ACTIVATION OF LIPID PEROXIDATION AND ITS PREVENTION BY IONOL IN MECHANICAL ASPHYXIA FOLLOWED BY RESUSCITATION

F. Z. Meerson, V. T. Dolgikh, and V. E. Merzhinskii

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Overactivation of lipid peroxidation (LPO) is important, and sometimes decisively important, in the development of many diseases [3, 6, 15]. The possibility cannot be ruled out that a key role in the pathogenesis of the postresuscitation syndrome may also belong to activation of LPO, induced by hypoxia, ischemia, and subsequent resuscitation with reoxygenation.

Accordingly, in the investigation described below, the state of LPO was studied in different organs and tissues of rats resuscitated after mechanical asphyxia, and the possibility of using ionol, an inhibitor of free-radical lipid oxidation under these circumstances to prevent overactivation of LPO.

EXPERIMENTAL METHOD

Experiments were carried out on 210 male rats weighing 180-200 g, anesthetized with ether. The animals were divided into four groups: 1) control, 2) asphyxia, 3) ionol + control, 4) ionol + asphyxia. From 10 to 12 animals were used in each group. Mechanical asphyxia was produced by compressing the intubation tube for 8 min. Resuscitation was carried out by closed cardiac massage and artificial ventilation of the lungs. Ionol was injected intraperitoneally in a dose of 30 mg/kg 1 h before the experiment, because the maximal concentration of ionol in the blood and internal organs is observed 1 h after its injection [5]. Lipids were extracted from the brain, heart, liver, lungs, and skeletal muscle by the method in [12]. Accumulation of primary molecular products of LPO (lipid hydroperoxides) was estimated by the characteristic absorption for diene conjugates at 232 nm [9]. End products of LPO (Schiff bases) were determined by measuring fluorescence of the lipids in chloroform [10] on a "Bian-130" fluorometer, which was calibrated before each series of measurements with 1% quinine sulfate solution in 0.1 N H_2SO_4. Concentrations of ATP, ADP, and AMP were determined with the Fast Combination Kit (from Boehringer Mannheim, West Germany), creatine phosphate by the method in [1], and free fatty acids (FFA) by the method in [11].

EXPERIMENTAL RESULTS

By the end of the 8th minute of mechanical asphyxia the level of lipid hydroperoxides and Schiff bases was found to be significantly raised in all the organs and tissues studied, especially in such vitally important organs as the brain, heart, and liver (Table 1), due evidently to blocking of the mitochondrial respiratory chain and an excess of catecholamines [8]. These two phenomena are known to give rise to ATP deficiency, inhibition of the Krebs cycle, of glycolysis, and of ATP-dependent cationic pumps, and FFA accumulation [7]. Asphyxia for 8 min was shown to lead, first, to a decrease in ATP production by mitochondria of heart and brain by more than 80% and, second, to intensified ATP breakdown, accompanied by an increase in ADP and AMP concentrations (Table 2). A reflection of changes in the level of these metabolites was lowering of the energy potential of the adenine-nucleotide system of the brain from 0.82 ± 0.02 to 0.44 ± 0.01 and of the heart from 0.86 ± 0.02 to 0.56 ± 0.01. The FFA level rose in all organs, more especially in the lungs (by 164%) and liver (by 80%), and less so in

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Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Central Research Laboratory, Omsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 11, pp. 33-36, November, 1983. Original article submitted February 1, 1983.

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TABLE 1. Effect of Preliminary Administration of Ionol on Content of LPO Products in Various Organs and Tissues of Rats Subjected to Mechanical Asphyxia (M ± m)

Parameter	Test object	Control	Control + ionol	8 min of asphyxia	Time after resuscitation, min			
					5 min	30 min	90 min	Ionol + 30 min
Concentration of lipid hydroperoxides, nmoles/mg lipids	Heart	5,1±0,33	4,2±0,32	6,4±0,40*	7,0±0,62*	7,5±0,55**	7,9±0,62**	5,2±0,40[6]
	Brain	4,9±0,35	4,1±0,31	6,3±0,47*	10,8±0,83***	13,9±1,12***	17,2±1,35***	7,3±0,50[B]
	Liver	6,6±0,42	5,9±0,45	10,6±0,92**	13,5±1,20***	12,6±0,98***	16,7±1,40***	6,2±0,42[B]
	Lungs	3,9±0,29	3,6±0,32	6,7±0,53**	7,1±0,66***	8,2±0,71***	10,9±1,02***	4,4±0,35[B]
	Muscle	5,1±0,40	4,6±0,41	6,6±0,51*	7,4±0,57**	8,7±0,68***	9,0±0,80***	6,3±0,55[a]
Intensity of fluorescence of Schiff bases, relative units	Heart	3,0±0,16	2,7±0,20	4,6±0,27***	7,2±0,60***	6,8±0,52***	6,9±0,60***	4,0±0,36[B]
	Brain	2,8±0,20	3,0±0,17	4,2±0,30**	4,6±0,32***	4,6±0,32***	7,4±0,59***	4,2±0,29[B]
	Liver	3,8±0,31	3,7±0,30	6,6±0,42***	7,3±0,51***	9,5±0,60***	8,9±0,58***	5,2±0,39[B]
	Lungs	2,9±0,16	2,1±0,17	4,1±0,30**	4,0±0,26**	4,2±0,31**	4,3±0,27***	3,2±0,19[6]
	Muscle	2,3±0,13	2,2±0,20	2,5±0,21	3,2±0,19**	3,4±0,19***	5,6±0,45***	3,1±0,31[B]

Parameter	Test object	Time after resuscitation						
		6 h	1 days	3 days	7days	14 days	1month	3months
Concentration of lipid hydroperoxides, nmoles/mg lipids	Heart	8,0±0,49***	8,5±0,70**	8,4±0,55***	9,6±0,60***	8,3±0,72***	6,9±0,58*	5,7±0,55
	Brain	11,9±0,77***	14,2±1,10***	9,4±0,69***	14,9±1,13***	10,7±0,94***	6,1±0,49	5,6±0,44
	Liver	9,4±0,86**	10,7±0,94***	8,6±0,72*	12,6±1,01***	11,2±1,05***	7,1±0,67	6,9±0,65
	Lungs	11,1±1,12***	12,4±1,02***	9,2±0,81***	12,7±0,98***	10,2±0,91***	6,2±0,53**	4,0±0,27
	Muscle	8,7±0,60***	8,3±0,72**	7,0±0,63*	8,3±0,77**	8,4±0,70***	6,7±0,50*	5,5±0,41
Intensity of fluorescence of Schiff bases, relative units	Heart	7,0±0,59***	6,3±0,42***	3,9±0,30*	5,5±0,42***	8,0±0,57***	5,4±0,49***	4,4±0,32**
	Brain	5,1±0,38***	5,7±0,41***	4,1±0,27**	5,1±0,34***	7,4±0,42***	3,4±0,27	4,0±0,37*
	Liver	8,1±0,64***	8,6±0,59**	6,8±0,44***	8,5±0,69***	8,9±0,60***	5,6±0,42**	4,6±0,40
	Lungs	4,3±0,33**	7,6±0,45***	6,5±0,29***	4,5±0,27***	4,0±0,22***	3,1±0,20	3,2±0,22
	Muscle	4,6±0,31***	6,2±0,40***	3,6±0,35	5,3±0,36***	7,5±0,49***	2,9±0,30	3,1±0,37

Legend. Significance of differences relative to control: *P < 0.05; **P < 0.01; ***P < 0.001. Significance of differences between resuscitated animals and animals receiving ionol before asphyxia: a) P < 0.05, b) P < 0.01, c) P < 0.001.

TABLE 2. Effect of Preliminary Administration of Ionol on Concentrations of Creatine Phosphate, Adenine Nucleotides, and FFA during Mechanical Asphyxia (M ± m)

Parameter		Test object	Control	Control + ionol	8 min of asphyxia	Time after resuscitation, min			
						5	30	90	ionol +90
Creatine phosphate	μmoles/g tissue	Heart	4,50±0,27	4,04±0,47	0,73±0,09***	1,56±0,35***	4,22±0,61	3,80±0,45	3,97±0,34
		Brain	1,77±0,16	2,64±0,22**	0,34±0,06***	1,33±0,16	1,36±0,16	2,67±0,26**	2,32±0,19
ATP		Heart	2,01±0,09	2,70±0,11***	0,39±0,06***	0,89±0,09***	1,24±0,10***	1,41±0,07***	2,36±0,13[B]
		Brain	2,11±0,17	2,06±0,04	0,31±0,06***	0,75±0,11***	1,40±0,10**	1,42±0,06***	2,55±0,13[B]
ADP		Heart	0,41±0,02	0,52±0,04**	0,82±0,12***	0,78±0,07***	0,67±0,06***	1,22±0,06***	0,47±0,02[B]
		Brain	0,66±0,06	0,31±0,02***	0,43±0,03***	0,59±0,02	0,59±0,04	0,41±0,03**	0,30±0,02[b]
AMP		Heart	0,14±0,01	0,18±0,01**	0,22±0,03**	0,12±0,02	0,18±0,04	0,28±0,03***	0,14±0,01[B]
		Brain	0,21±0,02	0,12±0,01***	0,25±0,01	0,30±0,02**	0,21±0,01	0,20±0,02	0,14±0,01[a]
Energy potential		Heart	0,86±0,02	0,87±0,02	0,56±0,01***	0,72±0,01***	0,75±0,02**	0,70±0,01***	0,87±0,02[B]
		Brain	0,82±0,02	0,89±0,02*	0,44±0,01***	0,64±0,01***	0,77±0,02	0,80±0,02	0,90±0,02[b]
FFA, meq/kg tissue		Heart	8,1±0,56	5,4±0,48**	9,35±0,70	9,9±0,74	9,6±0,70	10,6±0,79*	5,1±0,44[B]
		Brain	5,9±0,41	6,6±0,45	6,8±0,53	8,9±0,62***	11,2±0,90***	8,3±0,71*	8,0±0,61
		Heart	7,0±0,50	6,3±0,41	12,6±1,02***	10,9±0,59***	9,5±0,67**	6,1±0,52	6,9±0,37
		Brain	3,1±0,18	5,1±0,33***	8,2±0,41***	7,7±0,33***	6,2±0,25***	7,1±0,41***	4,2±9,23

Legend. Significance of differences relative to control: *P < 0.05; **P < 0.01; ***P < 0.001. Significance of differences between resuscitated animals and animals receiving ionol before asphyxia: a) P < 0.05, b) P < 0.01, c) P < 0.001.

the brain and heart (by 15%). Excess of FFA is known to have a pathogenic action. They uncouple oxidation from phosphorylation, block energy transport at the mitochondrial level, and have a detergent action on the cell membrane [14]. All these changes, in turn, give rise to a series of changes in the lipid bilayer of the cell membranes: activation of lipases and phospholipases and intensification of LPO against the background of reduced activity of antiradical and antiperoxide defensive enzymes and lowered antioxidative activity of membrane lipids [4].

With the beginning of resuscitation measures, and under conditions of increased tissue oxygenation, the concentrations of lipid hydroperoxides and Schiff bases rose considerably, in agreement with modern views on the so-called oxygen paradox. The essence of this phenomenon is that reoxygenation, if following hypoxia and ischemia, stimulates LPO and is accompanied by accumulation of large quantities of LPO products, capable of destroying biomembranes [13]. The greatest increase in hydroperoxides and Schiff bases is found in vitally important organs such as the brain, heart, and liver, in which the volume blood flow is doubled or trebled after the beginning of resuscitation measures and the partial pressure of oxygen is considerably increased.

Later the content of lipid hydroperoxides and Schiff bases rose steadily in the organs and tissues studied for 24 h, and after 90 min its values were 2-3 times higher than in the control. During the next 2 days the level of primary and secondary LPO products fell a little, but after 1 week it rose again, returning to the control level after 3 months.

After mechanical asphyxia for 8 min LPO activation continued for quite a long time, and could play an essential, or possibly key role in the formation of manifestations of the postresuscitation syndrome such as encephalopathy and cardiac and hepatic failure.

Preliminary administration of the synthetic antioxidant ionol significantly reduced LPO activation in all organs and tissues studied from rats exposed to mechanical asphyxia, and improved the energy supply to the brain and heart. The mortality among animals receiving ionol before asphyxia was reduced by almost two-thirds.

LPO activation is thus a decisive factor in the pathogenesis of the postresuscitation syndrome. However, this can be prevented to some degree by preliminary administration of the synthetic antioxidant ionol, which is an effective trap for peroxide radicals [2].

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