCUSSION

Fig. 1 shows that addition of CuOOH to Ca^{2+} loaded mitochondria induced the fall of $\Delta\phi$. This fall was associated with rapid accumulation of lipid peroxidation products. It should be mentioned, that in the absence of prooxidant mitochondria can retain $\Delta\phi$ for a long time (data not shown).

CuOOH-induced deenergization of mitochondria was found to be Ca^{2+}-dependent, since addition of EGTA in the incubation medium prevented deenergization without any effect on lipid peroxidation. Taking into account the ability of Ca^{2+} to activate mitochondrial phospholipase A_2, it can be supposed that prooxidant-induced uncoupling of mitochondria is mediated by activation of enzyme. To clarify the role of phospholipase A_2, we inhibited enzyme by addition of 10 μM *p*-BrPhBr prior to CuOOH. It has been shown [9] that higher concentrations also inhibited lipid peroxidation.

As can be seen from Fig. 2, *p*-BrPhBr completely prevented the drop of $\Delta\phi$ and Ca^{2+} release from mitochondria (until uncoupler CCCP was added) without any decrease in MDA production.

Inhibition of phospholipase A_2 abolished prooxidant-induced disturbance of oxidative phosphory-

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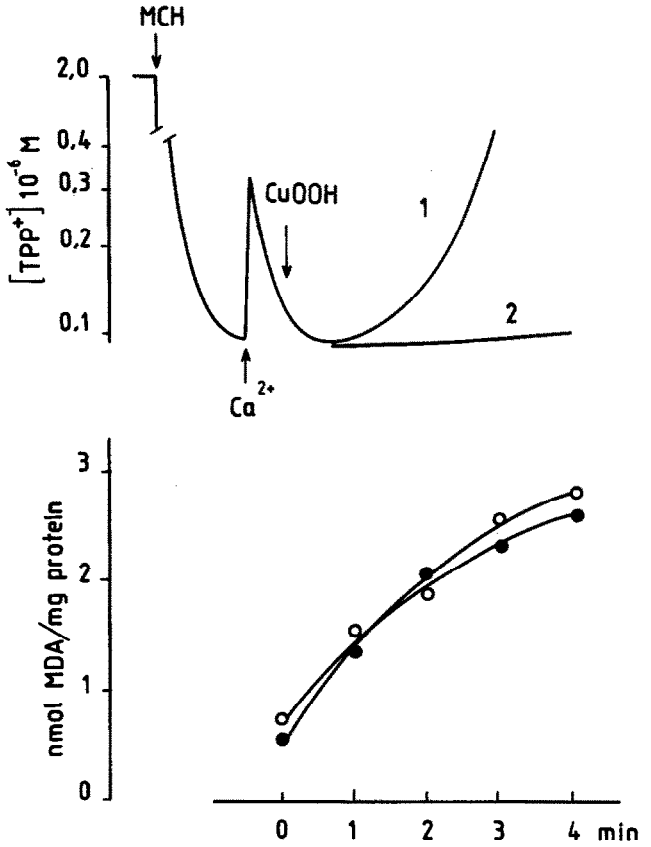

Fig. 1. Effect of EGTA on the CuOOH-induced fall of $\Delta\phi$ and accumulation of lipid peroxidation products. Mitochondria (MCH), 1 mg/ml; Ca^{2+}, 60 μM; CuOOH, 230 μM; EGTA (curve 2, black symbols), 0.2 mM.

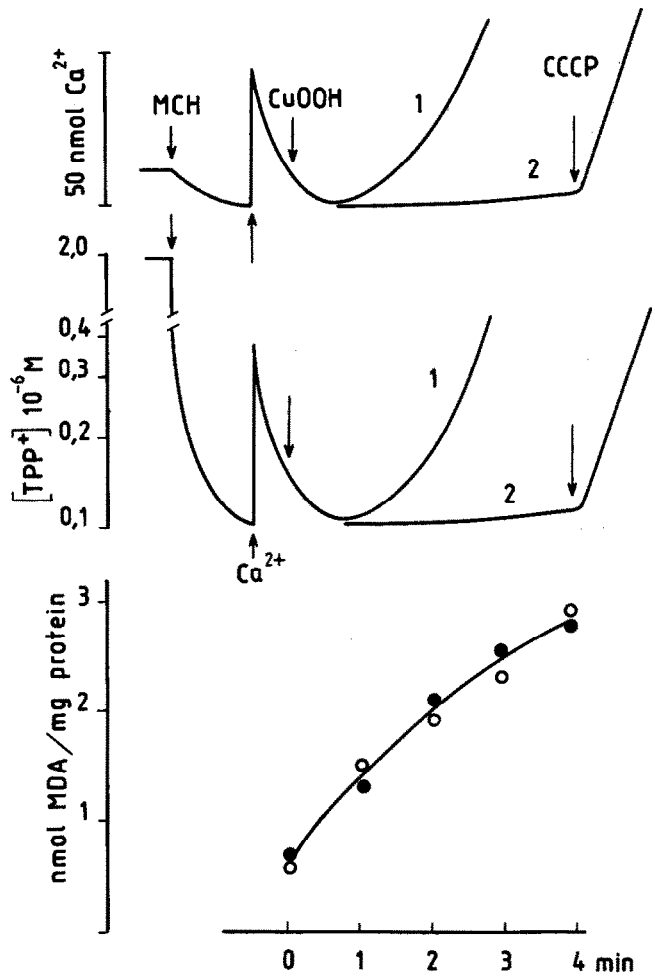

Fig. 2. Effect of *p*-BrPhBr on the CuOOH-induced fall of $\Delta\phi$, Ca^{2+} efflux and accumulation of lipid peroxidation products. MCH, 1 mg/ml; Ca^{2+}, 60 μM; CuOOH, 230 μM; *p*-BrPhBr (curve 2, black symbols), 10 μM.

lation. As seen from Table I CuOOH activated State 4 respiration and reduced respiratory control coefficient in Ca^{2+}-loaded mitochondria. Addition of *p*-BrPhBr partially restored respiratory control and prevented CuOOH-induced acceleration of State 4 respiration.

A number of studies have demonstrated an association between lipid peroxidation and phospholipase A_2 activity [5,9,10]. It has been shown that activity of enzyme is dependent on the physico-chemical state of the membrane lipid phase [11]; lipid peroxidation induces alteration of membrane lipids [12], enhancing the

Table I

Effect of *p*-BrPhBr on CuOOH-induced alteration of mitochondrial respiration

Additions	Rate of respiration nmol O_2/min mg protein		Respiratory control coefficient
	V_3	V_4	
Ca^{2+}	53.6 + 2.8	15.2 + 1.4	3.5 + 0.3
Ca^{2+} + *p*-BrPhBr	55.1 + 2.2	16.8 + 1.2	3.3 + 0.2
Ca^{2+} + CuOOH	59.2 + 1.8	30.4 + 2.4*	1.9 + 0.2*
Ca^{2+} + CuOOH + + *p*-BrPhBr	58.2 + 2.5	19.2 + 1.4	2.7 + 0.3

Mitochondria, 1 mg/ml; Ca^{2+}, 60 μM; CuOOH, 230 μM; *p*-BrPhBr, 10 μM; ADP, 300 μM. Results are mean ± SE.

* Statistically significant difference from other values, $P<0.05$

susceptibility of phospholipids to phospholipase A_2 attack [13].

Since addition of *p*-BrPhBr in incubation medium keeps mitochondria intact in spite of TBA-active product accumulation, it can be concluded that activation of phospholipase A_2 plays a key role in the prooxidant-induced fall of $\Delta\phi$ and Ca^{2+} efflux from mitochondria. The process of peroxidation appears to facilitate phospholipid hydrolysis, while Ca^{2+} is necessary for phospholipase A_2 activation.

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