STUDY OF LIPID OXIDATION IN VARIOUS RAT TISSUES DURING AND AFTER HYPEROXIA

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Peroxidation processes in the tissues are limited because of the low physiological oxygen concentrations in them [1]. Saturation of the body with oxygen initiates rapid intracellular lipid oxidation [10, 11, 13]. An increase in the content of lipid peroxides in the body is one of the most important aspects of the general pathogenetic mechanism of oxygen poisoning. That is why, with a view to ensuring survival of animals exposed to hyperoxia it is essential to take appropriate steps to restore the normal body level of lipid peroxides as quickly as possible.

With this aim, in the investigation described below the principles governing the dynamics of the lipid peroxide content in various tissues of albino rats were studied immediately after exposure to hyperbaric oxygen and in the posthyperoxic period.

EXPERIMENTAL METHOD

Experiments were carried out on 48 mature albino rats of both sexes weighing 150-200 g, kept on an ordinary diet. The animals were exposed in a pressure chamber to the action of oxygen under a pressure of 5 atm, in which they were kept until the onset of convulsions or the terminal stage of oxygen poisoning. To study the aftereffects of hyperoxia on the lipid peroxide level, the animals were used in the experiments 1, 24, and 72 h after the onset of a distinct picture of oxygen poisoning. Intact animals kept under ordinary conditions in the animal house were used as the control.

Lipid peroxides were determined in the brain, liver, heart, spleen, skeletal muscle, and blood, which were removed without delay in the cold. From the above tissues 0.5% homogenates were prepared in Krebs–Ringer solution in the presence of ascorbic acid, in a glass homogenizer with Teflon head, in the cold at 500 rpm; the homogenates were transferred to conical flasks, saturated with oxygen for 3 min, after which the flasks were tightly sealed and incubated in a waterbath at 37°C for 60 min with constant shaking. The degree of peroxidation was judged from the accumulation of malonic dialdehyde (MDA) during incubation of the homogenates in the presence of ascorbate. In the writers' opinion, this parameter characterizes the quantity of lipid peroxidation products.

To determine MDA quantitatively, a 0.65% solution of thiobarbituric acid (TBA) was added to samples previously fixed with an equal volume of a 10% solution of TCA. The reaction of TBA with MDA at a high temperature and in medium with acid pH is accompanied by the formation of a colored trimethine complex, which can be estimated photometrically at a wavelength of 532 nm [1]. The content of lipid peroxides was expressed in nanomoles MDA/mg protein, determined by the method of Lowry et al. [12], using a molar extinction coefficient of 1.56×10^5 $M^{-1} \cdot sec^{-1}$. The results were subjected to statistical analysis [8].

EXPERIMENTAL RESULTS

The results (Table 1) showed activation of peroxide formation in all the tissues studied and, in particular, in the brain, whereas the smallest amount of lipid peroxides was found in the blood of the intact animals. As Table 1 shows, during oxygen poisoning there was a sharp rise in the concentration of lipid peroxides in the

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TABLE 1. Content of Lipid Peroxides (in nmoles MDA/mg protein) in Rat Tissues during Exposure to Oxygen under a Pressure of 5 atm and in Posthyperoxic Period (mean results of eight experiments)

Tissue	Control	Immediately after hyperoxia		In posthyperoxic period		
		convulsive state	terminal state	1 h	24 h	72 h
Brain	9,06±0,35	12,97±0,63	14,18±0,53	8,71±0,28	5,02±0,36	8,09±0,51
P		<0,001	<0,001	>0,5	<0,001	>0,10
Blood	1.86±0,16	3,35±0,18	3,51±0,30	3,73±0,10	2,24±0,16	2,06±0,20
P		<0,001	<0,001	<0,001	>0,20	>0,40
Heart	2.04±0,22	5,08±0,39	6,30±0,36	4,60±0,19	2,32±0,19	2,20±0,19
P		<0,001	<0,001	<0,001	>0,40	>0,50
Liver	6,74±0,22	8,31±0,32	8,80±0,64	8,06±0,30	4,20±0,20	6,62±0,22
P		<0,001	<0,01	<0,005	<0,001	>0,50
Spleen	2,30±0,14	4,40±0,15	4,96±0,22	3,71±0,14	2,30±0,11	2,33±0,20
P		<0,001	<0,001	<0,001	>0,50	>0,50
Kidneys	4,64±0,21	5,96±0,26	5,83±0,34	6,05±0,16	4,72±0,24	3,37±0,30
P		<0,005	<0,01	<0,001	>0,60	<0,005
Skeletal muscles	4,40±0,29	6,82±0,48	8,09±0,40	5,64±0,25	5,54±0,16	5,39±0,34
P		<0,001	<0,001	<0,01	<0,005	<0,05

tissues examined. In both convulsive and terminal stages these changes were most marked in the myocardium, followed (in descending order) by the spleen, blood, skeletal muscles, brain, kidneys, and liver.

The experiments to study the dynamics of the lipid peroxide content in the posthyperoxic period show the relative rapidly beginning (during the first hour) normalization of the brain vessels of these compounds. The intensity of the normalizing effect on these compounds in the other organs did not conform to a single plan, and during the period specified the concentrations of lipid peroxides in them remained fairly high. It is an interesting fact that 24 h after exposure to oxygen in a pressure of 5 atm their content reached control values in the blood, heart, spleen, and kidneys, whereas in the brain and liver it was 44.6 and 37.7% respectively below normal. The data show that skeletal muscles were the exception, for the lipid peroxide level continued to remain high in these tissues throughout the 72 h of the posthyperoxic period. During this same period the lipid peroxide content in the brain, blood, heart, liver, and spleen succeeded in falling to the control level, and in the kidneys it was significantly reduced (Table 1).

It can thus be concluded that the substantial increase in the content of lipid peroxides taking place under the influence of hyperoxia in all the tissues studied gives way in the posthyperoxic period to the development of the opposite effect. The rapid onset of normalization of the lipid peroxide level in the tissues (especially in nerve tissue) must in all probability be regarded as a true indicator of the ability of the animals to survive after exposure to hyperoxia. The phenomenon described above, which can be regarded as a manifestation of protective and adaptive reactions of the body to the unusual conditions of existence created for it, is based, it may be considered, on the action of powerful antioxidant systems of nonenzymic and enzymic nature. As a triggering mechanism in the development of many pathological disturbances, lipid peroxidation aggravates the toxic effects of hyperoxia by initiating disturbances of permeability of biological membranes [7, 13], of enzyme activity, and of activity of other regulatory systems of metabolic processes [2-5, 9, 14, 15]. However, besides the comparatively rapid onset of normalization of lipid peroxide levels, the results of the aftereffect of these poisons still remain demonstrable because of the profound pathobiochemical changes that affect the course of tissue metabolism.

It is intended to pursue this research in the direction of a study of the role of peroxides of individual phospholipids in order to examine certain aspects of the pathogenesis of membrane disturbances under hyperoxic conditions and to seek effective ways of preventing toxic lipid oxidation through the use of various natural and artificial antioxidants.

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