ACTIVATION OF LIPID PEROXIDATION AND CHANGES IN VITAMIN E LEVELS IN THE LUNGS DURING OXIDATIVE STRESS

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The lung cells are among the most sensitive to stress-induced damage [8]. It is generally accepted that stress-induced damage may at least partly be the result of so-called oxidative stress. This type of stress is manifested as activation of free-radical reactions and, in particular, of lipid peroxidation (LPO) [7, 8].

It is well known that a state of akinesia in animals is accompanied by oxidative damage to the tissues by reduction of the quantity of antioxidants and activity of antioxidative enzymes, an increase in the content of LPO products, a decrease in the glutathione content, destruction of polyene phospholipids, and induction of lysosomal enzymes [5, 7, 8]. Because of the large membrane surface of the respiratory portion of the lungs, intensive phospholipid metabolism in lung tissue, and the presence of lung surfactant (92% of the composition of which is accounted for by lipids [4, 11]), the aim of the present investigation was to study changes in concentrations of endogenous LPO products (conjugated dienes. fluorescent products) and the vitamin E content in lung tissue and broncho-alveolar washings in animals subjected to experimental akinesia.

EXPERIMENTAL METHOD

Male Wistar rats (170-200 g) were used. The state of akinesia was induced by the method of Malikova and Arefalov (modification) [1]. The animals were divided into six groups: 1) control, 2) immobilized for 3 h, 3) immobilized for 12 h, 4) immobilized for 24 h, 5) immobilized for 30 h, 6) immobilized for 30 h + unrestrained 5 h (in order to study reversibility of the oxidative lesions). The rats were immobilized in special constraining cages. They were deprived of food and water during the 6 h before being placed in the constraining cages. At definite time intervals the animals were killed by decapitation and the lungs isolated. The lung tissue was homogenized in 0.1 M K,Na-phosphate buffer, pH 7.4 (4°C). Broncho-alveolar washings were obtained by injecting 10 portions, each consisting of 5 ml of physiological saline, into the tracheobronchial tree through the cannulated trachea, followed by respiration, in rats anesthetized with 10% urethane [9]. The fluid thus obtained was centrifuged at 25,000*g* for 10 min (4°C). The supernatant was used in the study. The content of fluorescent products in the biological test objects was determined as in [8]. Conjugated dienes were estimated as in [3]. Vitamin E was determined by a fluorescent method after preliminary extraction of substances from the lung homogenate or washings with the aid of n-hexane [10]. Lipids were determined by the method in [2]. The following reagents were used: K_2HPO_4, NaH_2PO_4, and H_2SO_4 from "Merck," KOH, chloroform, and n-hexane from "Reachim," ascorbate from "Reanal"; methanol, ethanol (absolute).

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TABLE 1. Content of Secondary LPO Products in Lung Tissue of Immobilized Rats

Duration of immobilization, h	Fluorescent products, 1 fL at 235 nm/mg lipid	Conjugated dienes, μmoles/mg lipid
0	16,28±0,92	2,11±0,04
3	17,71±0,50	2,16±0,03
12	23,32±1,47	2,66±0,04
24	27,08±1,21	2,52±0,09
30	37,02±1,41	2,80±0,20
30 h Immobilization + 5 h mobility	42,17±1,97	2,90±0,14

Legend. Number of animals in experimental group 15, in control 12, $p < 0.001$.

TABLE 2. Content of Secondary LPO Products in Broncho-Alveolar Washings from Immobilized Rats

Duration of immobilization, h	Fluorescent products, 1 fL at 235 nm/mg lipid	Conjugated dienes, μmoles/mg ligand
0	350±33	6,14±0,11
3	486±50	6,98±0,11
12	725±79	8,15±0,28
24	1087±57	10,39±0,11
30	1120±46	10,55±0,10
30 h Immobilization + 5 h mobility	1346±117	12,06±0,22

Legend. Number of animals in experimental group 10, in control 12, $p < 0.001$.

EXPERIMENTAL RESULTS

The results given in Table 1 illustrate changes in concentrations of secondary LPO products in the lung tissue of rats kept under conditions of akinesia. With an increase in the duration of immobilization of the animals the content of fluorescent products and of conjugated dienes in the tissue increased. The kinetics of the change in content of secondary LPO products clearly reaches a plateau. There was virtually no difference in the content of fluorescent products and conjugated dienes after immobilization of the rats for 24 and 30 h.

The expected effect of reversibility of the oxidative changes in the membrane lipids 5 h after the end of 30 h of immobilization was not in fact recorded. On the contrary, in the case of immobilization for 30 h + 5 h of unrestrained mobility the content of LPO products in the lung tissue was significantly higher than that observed in tissues studied immediately after immobilization of the animals for 30 h. The results suggest that a state of akinesia leads to induction of LPO in the lungs. Oxidative changes in the lipid composition of the membranes evidently reach their culmination not immediately after the end of immobilization, but a few hours thereafter (Table 1). It can be tentatively suggested that identical results obtained after 24 and 30 h of akinesia can be explained by adaptation of the animals to the conditions of immobilization.

Similar results were obtained in the experiments on broncho-alveolar washings (Table 2). Here also the results correspond to the three tendencies listed above: a) an increase in the content of fluorescent products and conjugated dienes with an increase in the duration of akinesia, reflecting the phase of alarm; b) rising to a plateau in the kinetics of the change in content of endogenous LPO products 24 h after the beginning of immobilization stress, reflecting the phase of adaptation; c) intensification of oxidative changes after the end of immobilization. It will be clear from Table 2 that the

TABLE 3. Vitamin E Content in Lung Tissue and Broncho-Alveolar Washings from Immobilized Rats

Duration of immobilization, h	Vitamin E content	
	lung tissue, μg/mg lipid	broncho-alveolar washings, $\times 10^{-3}$ μg/mg lipid
0	0,132±0,006	0,346±0,016
3	0,118±0,004	0,318±0,022
12	0,092±0,003	0,324±0,022
24	0,072±0,005	0,346±0,016
30	0,077±0,006	0,335±0,016
30 h Immobilization + 5 h mobility	0,064±0,006	0,374±0,016

Legend. Number of animals in experimental groups 12-15. Number of animals in control group 10-12, $0.001 < p < 0.01$.

content of fluorescent products and conjugated dienes after immobilization for 30 h + 5 h of unrestrained mobility was significantly higher than that observed after immobilization for 30 h alone. This suggests that the process is irreversible and passes into the phase of exhaustion.

Comparative analysis of the results given in Tables 1 and 2 shows that changes in the content of the two tested products of peroxide degradation of lipids were more conspicuous in the lung washings than in the lung tissue. This can be explained by the higher concentration of lipids in the washings [4, 11], permitting a higher velocity of LPO in this lung fraction.

Negative correlation exists in lung tissue between changes in the content of fluorescent products and conjugated dienes, on the one hand, and the vitamin E content on the other hand (Tables 1 and 3). The results given in Table 3 show that the vitamin E content in lung tissue decreases with an increase in the duration of immobilization; the vitamin E levels, moreover, are about equal after akinesia for 24 and 30 h. Allowing the rats unrestrained mobility for 5 h after immobilization for 30 h leads to utilization of vitamin E, indirect proof of the development of oxidative lesions in the tissues after the end of akinesia.

Changes in the vitamin E content in the lung washings were not observed (Table 3). The vitamin E concentration in washings from control animals was much lower than in lung tissue (by 100 times). This can be explained by the fact that vitamin E is located in the hydrophobic phase of the membranes and forms a complex with neighboring fatty-acid residues of phospholipids [5]. This makes it unlikely that any vitamin would be removed from the surface of the cells of the tracheobronchial tree by the procedure which we used to obtain broncho-alveolar washings. It can be concluded that a state of akinesia in rats leads to an increase in the vitamin E concentration, i.e., to changes indicative of the development of oxidative stress in animals.

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