STATE OF THE LUNG SURFACTANT AND ULTRASTRUCTURE OF THE AIR—BLOOD BARRIER IN ACUTE HYPOXIA

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KEY WORDS: lungs; hypoxia; surfactant; air—blood barrier.

A combined study of the state of the lung surfactant (LS) and the ultrastructure of the air—blood barrier in hypoxia of different types is important to clarify and supplement data on the pathogenesis of acute respiratory failure and on the "shock lung," and it is also of practical importance in connection with the improvement of methods of diagnosis, prevention, and treatment of post-traumatic and postoperative pulmonary complications. However, the study of LS and of morphological changes in the lungs in hypoxia has largely been undertaken separately [1, 2, 7], making it impossible to evaluate the degree of damage to components of the air—blood barrier, one of which is the surfactant alveolar complex, as a whole.

This paper describes a combined study of the state of LS and the ultrastructure of ABB in acute hypoxia by means of physical, biochemical, histological, and electron-microscopic methods.

EXPERIMENTAL METHODS

Experiments were carried out on eight mongrel dogs of both sexes weighing from 13 to 25 kg. To create acute hypoxia of respiratory type, the animals were anesthetized with morphine and hexobarbital, the trachea was intubated, and natural breathing was blocked by listhenon. Artificial respiration was carried out by means of a type RO-6 apparatus for artificial ventilation of the lungs, at the rate of 3-4 cycles/min, giving a respiratory minute volume of 40% of normal. All the animals died 1.5 h after the beginning of bradypnea. Four intact dogs killed by injection of succinylcholine (ditilin) served as the control. The lungs were weighed, the lung coefficient (LC) calculated, paraffin sections of the lungs were stained with hematoxylin and eosin, semithin sections with toluidine blue, and ultrathin sections were examined in the ÉVM-100L and IEM 100CX electron microscopes.

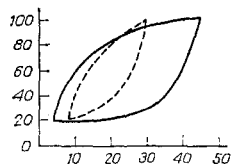

Fig. 1. Dependence of ST on change in surface area of bronchoalveolar washings. Abscissa, ST (in dynes/cm); ordinate, relative area (in % of maximal area of cuvette). Continuous line — control, broken line — hypoxia.

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TABLE 1. Parameters of State of LS in Dogs with Acute Hypoxia (M ± m)

Experimental conditions	CS	ST_{min}, dynes/cm	IS	PC	PEA	PI	LPC	SPM
Control	0,92±0,07	2±1	1,85±0,3	78±1	16±3	0,8±0,04	4,03±0,3	0,7±0,04
Hypoxia	0,87±0,04	10±3	0,92±0,12	68±1,9	12±2	1,0±0,8	10,5±2,2	0,5±0,1
P	>0,05	<0,05	<0,05	<0,05	>0,05	>0,05	<0,05	>0,05

Legend. Ratio between fraction in control and experimental groups shown in percent

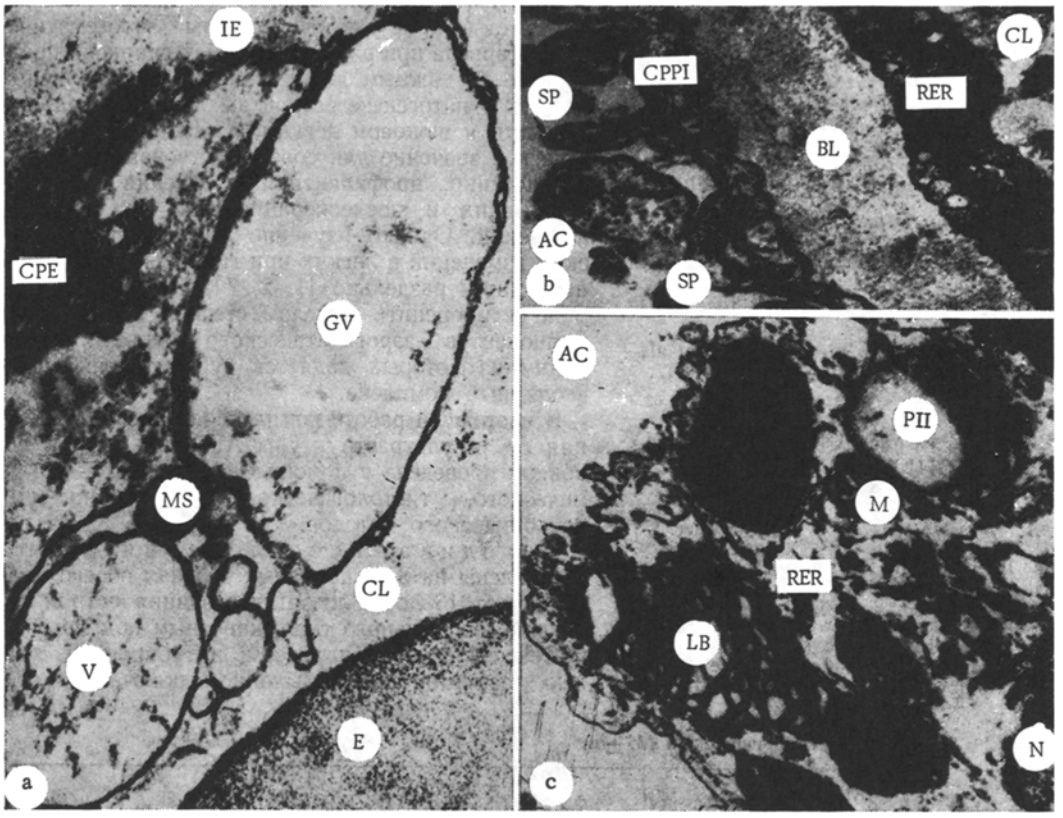

Fig. 2. Ultrastructural changes in ABB in acute hypoxia: a) giant vacuole (GV) in cytoplasmic process of endotheliocyte (CPE). Another vacuole (V) located in capillary lumen (CL). Interstitial edema (IE), E) erythrocyte, MS) myelinated structure, 30,000 ×; b) sail-like projections (SP) on surface of cytoplasmic processes of type I pneumocytes (CPPI). AC) Alveolar cavity, BL) basal layer, CPE) cytoplasmic process of endotheliocyte, 20,800 ×; c) edema and disorganization of endoplasmic reticulum of type II pneumocyte (PII) located in alveolar cavity. LB) Lamellar body, M) mitochondrion, RER) rough endoplasmic reticulum, N) nucleus of type II pneumocyte, 15,400 ×.

The state of the surface activity of the lung tissue was assessed by a microscopic method after determination of the coefficient of stability (CS) of air bubbles expelled from the lungs [17], by measurement of the surface tension (ST) of bronchoalveolar washings from the lung tissue on modified Wilhelmy scales [14], and demonstration of individual phospholipid fractions in them by two-dimensional thin-layer chromatography [15, 16]. When determining ST, the maximal (ST_{max}) and minimal (ST_{min}) values of ST and the index of stability (IS) were taken into account and the shape of the hysteresis loop was analyzed. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

All experimental dogs died 1.5-2 h after the beginning of bradypnea with signs of disturbance of the hemodynamics and serious metabolic disorders. Shifts in the parameters of the acid–base balance and respiratory function of the blood reflected increasing respiratory and metabolic acidosis and also respiratory hypoxia.

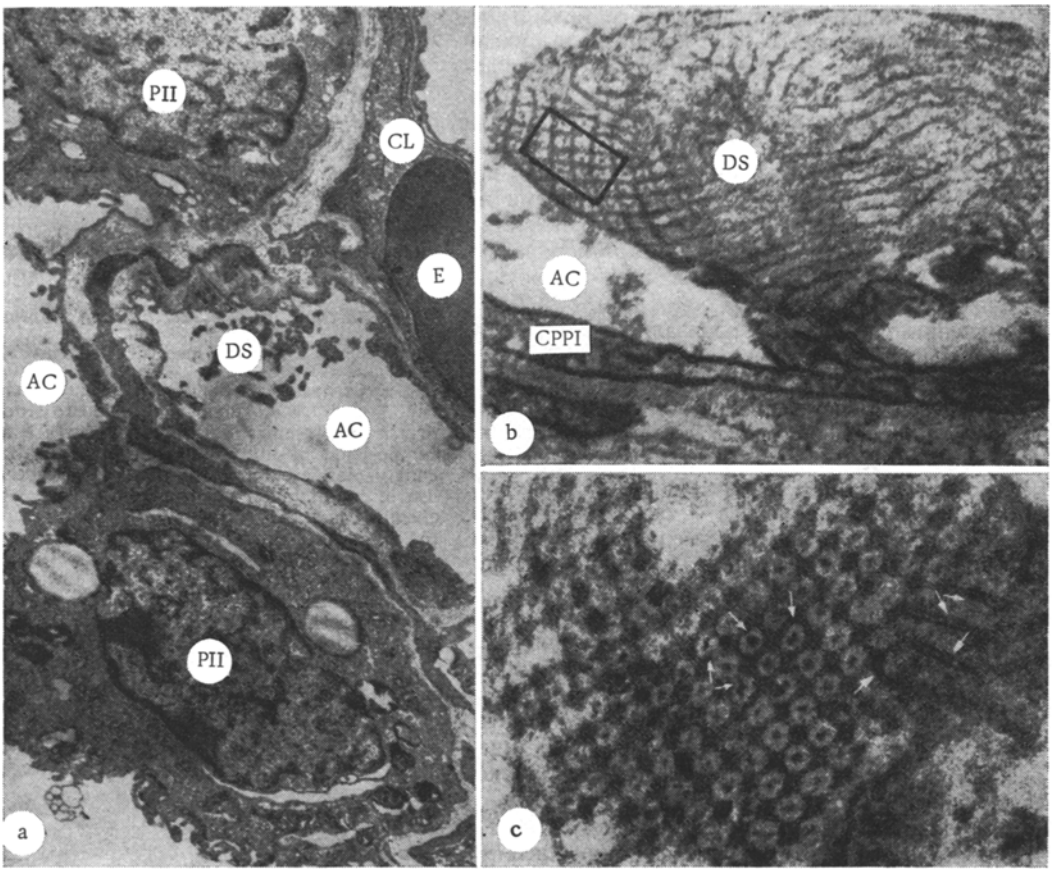

Fig. 3. Changes in extracellular component of LS in hypoxia: a) general view of interalveolar septum with disintegrated surfactant (DS) in alveolar cavity (AC). PII) Type II pneumocyte, E) erythrocyte, CL) capillary lumen, 4000 ×; b) disintegrated surfactant (DS), regular membrane formations in alveolar cavity (AC). CPPI) Cytoplasmic process of type I pneumocyte, 15,400 ×; c) detail of Fig. 3b. Transverse and longitudinal section (arrows) of membranous structures of surfactant, 50,000 ×.

Comparison of the values of LC in the control (0.90) and experimental (1.05) groups showed that although differences between them were not significant ($P > 0.05$) there was a tendency for this coefficient to be higher in dogs with hypoxia. Compared with the control CS showed a tendency to fall and ST_{min} of the bronchoalveolar washings was increased five-fold and IS was reduced by half (Table 1). The shape of the hysteresis loop of the surfactant also was changed, becoming narrower than the wide hysteresis loop in the control (Fig. 1). Seven phospholipid fractions were found in the bronchoalveolar washings from intact dogs: phosphatidylcholine (PC), phosphatidylethanolamine (PEA), lysophosphatidylcholine (LP), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SPM), and polyglycerophosphatides. In animals dying from hypoxia the PC content was lower and the LPC content higher than in the control. No significant differences between the groups were found in the content of the other fractions (Table 1). Statistical analysis revealed distinct correlation between the composition of phospholipids and ST_{min} (coefficient of correlation 0.85, $P < 0.001$).

Histologically only a few small areas of emphysema, dystelectasis, and atelectasis were discovered in the lungs. The interalveolar septa were thickened because of the highly dilated capillaries, congested with blood. In the aerated parts of the lungs, single cells of the alveolar epithelium and erythrocytes could be seen in the cavities of some alveoli.

Electron-microscopic investigation of the lungs of the experimental dogs showed gross dilatation of the lumen of the capillaries of the interalveolar septa, which contained many erythrocytes closely packed together. Initial signs of erythrocytes and leukocytes, accompanied by partial destruction of the cytolemma of the cells and by a decrease in the number of

lysosomes in the leukocytes, were observed. Giant vacuoles were seen in the capillary lumen, with electron-dense finely granular material inside them. The number of pinocytotic vesicles in the cytoplasmic processes of the endotheliocytes was increased and some of them contained large vesicles (Fig. 2a). The basal layer was loose in texture. Focal dispersion of collagen fibrils and elastic fibers, accompanied by the formation of electron-translucent cavities, was observed in the thickened part of the septal space. On the surface of the cytoplasmic processes of the type I pneumocytes microvilli and sail-like projections of the cytoplasm were observed (Fig. 2b). Destruction and homogenization of the mitochondria in the type II pneumocytes were combined with gross dilatation of the tubules of the rough endoplasmic reticulum. Lamellar bodies of the type II pneumocytes were represented by homogeneous material of high electron density or by parallel osmiophilic lamellae. Cells of a similar type were found in the cavities of the alveoli (Fig. 2c). In semithin sections they were clearly detected in the alveolar septa as large cells, projecting into the lumen of the alveoli, with small granules in their cytoplasm, staining intensively with basophilic dyes. Disturbances of the integrity of the cytoplasmic processes of the type I pneumocytes and seepage of blood plasma into the alveolar cavity were not observed. Meanwhile on the surface of the alveoli and in the alveolar cavities myelinated structures and regular membranous formations were found in the form of reticula — these were disintegrated surfactant (Fig. 3a, c).

The results showed that in acute hypoxia of respiratory type in dogs a complex series of mainly nonspecific changes arises in the lungs, consisting of compensatory-adaptive and pathological processes. In the modern view [1, 2], a significant decrease in the content of PC, the main component of LS, in the bronchoalveolar washings and a simultaneous twofold increase in the content of LPC are evidently due to intensification of lipid peroxidation processes and activation of phospholipases, which facilitate conversion of PC into LPC. Accumulation of highly toxic lysophosphatides, with low surface-active properties, is evidently reflected in values of the surface tension of the bronchoalveolar washings of the dogs' lungs and leads to the development of atelectases. However, injury to LS in hypoxia is also connected with disturbance of the permeability of the ABB, edema of the septal space, and disturbance of the functin of the type II pneumocytes. These results clarify and supplement data on the pathogenesis of acute respiratory failure and they are also of applied importance in connection with the search for effective methods of its prevention and treatment.

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FORMATION OF A GENERATOR OF PATHOLOGICALLY ENCHANCED EXCITATION IN THE CAUDATE NUCLEUS IN AN EXPERIMENTAL PARKINSONIAN SYNDROME

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KEY WORDS: tetanus toxin; generator of pathologically enhanced excitation; parkinsonian syndrome; caudate nucleus.

Local disturbance of inhibitory mechanisms in the rostral part of the two caudate nuclei after microinjection of tetanus toxin (TT) leads to the development of a number of neuropathological syndromes in animals [1, 3, 4] which are based on the appearance of corresponding hyperactive determinant structures [3]. In the late stages of the pathological process the animals develop a parkinsonian syndrome. The investigations so far undertaken have suggested that the character of the neurochemical organization of the hyperactive determinant structure in this syndrome is linked with activation of the cholinergic mechanisms of the caudate nuclei in connection with a disturbance of dopaminergic control under the influence of TT.

Disinhibited cholinergic interneurons of the caudate nuclei may perhaps form a generator of pathologically enhanced excitation [3], which may be the working neuropathological component of the determinant structure of this syndrome. To test this hypothesis, the character of unit activity in the rostral part of the caudate nuclei was studied in rats with such an experimental parkinsonian syndrome.

EXPERIMENTAL METHODS

Experiments were carried out on male albino rats weighing 250-270 g. Microinjections of TT were given into both caudate nuclei in accordance with coordinates taken from the stereotaxic atlas [9]: AP −2.0, L 2.5, H 4.0 mm, by the method described previously [4]. Spontaneous activity of the neurons was investigated under chloral hydrate anesthesia (350 mg/kg). To record unit activity metal electrodes with viniflex insulation (diameter of tip 10-15 μ) were used. Spontaneous unit activity was investigated in three tracks (Fig. 1A) in the caudate nuclei on the right side, located in frontal plane AP −2.0 mm, extending in depth from 3.5 to 4.9 mm and with a distance apart in the mediolateral direction of 200 μ coordinates L 2.3, L 2.5, L 2.7 mm). In each track spontaneous unit activity was evaluated at 15 points situated 100 μ apart in the vertical direction. Passage from one point to the next occupied 20 sec, and during the next 50-55 sec the presence or absence of spontaneous activity (single, grouped, rhythmic) was determined. If activity was present the point was considered to be active and potentials were recorded on magnetic tape for 180 sec. In each series (animals with experimental parkinsonian syndrome, intact rats, and control animals receiving an injection of inactivated TT 5 days before the investigation, at the same times as the experimental animals) 180 points were tested. To characterize the changes in spontaneous activity the number of active points, their distributions depending on depth in the track, the character and frequency of the discharge of single neurons, the character of distribution of the neurons depending on frequency, and the mean firing rate of the neurons in each series of the investigation were determined. A M-4 cathode follower, VC-9 oscilloscope, ATAC 501-20 analyzer (from Nihon Kohden, Japan), and a Jupiter tape recorder were used.

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