LIPID PEROXIDATION IN SYNAPTOSOMAL AND MITOCHONDRIAL FRACTIONS OF INDIVIDUAL BRAIN STRUCTURES DURING HYPOXIA

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A special place in the development of cell damage during hypoxia and ischemia is played by intensification of lipid peroxidation (LPO) [5]. Data in the literature on the study of functional states of individual brain structures under the influence of various extremal factors (hyperoxia, vitamin E deficiency, the action of phospholipase A_2, fatty acids, etc.) show that the synaptosomes and mitochondria are the more senstiive subcellular fractions [1-4, 6, 7]. It can therefore be postulated that intensification of LPO in synaptosomes and mitochondria may play an essential role also during the action of hypoxia.

It was accordingly decided to study changes in the intensity of LPO in different parts of the brain and in their synaptosomal and mitochondrial fractions in acute hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on 520 Wistar albino rats. A severe degree of hypoxia, determined by the method of Khvatova and co-workers [8], was created by replacing the air completely by nitrogen in an airtight chamber with a capacity of 0.12 m^3 for 14-15 min. Reoxygenation was carried out by ventilating the chamber with oxygen under low pressure from a cylinder for 15 min. Vitamin E was injected intramuscularly in a dose of 300 mg/kg, and ionol intraperitoneally in a dose of 80 mg/kg 16 h before exposure to hypoxia. Immediatley after hypoxia and a combination of hypoxia with reoxygantion, the animals were decapitated and various parts of the brain removed; the medulla, cerebellum, and visual and sensomotor cortex. The fraction of unpurified synaptosomes and mitochondria was obtained from brain tissue by the method in [10]. The intensity of LPO was judged by the change in concentrations of hydroperoxides (HP) and malonic dialdehyde (MDA), determined by the method in [9]. Total protein was determined as in [11].

TABLE 1. Accumulation of HP (in relative units) and MDA (in mmoles/mg protein) in Different Parts of the Brain during Hypoxia (M ± m)

Experimental conditions	Medulla		Cerebellum		Visual cortex		Sensomotor cortex	
	HP	MDA	HP	MDA	HP	NDA	HP	MDA
Control	3,5±0,26	2,87±0,21	3,48±0,3	2,89±0,17	4,19±0,31	3,54±0,28	3,75±0,25	3,31±0,23
Hypoxia	4,67±0,34*	3,58±0,29	4,5±0,36*	3,82±0,3*	5,26±0,41*	4,65±0,34*	5,35±0,46*	3,98±0,31
Hypoxia + reoxygenation	5,05±0,43	4,34±0,36	4,68±0,39	4,12±0,30	6,37±0,52**	5,12±0,45	6,19±0,49	5,67±0,43**
Vitamin E + hypoxia	4,27±0,33	3,37±0,27	4,00±0,26	3,18±0,23	3,99±0,28**	3,04±0,21**	3,86±0,3**	3,52±0,29
Ionol + hypoxia	3,53±0,26**	2,65±0,18**	3,44±0,23**	2,95±0,20**	4,02±0,31**	2,95±0,19**	4,28±0,32**	3,20±0,27**
Vitamin E + hypoxia + reoxygenation	4,48±0,36	3,54±0,26***	4,09±0,30	4,30±0,33	4,23±0,36***	3,93±0,36***	3,88±0,32***	3,64±0,28**
	3,82±0,30***	4,20±0,35	4,06±0,36	3,40±0,25	4,46±0,37***	3,15±0,26***	5,29±0,41	3,72±0,30

Legend: *p < 0.05 compared with control, **p < 0.05 compared with hypoxia, ***p < 0.05 compared with hypoxia + reoxygenation.

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TABLE 2. Accumulation of HP (in relative units) and MDA (in mmoles/mg protein) in Synaptosomes and Mitochondria of Different Parts of the Brain during Hypoxia (M ± m)

Experimental conditions	Medulla				Cerebellum			
	synaptosomes		mitochondria		synaptosomes		mitochondria	
	HP	MDA	HP	MDA	HP	MDA	HP	MDA
Control	1,7±0,13	2,11±0,17	2,22±0,14	2,21±0,18	2,73±0,20	1,84±0,09	2,44±0,21	2,03±0,16
Hypoxia	3,8±0,30*	3,37±0,32*	2,97±0,21**	3,56±0,29**	4,52±0,33*	3,43±0,35*	4,14±0,33**	3,26±0,25**
Hypoxia + reoxygenation	2,55±0,17	4,64±0,35**	3,42±0,28	3,32±0,27	4,11±0,30	2,86±0,17	3,05±0,26	2,46±0,19
Vitamin E + hypoxia	2,04±0,16*	1,89±0,09*	1,50±0,09*	2,99±0,21	2,88±0,19*	2,13±0,15*	2,67±0,19*	2,41±0,35**
Ionol + hypoxia	2,01±0,15*	1,64±0,11*	2,02±0,15**	1,68±0,14*	—	—	—	—
Vitamin E + hypoxia + reoxygenation	2,45±0,23	2,78±0,21***	2,10±0,15***	2,22±0,11***	—	—	—	—
Ionol + hypoxia + reoxygenation	2,53±0,13	2,59±0,16***	2,15±0,15***	2,62±0,21	3,91±0,28	2,06±0,15***	2,47±0,16	1,98±0,14

Table 2 (continued)

Experimental conditions	Visual cortex				Sensomotor cortex			
	synaptosomes		mitochondria		synaptosomes		mitochondria	
	HP	MDA	HP	MDA	HP	MDA	HP	MDA
Control	3,35±0,28	2,88±0,36	4,72±0,36	3,69±0,23	2,41±0,19	1,74±0,10	1,93±0,17	1,50±0,09
Hypoxia	5,80±0,39**	4,82±0,40*	7,24±0,57*	5,67±0,45**	3,95±0,31**	2,83±0,20**	3,27±0,24**	2,82±0,20*
Hypoxia + reoxygenation	5,30±0,41	4,87±0,35	6,80±0,57	6,15±0,47	4,92±0,35	3,93±0,25**	3,33±0,27	2,65±0,20
Vitamin E + hypoxia	2,70±0,20**	2,39±0,16*	5,32±0,43**	4,04±0,31**	2,60±0,19**	1,80±0,13*	2,48±0,19**	1,87±0,13**
Ionol + hypoxia	3,26±0,26**	2,53±0,21*	4,25±0,35**	3,13±0,26*	—	—	—	—
Vitamin E + hypoxia + reoxygenation	3,96±0,25***	3,6±0,24***	4,78±0,38*4	3,47±0,28*4				
Ionol + hypoxia + reoxygenation	3,75±0,27***	3,07±0,20***	6,70±0,5	4,98±0,31***	4,07±0,28	3,18±0,20	2,60±0,18	2,09±0,15

Legend. *$p < 0.01$, **$p < 0.05$ compared with control or hypoxia, ***$p > 0.05$, *4) $p < 0.91$ compared with hypoxia + reoxygenation.

EXPERIMENTAL RESULTS

The results showed that the action of acute hypoxia leads to an increase in concentrations of LPO products in all structures studied (Table 1); the change in the HP and MDA levels in different regions of the brain, moreover, were different in degree: the highest HP level was observed in the sensomotor cortex (42%), the highest MDA level in the cerebellum (32%).

Reoxygenation after hypoxia caused a more significant increase in the concentrations of LPO products in the visual and sensomotor cortex (Table 1). Hoowever, the degree of this increase was greater in regions of the cortex than in the cerebellum and medulla. In the modern view, a greater increase in the intensity of LPO during reoxygenation after ischemia and hypoxia is the result of accumulation of various reduced metabolites (AMP, lactate, NADPH, fatty acids, metals of variable valency, and so on) which, in the presence of oxygen, lead to intensification of LPO [5, 8]. Under these circumstances, more intensive utilization of endogenous antioxidants evidently takes place. Taking this into account, we attempted to study whether stabilization of the lipid component of the membrane by antioxidants is possible in hypoxia and a combination of hypoxia with reoxygenation. The results showed that preliminary injection of antioxidants (vitamin E and ionol) into the animals prevented the accumulation of LPO products (Table 1), and stabilization was more marked in the case of ionol.

In the study of the effect of acute hypoxia on the intensity of LPO in synaptosomal and mitochondrial fractions, hypoxia in these membrane formations also was found to induce a marked increase in the intensity of LPO. However, by contrast with the intact structure, intensification of LPO in synaptosomes and mitochondria during hypoxia was more marked and, in some cases, it was twice the control level (Table 2). In this case reoxygenation against the background of hypoxia did not bring about any significant changes in the intensity of LPO. The reason evidently was that during isolation of the synaptosomal and mitochondrial fractions the membranes come into contact with oxygen, and this contributes to further intensification of oxidative processes. Preliminary administration of antioxidants leads to considerable stabilization of LPO in the cellular structures studied under the conditions of hypoxia alone and under a combination of hypoxia with reoxygenation (Table 2).

Acute hypoxia thus leads to intensification of LPO both in individual brain structures and in their synaptosomal and mitochondrial fractions, and this may be one cause of the structural and functional disturbances of the nervous system during ischemia and hypoxia.

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