Effects of Hyperbaric Oxygen on Lipid Peroxidation and Content of Phospholipids in Rat Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 9, pp. 261-263, September, 1999
Original article submitted March 1, 1999

Exposure to absolute oxygen pressure of 3 and 5 bar revealed hemispheric and interhemispheric differences in lipid peroxidation and the content of phospholipids in rat brain.

Key Words: *synaptosomes; phospholipids; hyperbaric oxygen; brain asymmetry*

Generation of reactive oxygen species (O^{-}_2, HO^{-}, and H_2O_2) inducing peroxidation of lipids, proteins, and nucleic acids and, therefore, leading to membrane destruction and nervous cell death, accounts for neurotoxic effects of hyperbaric oxygen (HBO_2) [3,8]. Changes in lipid peroxidation (LPO) and lipid composition of cell membranes in symmetrical brain structures induced by stress, ischemia, and other factors are different in rats demonstrating various patterns of behavior [4,5]. Therefore, HBO_2 can cause different cytostructural damages to right and left brain structures.

Here we studied interhemispheric asymmetry in LPO and content and composition of phospholipids (PL) in synaptosomal fraction of brain hemispheres of rats exposed to HBO_2.

MATERIALS AND METHODS

Experiments were performed on 26 outbred male rats weighing 150-200 g. The animals were divided into 4 groups. Group 1 rats (n=8) served as the control (open decompression chamber) and were exposed to air at atmospheric pressure. Animals of groups 2 (n=6) and 3 (n=6) were exposed to absolute oxygen pressure of 3 and 5 bar, respectively. Group 4 rats (n=6) were intravenously injected with superoxide dismutase (Cu/Zn-SOD, 700 U/kg) immediately before compression of 5 bar.

Compression was performed in a pressure chamber (400 liters) at a rate of 1 bar/min for 30 min. After decompression, the animals were decapitated, and the right and left hemispheres were removed. Synaptosomes were isolated as described elsewhere [10], and protein concentration was determined [12]. Total PL were extracted [9] and fractionated to phosphatidylserine, phosphatidylinositol, sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine by two-dimensional microthin layer chromatography using chloroform:methanol:28% ammonia (65:35:5) and chloroform:methanol:acetone:acetic acid:H_2O (50:10:20:10:5) systems. KSK silica gel (5-μ fraction) containing 10% gypsum served as the sorbent. Quantitative analysis of PL was performed as described previously [13]. The intensity of LPO was evaluated by the content of MDA [1]. The results were analyzed using Student's t test and STATGRAPHICS 3.0 software.

RESULTS

HBO_2 exposure enhanced anxiety and ambulatory activity (without convulsions) in all rats. Changes in the content of MDA and composition of PL were primarily observed in the left hemisphere (Table 1). In group 2 rats, the exposure to 3 bar increased the content of MDA by 50.5% and decreased the content of phosphatidylcholine by 8.4% (Table 2). Exposure to 5 bar

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0007-4888/99/0009-0885$22.00

TABLE 1. Contents of MDA and Total PL in Rat Brain after HBO_2 Exposure ($M \pm m$)

Group	Hemisphere	MDA, nmol/mg protein	Total PL, nmol/mg protein
1	Right	1.25±0.10	0.37±0.04
	Left	1.07±0.03	0.48±0.03°
2	Right	1.14±0.07	0.59±0.04*
	Left	1.61±0.09*°	0.38±0.01*°
3	Right	0.98±0.09	0.60±0.01*
	Left	1.09±0.03	0.42±0.03°
4	Right	1.16±0.09	0.43±0.02
	Left	1.73±0.09*°	0.49±0.01°

Note. Here and in Table 2: *$p<0.05$ compared with the control, °$p<0.05$ compared with the right hemisphere.

had no significant effect on MDA concentration but changed the ratio of individual PL (except for sphingomyelin) in group 3 animals. The contents of phosphatidylserine, phosphatidylinositol, and phosphatidylcholine increased by 72.6, 95.2, and 17.9%, respectively, while the content of phosphatidylethanolamine decreased by 52% (Table 2). In group 4 rats, the concentration of MDA increased by 61.7%, the content of phosphatidylserine reached the control levels, the concentration of phosphatidylethanolamine considerably increased, and the amount of phosphatidylcholine decreased compared with this parameter in group 3 rats. In group 2 rats, HBO_2 exposure (3 bar) increased the content of total PL in the right hemisphere by 59.5% and decreased this parameter in the left hemisphere by 20.8%. Exposure of group 3 animals to HBO_2 at 5 bar increased the content of PL in the right hemisphere by 62.2% and had no effect on this parameter in the left hemisphere (Table 1).

The increase in the content of total PL in the right hemisphere was probably due to intensification of their synthesis in response to acute stress (HBO_2), because stress is accompanied by enhancement of energy consumption and transition from the carbohydrate to lipid energy metabolism (mobilization of resources) [6]. The reaction of the organism to HBO_2 corresponds to general adaptive syndrome phases [2]. Therefore, accumulation of total PL in the right hemisphere reflects energy stores in the form of PL fatty acids and performs a compensatory function in stress. It is difficult to explain why subdominant and dominant role of the right and left hemispheres in relation to brain content of total PL are inverted in stress. As a whole, this reflects some differences in their biochemical reactions to stress. At the same time, these reactions differed not only during HBO_2 exposure, but also depended on its intensity. Biochemical changes in the right hemisphere were the same during exposure to 3 and 5 bar and were characterized by an increase in the content of total PL, while other parameters remained unchanged. By contrast, the concentration of MDA increased in the left hemisphere of rats exposed to 3 bar, but did not change with pressure rise to 5 bar (group 3) indicating inhibition of LPO processes. Modification of PL composition may account for LPO inhibition (for example, the decrease in the content of phosphatidylethanolamine suppresses phospholipase A_2 activity) [7]. The 2-fold decrease in the content of phosphatidylethanolamine observed in our experiments was probably due to inhibition of phosphatidylserine decarboxylation and stimulation of phosphatidylcholine synthesis through methylation of phosphatidylethanolamine. Phosphatidylcholine synthesis during stress is induced by accumulation of dopamine (and total catecholamines) in the left hemisphere stimulating phosphatidylethanolamine methyltransferase and, therefore, phosphatidylcholine production [11]. Intensification of LPO caused by SOD was probably due to the fact that SOD considerably enhances cerebral blood flow in rats exposed to HBO_2 and increases oxygenation of brain tissues, thus promoting generation of reactive oxygen species [14].

TABLE 2. Composition of PL (% of Total PL) in Rat Brain after HBO_2 Exposure ($M \pm m$)

Group	Hemisphere	PS	PI	SM	PC	PEA
1	Right	10.6±0.9	4.6±0.4	5.7±0.3	41.2±0.6	36.7±0.8
	Left	11.3±0.4	4.2±0.3	5.0±0.3	41.8±0.4	37.0±0.7
2	Right	8.9±1.8	5.7±0.9	6.2±1.3	43.5±1.4	35.5±1.9
	Left	15.8±3.5	6.7±1.0	4.8±0.3	38.3±1.3*°	34.7±3.5
3	Right	10.4±0.5	4.7±0.8	6.4±1.2	39.1±1.5	39.5±0.8
	Left	19.5±0.7*°	8.2±1.3*°	5.1±0.6	49.3±0.9*°	17.8±2.4*°
4	Right	11.9±0.9	5.8±1.8	6.0±1.1	41.6±1.5	34.9±0.8
	Left	9.2±0.9	5.5±0.9	3.5±0.7	47.8±1.6*°	34.1±0.6*

Note. PS: phosphatidylserine; PI: phosphatidylinositol; SM: sphingomyelin; PC: phosphatidylcholine; and PEA: phosphatidylethanolamine

Hence, HBO_2 changed all studied parameters in rat brain and led to interhemispheric asymmetry in the intensity of LPO and the content and composition of PL.

The study was supported by the Russian Foundation for Basic Research (grant No. 98-04-49875).

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