Volume 287, number 1,2, 139–141 FEBS 10000 August 1991

ADONIS 001457939100713P

The role of lipid peroxidation products in cumene hydroperoxide-induced Ca^{2+} efflux from mitochondria

V.G. Gogvadze and A.A. Zhukova

Institute of Theoretical and Experimental Biophysics, USSR Academy of Sciences, Pushchino 142292, USSR

Received 24 May 1991

Cumene hydroperoxide-induced calcium release from mitochondria has been studied. Activation of lipid peroxidation by increasing concentrations of cumene hydroperoxide does not enhance calcium efflux induced by low (up to 50 μM) concentration of cumene hydroperoxide. It is concluded that cumene hydroperoxide-induced calcium release depends mainly on processes coupled to hydroperoxide reduction by an endogenous enzyme system.

Mitochondria; Lipid peroxidation; Calcium; Cumene hydroperoxide

1. INTRODUCTION

It is now widely believed that organic hydroperoxides can alter Ca^{2+} retention by mitochondria (for a recent review see [1]). When hydroperoxides are added to mitochondria or submitochondrial particles at submillimolar concentration, lipid peroxidation occurs and MDA formation is measurable [2]. This brings about a drop in $\Delta\psi$ and the efflux of K^+ and Ca^{2+} from mitochondria. Antioxidant BHT suppresses lipid peroxidation and prevents hydroperoxide-induced decrease of $\Delta\psi$ and Ca^{2+} release, that points out free-radical participation in the ion flux regulation [3,4].

On the other hand, it has been shown that small amounts of hydroperoxides also induce Ca^{2+} release from mitochondria (without any significant increase of MDA content), owing to enzymatic reduction of hydroperoxides to corresponding alcohols, as a consequence of pyridine nucleotide oxidation by glutathione peroxidase, glutathione reductase, and energy-linked transhydrogenase. Oxidized nicotinamide nucleotides undergo hydrolysis with the production of nicotinamide and ADP-ribose, the latter binding covalently to a protein of the inner mitochondrial membrane, possibly involved in Ca^{2+} release [5].

The aim of this paper was to clarify the significance of each process mentioned above in hydroperoxide-induced Ca^{2+} release, and to elucidate their possible interrelation. The results obtained suggest that hydroperoxide-induced Ca^{2+} release depends mainly on processes coupled to hydroperoxide metabolism by an endogenous enzyme system. Activation of lipid peroxidation by increasing concentrations of CuOOH does not enhance Ca^{2+} efflux induced by low concentrations of CuOOH.

Abbreviations: CuOOH, cumene hydroperoxide; MDA, malonic dialdehyde; TBA, 2-thiobarbituric acid; NEM, *N*-ethylmaleimide; AΨ, mitochondrial inner membrane potential; BHT, butylated hydroxytoluene.

Correspondence address: V.G. Gogvadze, Institute of Theoretical and Experimental Biophysics, USSR Academy of Sciences, Pushchino 142292, USSR.

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 of protein/ml. The standard incubation medium contained 0.1 M sucrose, 0.1 M KCl, 2 mM KH_2PO_4, 5 mM potassium succinate and 5 mM Tris-HCl. The changes in Ca^{2+} in the incubation medium were recorded using a Ca^{2+}-selective electrode (Orion 93-90, USA). Lipid peroxidation was estimated by accumulation of MDA using the TBA-test [6]. Protein concentration was determined by Lowry's method [7]. All incubations were carried out at 24°C.

3. RESULTS AND DISCUSSION

As can be seen from Fig. 1 (trace 1) the addition of a small amount of CuOOH (60 nmol/mg of protein) to Ca^{2+}-loaded mitochondria induces rapid enough Ca^{2+} release. According to the literature to date, Ca^{2+} efflux from mitochondria is associated with the NADH oxidation and following hydrolysis as a result of hydroperoxide reduction by GSH/GSH-peroxidase system [5]. NEM inhibits the reduction of CuOOH by interacting with reduced glutathione [8] that prevents Ca^{2+} efflux (Fig. 1, trace 2). Rotenone, on the other hand, accelerates Ca^{2+} efflux from mitochondria (Fig. 1, trace 3) as a consequence of the inhibition of reversed electron flow from succinate to NADH, that brings about rapid oxidation and subsequent hydrolysis of endo-

Published by Elsevier Science Publishers B.V.

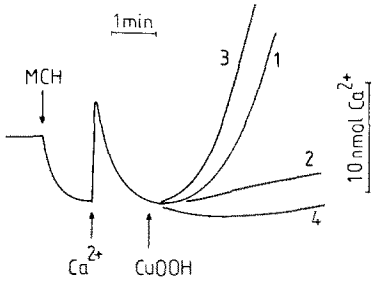

Fig. 1. The effect of NEM (2), rotenone (3) and BHT (4) on CuOOH-induced Ca^{2+} efflux (1) from mitochondria. Mitochondria (MCH), 0.5 mg/ml; NEM, 50 μM; rotenone, 2 μM; Ca^{2+}, 25 μM; CuOOH, 30 μM, and BHT, 25 μM.

genous NADH. Antioxidant BHT was reported previously [3,4] to prevent Ca^{2+} release from mitochondria, that may indicate the participation of free-radical reactions in Ca^{2+} efflux regulation (trace 4).

It is known that besides reduction, organic hydroperoxides can be metabolized by the cytochrome P-450 system. When hydroperoxide concentration exceeds the capacity of the detoxifying enzyme system, or reducing equivalents are exhausted and no further reduction is feasible, the remaining hydroperoxides undergo decomposition. This pathway yields radical intermediates, that can initiate lipid peroxidation and TBA active product accumulation [9].

To elucidate the relative significance of each way of hydroperoxide utilization in Ca^{2+} release from mitochondria, lipid peroxidation product formation as well as Ca^{2+} retention time was estimated as a function of CuOOH concentration. For this purpose different concentrations of hydroperoxide were added to Ca^{2+}-loaded mitochondria and 5 min later, aliquots of 0.2 ml were taken for TBA-active product content determination. Ca^{2+} retention time was measured as the time interval between CuOOH addition and Ca^{2+} half-maximum release.

Exposure of mitochondria to increasing concentrations of CuOOH results in a progressive activation of lipid peroxidation (Fig. 2, filled circles), but at the same time, as can be seen (open circles) the most pronounced fall of Ca^{2+}-retention time occurs at low (up to 50 μM) concentrations of CuOOH, without any significant accumulation of TBA-active products. On the other hand, intensive accumulation of lipid peroxidation products at higher concentrations of CuOOH does not enhance Ca^{2+} efflux.

It can be concluded from results presented here, that Ca^{2+} efflux from mitochondria is attributed to a considerable extent to the hydroperoxide metabolism by an endogenous enzyme system rather than to lipid peroxidation product accumulation, induced by free radicals formed in consequence of hydroperoxide decomposition on cytochrome P-450.

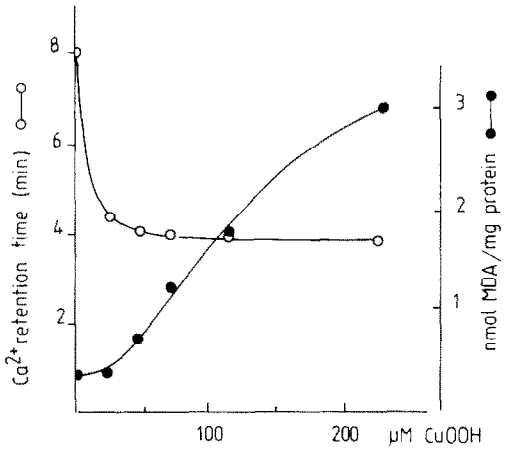

Fig. 2. The dependence of TBA-active product formation and Ca^{2+} retention time on added CuOOH concentration. Mitochondria, 0.5 mg/ml, Ca^{2+}, 20 μM. For explanation, see text.

This conclusion, however, contradicts the result demonstrating the ability of BHT to prevent CuOOH-induced Ca^{2+} release from mitochondria, since it makes questionable the role of free-radical-induced lipid peroxidation reactions in Ca^{2+} efflux regulation. To elucidate this discrepancy, the protective effect of BHT at different concentrations on Ca^{2+}-retention and TBA-active product accumulation has been studied. BHT at different concentrations was added to Ca^{2+}-loaded mitochondria. Lipid peroxidation was initiated by 230 μM CuOOH and Ca^{2+} release as well as TBA-active product content was registered. Fig. 3 represents the dependence of CuOOH-induced TBA-active product accumulation and Ca^{2+} retention time on added BHT concentration.

As can be seen from Fig. 3, low concentrations of BHT (up to 5 μM) significantly suppress lipid peroxidation, having no effect on Ca^{2+} efflux from mitochondria. Exposure of mitochondria to increasing concen-

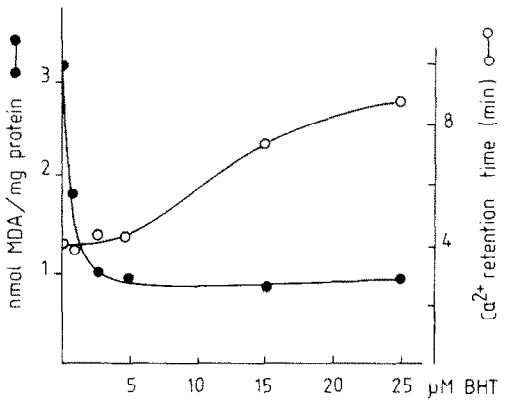

Fig. 3. The dependence of TBA-active product formation and Ca^{2+} retention time on BHT concentration. Mitochondria, 0.5 mg/ml; Ca^{2+}, 20 μM; CuOOH, 230 μM. For explanation, see text.

trations of antioxidant results in progressive prevention of Ca^{2+} release, but at the same time further decrease of TBA-active products content is not detected.

It follows from the above data, that the prevention of Ca^{2+} release from mitochondria by BHT does not correlate with antioxidant activity and is probably attributed to its unspecific action. Our results support the data reported by Novgorodov et al. [10], that the inhibition of lipid peroxidation by different antioxidants does not correlate with their capacity to prevent the fall of $\Delta\psi$ induced by CuOOH as well as Ca^{2+} plus phosphate.

An inhibitory effect of BHT at a concentration of 50 μM on the increase of mitochondrial membrane permeability induced by Ca^{2+} plus phosphate [11,12], SH-reagents [12,13], and *t*-butyl hydroperoxide [12] have already been reported. Since BHT is a well-known free-radical scavenger, its protective effect suggests that all these agents act via free-radical formation. Taking into account the results presented here, that BHT suppresses lipid peroxidation at a considerably lower concentration, which does not influence Ca^{2+} release, it can be supposed, that the inhibition of Ca^{2+} release induced by the agents mentioned above may also be explained by an unspecific effect of BHT.

At the same time it should be mentioned that the absence of quantitative correlation between the inhibition of lipid peroxidation product accumulation and Ca^{2+} release prevention by BHT (Fig. 3) may be explained on the basis of the assumption [10] that hydroperoxide-induced unspecific permeability can be initiated directly by free-radicals or mediated by the very initial steps of lipid peroxidation and is not dependent on the accumulation of the end products of lipid peroxidation.

In summary, it is important to emphasize, that CuOOH-induced lipid peroxidation practically does not influence Ca^{2+} efflux from mitochondria; induction of ion fluxes is mediated by the reduction of added hydroperoxide at the expense of NADH oxidation and following hydrolysis [5]. Formation of lipid peroxidation products can be considered as a process accompanied by Ca^{2+} release when a relatively high concentration of CuOOH is used.

REFERENCES

[1] Bindoli, A. (1988) Free Radical Biol. Med. 5, 247–261.
[2] Bindoli, A., Cavalini, L. and Jocelyn P. (1982) Biochim. Biophys. Acta 681, 496–503.
[3] Marshansky, V.N., Novgorodov, S.A. and Yaguzhinsky, L.S. (1983) FEBS Lett. 158, 27–31.
[4] Novgorodov, S.A., Gudz, T.I., Mohr, Yu.E., Goncharenko, E.N. and Yaguzhinsky, L.S. (1989) FEBS Lett. 247, 255–258.
[5] Richter, C. and Frei, B. (1988) Free Radical Biol. Med. 4, 365–375.
[6] Ohkava, H., Onishi, N. and Yagi, K. (1979) Anal. Biochem. 95, 351–358.
[7] Lowry, O., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[8] Jocelyn, P.C. and Dickson, J. (1980) Biochim. Biophys. Acta 590, 1–12.
[9] Cadenas, E. and Sies, H. (1982) Eur. J. Biochem. 138, 459–463.
[10] Novgorodov, S.A., Gudz, T.I., Kushnareva, Yu.E., Roginsky, V.A. and Kudryashov, Yu.B. (1990) Biologicheskie Membrany (in Russian) 7, 945–955.
[11] Novgorodov, S.A., Gogvadze, V.G., Medvedev, B.I. and Zinchenko, V.P. (1989) FEBS Lett. 248, 179–181.
[12] Carbonera, D. and Azzone, G. (1988) Biochim. Biophys. Acta 943, 245–255.
[13] Novgorodov, S.A., Kultayeva, E.V., Yaguzhinsky, L.S. and Lemeshko, V.V. (1987) J. Bioener. Biomembr. 19, 191–202.