EFFECT OF ACUTE HYPOXIC HYPOXIA ON LUNG SURFACTANT SYSTEM IN RATS

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A fall in the partial pressure of oxygen considerably alters the surface activity (SA) of the lung surfactant system (LSS) [5, 7], and during a stay at an "altitude" of 6000 and 9000 m for 2 and 6 h the SA of a lung tissue extract falls sharply [2]. A longer exposure to acute hypoxia leads to the development of compensatory processes, in the form of an increase in SA of the surfactants [4]. The dynamics of SA and its dependence on altitude and duration of hypoxia and the correlation between its changes in lung tissue extracts and endotracheobronchial washings have not been studied. Morphological changes have not been compared with the state of LSS.

The object of this investigation was to study the state of LSS in rats by various methods during exposure to acute hypoxia in a pressure chamber at different "altitudes" and for different exposures.

EXPERIMENTAL METHOD

Altogether 125 albino rats, native to Frunze (altitude 720 m above sea level) and weighing 150-250 g, were used. The animals of group 1 (23 rats) served as the control. Animals of group 2 (72) were exposed to hypoxia in an open type pressure chamber with a capacity of 0.2 m^3 for 6 h at an "altitude" of 2000, 4000, 6000, and 7500 m above sea level (6-12 rats in each series). The animals were kept at an "altitude" of 11,000 m until a state of agony (from 180 to 300 min). Animals of group 3 were exposed to hypoxia in the same pressure chamber at an "altitude" of 6000 m for 3 and 12 h and at an "altitude" of 9000 m for 1, 3, 5, and 12 h. The rats were decapitated immediately after "descent."

Endotracheobronchial washings were prepared from one large lobe of the lung. This lobe was then homogenized and centrifuged (8000 rpm, 30 min). The concentration of the washings and extract was 1 g/100 ml physiological saline. Surface tension (ST) was measured on modified Wilhelmy's scales with automatic recording of the hysteresis loops. The surface area of the cuvette was 60 cm^2 and the rate of change of surface area 0.8 cm^2/sec. SA of the surfactants was estimated from the change in minimal and maximal ST (ST_{min} and ST_{max}) and the stability index (SI) after [6].

Sections were cut from the second lobe of the lung and stained with Rhodamine 6G, according to [8] and [3]. The intensity of luminescence of the inner layer of the alveoli and of lipids was measured on a microfluorometer and expressed in conventional units. Five hanging drops were prepared from the third lobe of the lung in order to measure the coefficient of stability of Pattle's bubbles (PC) by the method in [1]. Histological sections from the two remaining lobes were stained with hematoxylin–eosin by van Gieson's method.

EXPERIMENTAL RESULTS

Analysis of changes in LSS (Table 1) showed that its activity falls steadily up to an altitude of 6000 m, rises a little at 7500 m, and returns to the control level at 9000 m. At an "altitude" of 11,000 m SA again falls sharply. Changes in the endotracheobronchial washings were more demonstrative than those in the homogenates, but they followed a parallel course ($r = +0.85$; $P < 0.02$). The changes in SA were confirmed morphologically by corresponding changes in the intensity of luminescence of the surfactant in the inner layer of the alveoli ($r = +0.96$; $P < 0.01$). Changes in PC were the same as those in SI ($r = +0.78$; $P < 0.1$). The absence of changes in the parameters in some cases can evidently be explained by the low sensitivity of Pattle's method.

The intensity of luminescence of lipids in lung sections increased gradually up to 9000 m. Lipids in the lungs of the control animals were uniformly distributed in cells of the alveolar septa. With an increase in hypoxia the lipids accumulated in large, intensively luminescent conglomerates, located at the periphery of the alveoli and in their lumen, a picture which was clearest at an "altitude" of 9000 m. At 11,000 m the topography of the lipids remained as before but the intensity of their luminescence was sharply reduced.

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TABLE 1. Parameters of State of LSS in Rats following a Single Exposure to Acute Hypoxia in a Pressure Chamber for 6 h (M ± m)

Altitude above sea level, m	Endotracheobronchial washings			Lung homogenate			PC	Intensity of luminescence of inner layer of alveoli, conventional units	Intensity of luminescence of lipids in
	ST_{min}, mN/m²	ST_{max}, mN/m²	SI	ST_{min}, mN/m²	ST_{max}, mN/m²	SI			
720	21,2±0,4	55,6±1,2	0,894±0,013	23,4±0,6	48,8±0,8	0,706±0,022	0,91±0,01	15,7±0,4	5,3±0,3
2 000	24,8±0,7	55,2±1,4	0,761±0,032*	26,4±0,6*	50,0±0,2	0,616±0,005*	0,86±0,02*	11,3±1,3*	6,1±0,3
4 000	25,8±0,7*	53,5±1,7	0,701±0,022*	26,8±0,7*	49,9±1,3	0,602±0,024*	0,90±0,01	11,1±0,7*	6,5±0,2*
6 000	29,0±0,7*	57,0±1,2	0,670±0,002*	29,6±0,7*	52,0±0,8*	0,550±0,014*	0,81±0,05	7,8±0,8*	7,0±0,2*
7 500	27,4±1,0*	57,0±1,2	0,685±0,029*	29,1±0,4*	52,5±0,5*	0,574±0,023*	—	11,0±0,9*	8,5±0,3*
9 000	20,2±0,9	51,9±1,1*	0,883±0,028	26,2±0,9*	48,7±1,0	0,610±0,020*	0,94±0,01*	13,3±1,0*	8,5±0,2*
11 000	30,7±0,4*	59,4±0,3*	0,637±0,001*	32,7±0,9*	55,7±0,7*	0,523±0,015*	0,67±0,04*	5,3±0,3*	5,1±0,1

Legend. Here and in Table 2, an asterisk indicates $P < 0.05$ compared with control.

TABLE 2. Parameters of State of LSS in Rats after a Single Exposure to Acute Hypoxia in a Pressure Chamber at "Altitudes" of 6000 and 9000 m (M ± m)

Altitude above sea level, m	Time, h	Endotracheobronchial washings			Lung homogenate			PC	Intensity of luminescence of inner layer of alveoli, conventional units	Intensity of luminescence of lipids in lungs, conventional units
		ST_{min}, mN/m²	ST_{max}, mN/m²	SI	ST_{min}, mN/m²	ST_{max}, mN/m²	SI			
720		21,2±0,4	55,6±1,2	0,894±0,013	23,4±0,6	48,8±0,8	0,706±0,022	0,91±0,01	15,7±0,4	5,3±0,3
6000	3	26,6±0,4*	55,4±0,7	0,702±0,018*	25,6±0,5*	49,8±0,9	0,641±0,016*	0,91±0,01	11,2±0,6*	5,9±0,4
	6	29,0±0,7*	58,2±0,5	0,670±0,012*	29,6±0,7*	52,0±0,8*	0,550±0,014*	0,81±0,05	7,8±0,2*	7,0±0,2*
	12	22,2±1,0	53,6±0,8	0,830±0,024	24,8±1,2	49,6±0,6	0,679±0,041	0,92±0,02	15,1±1,3	8,1±1,0*
9000	1	25,8±1,8*	52,4±2,2*	0,681±0,020*	22,4±1,0	48,8±0,7	0,740±0,028	0,86±0,05	13,4±1,2	7,0±0,1*
	3	27,4±0,7*	54,8±1,0	0,667±0,013*	26,4±0,9*	50,0±1,3	0,618±0,010*	0,83±0,03*	9,3±0,6*	6,9±0,8*
	5	25,0±1,8*	54,0±2,2	0,740±0,031*	25,4±0,9	49,2±0,6	0,639±0,023*	0,92±0,01	13,0±0,7*	7,0±0,6*
	6	20,2±0,9	51,9±1,1*	0,883±0,028	26,2±0,9*	48,7±1,0	0,610±0,020*	0,94±0,01*	13,3±1,0*	8,5±0,2*
	12	29,0±0,6*	55,0±4,1	0,618±0,028*	27,5±0,9*	50,2±0,5	0,585±0,031*	0,89±0,03	7,6±1,1*	5,2±0,3

Histologically, at "altitudes" of 2000 and 4000 m, moderate congestion, a few areas of atelectasis, and focal emphysema were found in the lungs. At an "altitude" of 6000 m the hemodynamic disturbances were more severe, the areas of atelectasis were wider, and the emphysema was diffuse in character. At 9000 m the hemodynamic disturbances took the form of congestion, stasis in the vessels of the microcirculation, and focal intraalveolar hemorrhages. The small-caliber bronchi were in spasm, and hypersecretion of mucus by their epithelium was observed. The areas of atelectasis were large and diffuse. Emphysema was predominantly subpleural. At an "altitude" of 11,000 m massive areas of atelectasis alternated haphazardly with emphysema. Both large and small bronchi were in spasm. Congestion and stasis, exudation of protein, hemorrhages, and focal edema of the alveoli were present.

In the experiments on the animals of group 3 changes in the state of LSS were dependent on the intensity and duration of hypoxia (Table 2). After an initial marked fall in SA which developed after 6 h at 6000 m and after 3 h at 9000 m, SA rose again. However, at 6000 m activation of LSS occurred after 12 h, compared with after 6 h at 9000 m. A sharp fall in SA was observed after 12 h at 9000 m but after only 2-3 h of exposure to hypoxia at 11,000 m (Table 1). At 6000 m, however, a similar fall in SA would evidently be expected to occur after a longer exposure to hypoxia.

The results of these experiments showed that changes in LSS during exposure to hypoxia depend on its intensity and duration. The initial fall in LSS activity is a reaction to subliminal stimulation by hypoxia. The increase in SA of the LSS must evidently be regarded as an emergency measure of compensation developing in response to an extremal, above-threshold stimulus. Activation of LSS is connected with structural changes in the lipids in lung tissue, manifested by accumulation of lipids in the lumen of the alveoli. Hypoxia has been shown [9] to delay lipid synthesis; the increase in the intensity of luminescence of lipids must evidently be regarded as the result of their phanerosis, expulsion into the lumen of the alveoli, and utilization by macrophages. The subsequent sharp decline in LSS activity must be regarded as a failure of compensation, under the influence of above-threshold hypoxic stimulation. This is shown by the decrease in the intensity of luminescence of the lipids in lung tissue and a change in their architectonics.

Changes in LSS activity are synchronous with changes in lung structure. The failure of compensation is manifested morphologically as spasm of the bronchi, massive areas of atelactasis, and marked hemodynamic disturbances.

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SPATIOTEMPORAL CHARACTERISTICS OF BETA-HYDROXYBUTYRATE DEHYDROGENASE ACTIVITY IN THE RAT LIVER LOBULE

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The spatiotemporal organization of metabolism in the liver lobule is discussed in reports of investigations into structural and functional heterogeneity of hepatocytes and biological rhythms of their functions [2-4, 7, 8]. Data are given in the literature on the spatial distribution of enzymes in the liver lobule and changes in their activity during the 24-h period [6, 10]. Meanwhile there have been no investigations in which a spatial and temporal approach has been made to study the structural and functional parameters in the liver lobule.

The object of the present investigation was to make a parallel study of the spatial and temporal principles governing activity of beta-hydroxybutyrate dehydrogenase (BDH) in the liver lobule of normal rats.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male albino rats weighing 180-200 g. For 2 weeks before sacrifice the animals were kept under conditions of 12 h daylight (9 a.m. to 9 p.m.) and 12 h of darkness; the intensity of illumination was 300 lx. The rats were decapitated at 1, 5, and 9 a.m. and 1, 5, and 9 p.m. Five animals were killed at each time. BDH activity in the hepatocytes was determined histochemically by the method of Nachlas et al. [5] in 12-μ frozen sections. Activity of the enzyme (EA) was determined quantitatively in five liver lobules of the same size from each animal on a modified scanning integrating digital microspectrophotometer [1], using a 10 × 10 frame with probe 0.5μ in diameter, in a monochromatic beam with wavelength 560 nm, and with a linear stepwise scanning time for the whole frame of 1-2 sec. EA was expressed in conventional units of total optical density of the test object. Lobules in which 18 cells were arranged along a line joining the central vein and the portal vessels (radius of the lobule) were analyzed. Cells along 2-4 radii were examined photometrically in each lobule. The radius was divided into three equal parts, with six cells in each part. These parts corresponded to circulatory zones 1, 2, and 3 of the hepatic acinus as described by Rappaport [9], in the direction from the periphery to the center of the lobule. Each of the above zones was subdivided into two equal subzones (three cells along the radius). The mean value of EA was calculated in the zones and subzones of the lobule, and also in cells occupying the same position on the radius. To describe changes in EA from cell to cell, the difference between the EA level in two neighboring hepatocytes was determined along the whole length of the radius of the lobule. This parameter was used to calculate the mean value of the change in EA for the same cell position in the whole lobule and its zones. The different parameters characterizing EA and its changes in the lobule were determined at each period of the experiment and on average for the 24-h period. The numerical results were subjected to statistical analysis by the Fisher–Student method, at the $P \leqslant 0.05$ level of significance.

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