it is better to use liposomes with concentrated solutions of the complexone, or to inject LP and FP simultaneouly. However, this latter procedure requires further experimental study.

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# LIPID PEROXIDATION IN OUTER AND INNER MITOCHRONDRIAL MEMBRANES DURING ANOXIA

A. I. Dzhafarov, N. M. Magomedov,
É. M. Kulieva, N. K. Neiman-zade,
R. F. Kerimov, T. A. Mamedkhanly,
É. G. Khasaeva, and A. G. Chogoshvili

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It has been shown recently that products of lipid peroxidation (LPO) participate in the mechanism of injury to membranes during hypoxia [3]. Analysis of experimental results obtained during a study of LPO in various organelles during anoxia *in vitro* and *in vivo* shows that mitochondria are particularly sensitive to anoxia and are liable to undergo oxidative destruction [2]. We know that the outer and inner mitochondrial membranes differ not only in their chemical composition, but also in the functional properties of their own ultrastructures [6].

The aim of the present invetigation was to study the character of development of LPO in the inner and outer mitochondrial membranes during anoxia, and also to examine whether labilization of membranes of other organelles by LPO products accumulating in the mitochondria is possible.

## EXPERIMENTAL METHOD

Experiments were carried out on 250 Wistar albino rats. Mitochondria were isolated from the liver of the rats by the method in [7]. The inner and outer membranes of the mitochondria were isolated by the method in [8]. Tissue anoxia *in vivo* was induced by ligation

Laboratory of Biophysics of Reception, A. I. Karaev Institute of Physiology, Academy of Sciences of the Azerbaidzhan SSR, Baku. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 433-435, October, 1985. Original article submitted March 13, 1985.

  

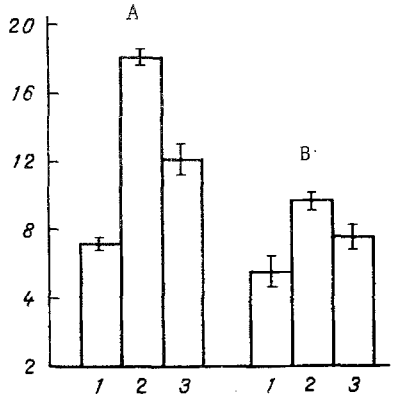


Fig. 1

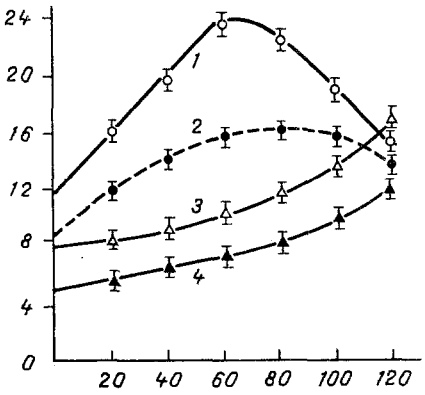


Fig. 2

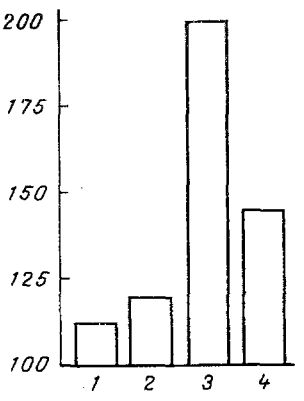


Fig. 3

Fig. 1. MDA concentration in outer (A) and inner (B) mitochondrial membranes before and after 30 min of anoxia. Ordinate, MDA level (in nmoles/mg protein). 1) Control, 2) tissue anoxia, 3) anoxia *in vitro*.

Fig. 2. Changes in MDA concentration in outer and inner mitochondrial membranes after anoxia *in vitro* for 30 min. Abscissa, duration of reoxygenation (in min); ordinate, MDA level (in nmoles/mg protein). 1, 2) Outer membrane, 3, 4) inner membrane, 2, 4) preliminary addition of cinchocaine to mitochondrial suspension (20 μM).

Fig. 3. Changes in free AP activity (in % of control) during combined incubation of lysosomal and mitochondrial fractions under different conditions. 1) Control lysosomes + control mitochondria, 2) lysosomes after anoxia *in vitro* for 30 min + control mitochondria; 3) control lysosomes + mitochondria exposed for 30 min to tissue anoxia, 4) lysosomes exposed for 30 min to tissue anoxia + control mitochondria.

of the vascular pedicle of the liver, and anoxia of the mitochondria *in vitro* was induced by in incubating a suspension of them in an atmosphere of helium.

The intensity of LPO was judged from the concentration of the secondary product of LPO, malonic dialdehyde (MDA), estimated by the reaction with 2-thiobarbituric acid [5]. Free acid phosphatase (AP) activity was measured by the method in [4]. Protein was determined by the biuret reaction.

## EXPERIMENTAL RESULTS

The experiments showed that after tissue anoxia *in situ* the intensity of LPO in the mitochondrial membranes was appreciably increased (Fig. 1). The rate of accumulation of MDA in the outer membrane in this case was higher than in the inner membrane. The high level of LPO in the outer membranes was probably due to the fact that these membranes contain more lipids (40% compared with 20% in the inner membranes) and are richer in polyunsaturated fatty acids [6]. Exposure of the mitochdonria to anoxia *in vitro* likewise was accompanied by activation of LPO of the mitochondrial membranes, especially the outer membrane, which is more sensitive to anoxia than the inner membrane. In the outer membrane, immediately after anoxia for 30 min, the MDA concentration was appreciably increased to 10 ± 0.55 nmoles/mg protein, and it continued to increase during the next 60 min of reoxygenation, when it reached 24 ± 0.8 nanomoles/mg protein. Unlike in the outer membrane, during the first 60 min of reoxygenation the MDA level in the inner membrane remained almost unchanged (Fig. 2). LPO in the outer and inner membranes during anoxia *in vitro* was sensitive to the action of cinchocaine, addition of which to the suspension caused marked inhibition of LPO, especially in the outer membrane. The reduction in the rate of MDA accumulation after addition of the phospholipase $A_2$ inhibitor is evidence of possible participation by this enzyme in the intensification of LPO in the outer mitochondrial membranes during anoxia.

After preliminary administration of antioxidants to the animals LPO was depressed in both kinds of mitochondrial membranes. After preliminary injection of α-tocopherol in a dose of 120 mg/kg and of the organic selenium compound (1-phenylseleno-4-phenyl-4-hexamethyleneiminobutine-2 hydrochloride) in a dose of 40 mg/kg the MDA level in the outer membrane fell from 7.2 ± 0.34 nmoles/mg protein (in the control) to 5.0 ± 0.4 and 3.8 ± 0.2 nanmoles/

mg protein ($n = 11$, $P < 0.01$). In the case of anoxia *in vitro*, after preliminary treatment with α-tocopherol the MDA concentration in the outer membrane fell to 11.5 ± 0.8 nmoles/mg protein ($n = 8$, $P < 0.001$) compared with 18.7 ± 0.57 nmoles/mg protein in the control, but after treatment with the selenium compound it fell to 9.1 ± 0.4 nmoles/mg protein ($n = 8$, $P < 0.001$).

It was interesting to discover whether LPO, located in the mitochondrial membranes, can extend to lysosomes during anoxia and cause labilization of their membranes. To study this problem mitochondrial and lysosomal fractions were incubated together. The degree of labilization of the lysosomal membranes was judged from the change in AP activity and MDA accumulation in the lysosomal membranes. During combined incubation of the control mitochondria, both with control lysosomes and with lysosomes exposed to anoxia *in vitro*, AP activity increased by only 13-15% and the MDA concentration in the lysosomes by only 8-12%. During combined incubation of the control lysosomes with mitochondria previously exposed to anoxia *in vitro*, AP activity was doubled and the MDA level rose by 3.15 times; during incubation of the control mitochondria with lysosomes exposed tissue anoxia *in situ*, the increases were by 1.3 and 1.5 times respectively compared with the control (Fig. 3).

Activation of LPO by contact with lysosomes after exposure of the mitochondria to anoxia thus brings about intensification of LPO and labilization of lysosomal membranes.

The higher rate of LPO and its earlier achievement of maximal values in the outer mitochondrial membranes than in the inner membranes during exposure to anoxia both *in situ* and *in vitro*, and also labilization of the membranes of other organelles in the course of their interaction indicate a leading role of the outer mitochondrial membranes in the mechanism triggering cell damage by peroxidation during ischemia.

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