SECRETORY ACTIVITY OF BRONCHEOLAR AND ALVEOLAR CELLS OF THE MOUSE LUNG AFTER PARTIAL "CHEMICAL SYMPATHECTOMY"

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The role of the sympathetic nervous system in regulation of synthesis and secretion of alveolar surfactant is not yet sufficiently clear and is a topic for discussion. The view has been expressed that the autonomic nervous system may exert a direct influence both on the secretion of phospholipids in the lung and on their synthesis in the type II alveolocytes [3, 4, 11]. It was shown previously that short-term chemical blocking of the sympathetic innervation of the lung has a protective action under conditions of formed hyperventilation [9], in hemorrhagic shock [10], and also when pathological changes arise in the lungs after head injury [8]. It can be postulated that the favorable effect of chemical desympathization on the lung is based on stimulation of the secretion of alveolar surfactant.

The aim of this investigation was to study the effect of "chemical sympathectomy" on the ultrastructural organization and secretory activity of type II alveolocytes, synthesizing alveolar surfactant.

EXPERIMENTAL METHOD

Partial sympathectomy was performed by daily intraperitoneal injection of the sympatholytic drug guanethidine (Isobarin, from Pliva, Yugoslavia) into newborn BALB mice in a dose of 15 mg/kg for 2 weeks. About 20% of neurons compared with the control mice of the same age still remained in the sympathetic ganglia two weeks after the last injection [5]. The lungs of four control and four experimental animals aged one month were fixed and prepared for transmission and scanning electron microscopy (TEM and SEM, respectively) [7]. For SEM the specimens were studied in a Hitachi S-500 microscope and photographed at an angle of 15°, whereas for TEM they were studied in the JEM-100B microscope. The total number of type II alveolocytes was counted in an area of 35,050 μ^2, under a magnification of 5000 and in 100 fields of vision, in the lungs of each animal; the secretory activity of these cells was judged by the state of their apical surface [6]. The height and width of the base of the apical part of the secretory broncheolar Clara cells were measured on photographs with a final magnification of 12,000. The functional state of the type II alveolocytes was assessed by TEM. Morphometry of the organelles was carried out by means of an arbitrary test system [1] on negatives of "central" sections through the cells (50 in the control, 30 in the experiment), containing the nucleus, under a magnification of 24,000. The relative bulk density of the organelles was then calculated relative to the area of the cell (Table 1). The results were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

With respect to their volume and external appearance the lungs of the experimental mice were indistinguishable from the control. The histological structure of the lungs of animals receiving guanethidine likewise corresponded to the control at the same age. These data are in agreement with the results of investigations by other workers [2], who observed no changes in the histological structure of the lungs after extirpation of the stellate or cervical sympathetic ganglia. In the experiment, however, a tendency was noted for the area of the

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TABLE 1. Quantitative Characteristics of Bronchiolar and Alveolar Epithelial Cells of BALB Mice after Partial Chemical Desympathization

Parameter	Control	Experiment	p
Secretory Clara cells (SEM, at an angle of 15°)			
Height of apical part, μ	3,56±0,33	4,99±0,43	<0,05
Width at base of apical part, μ	4,68±0,19	5,19±0,51	>0,05
Type II alveolocytes			
Number of cells examined	321	372	
Mean area of apical surface, μ^2	8,85±0,59	10,00±0,70	>0,05
Length of microvilli, μ	0,30±0,01	0,32±0,02	>0,05
Width of microvilli, μ	0,14±0,01	0,14±0,01	—
Cells, %			
without signs of secretion	25,50±3,10	21,30±4,50	>0,05
with signs of secretion	66,60±2,30	56,00±1,90	<0,05
with signs of completed secretion	7,90±1,50	22,70±3,00	<0,05
Mean number of cytophospholiposomes	13,50±0,95	15,03±1,79	>0,05
mitochondria	12,25±0,99	13,68±1,48	>0,05
Relative bulk density (relative to area of cell, %)			
of nucleus	33,20±1,95	38,46±2,60	>0,05
of cytoplasm	66,85±1,90	61,50±2,50	>0,05
of mitochondria	14,30±1,40	15,00±1,00	>0,05
	12,10±1,50	13,40±1,20	>0,05
Nucleo-cytoplasmic ratio	0,517±0,04	0,689±0,08	<0,05

lumen of the bronchioles and of the alveolar passages to increase, possibly connected with the "relaxing" effect of partial desympathization on the smooth musculature of the lung structures.

The cell composition of the bronchiolar epithelium of the lungs of the experimental mice was unchanged: just as in the control, secretory Clara cells predominated (Fig. 1). However, in the experimental series the height of the bronchiolar secretory cells was significantly increased by 45% compared with the control (Table 1). Cells with signs of apocrine secretion became more numerous (Fig. 1). The apical surface of these actively secreting cells was covered with "pools," evaginations, and folds. The ultrastructural organization of the apical surface of the type II alveolocytes in the lungs of mice receiving guanethidine differed comparatively little from the control in a number of features. The area occupied by these cells in the control averaged 2.3%, and in the experiment 2.7% of the total surface area of the alveolar lining. Meanwhile, definite changes in the relative proportions of type II alveolocytes in different phases of the secretory cycle took place in the lungs of the experimental mice. The number of cells in the phase of completed secretion (Fig. 2) was increased by more than 180% ($p < 0.05$) whereas the number of cells with no secretory pores on their apical surface was reduced. This is evidence of an increase in the number of simultaneously secreting cells. On the apical surface of the type II alveolocytes with evidence of completed secretion in the control, as a rule not more than one postsecretory pore was observed, whereas in the experiment, it was quite common to find cells whose apical surface contained several such pores, clearly visible (Fig. 2a-c). On parallel study by TEM, more secreting cells were discovered in the lungs of the experimental mice than in

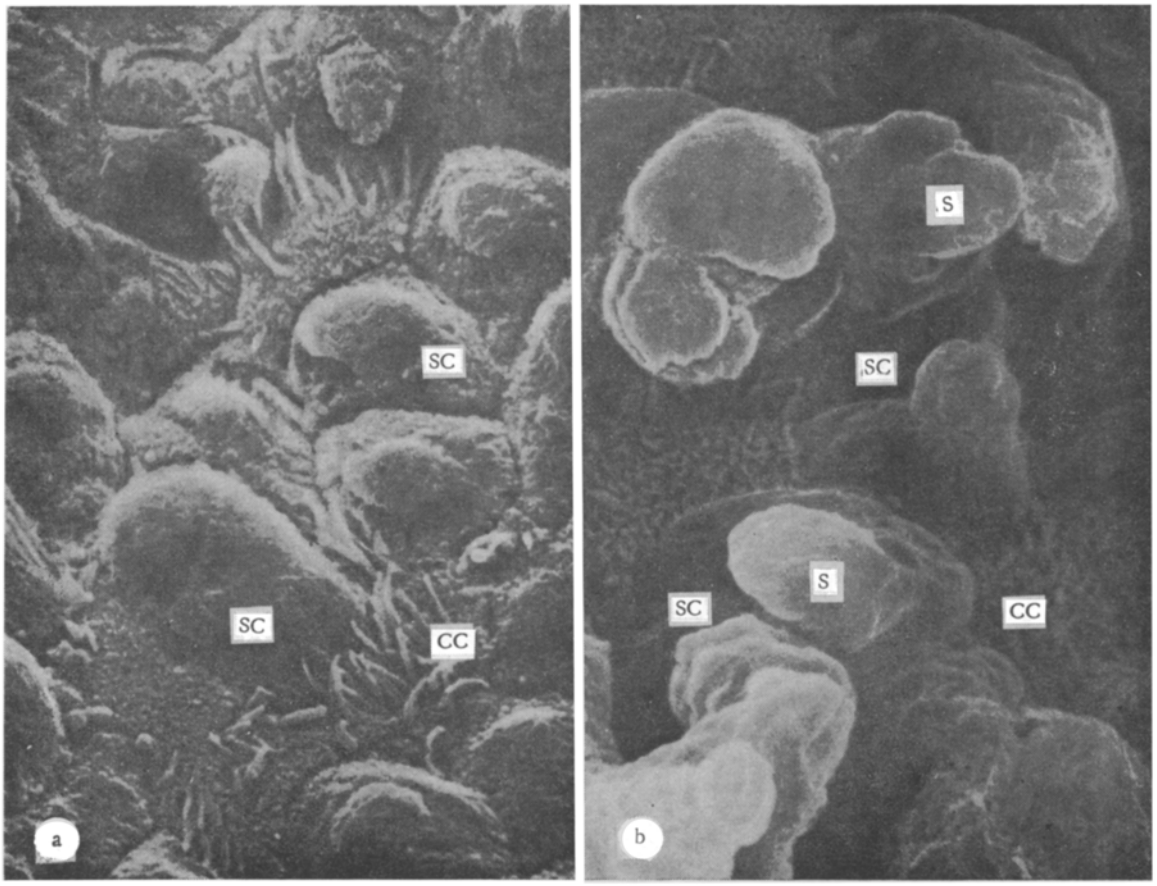

Fig. 1. Epithelial surface of bronchiole of a BALB mouse: a) control, b) experiment. After partial pharmacologic desympathization most of the secretory Clara cells are in a state of active secretion of apocrine type. CC) Ciliated cells, SC) secretory cells, S) pools of secretion. SEM. Magnification: a) 10,600, b) 12,000.

those of the controls. Whereas in the control, for 50 type II alveolocytes, only one secreting cell was observed, in the experimental series, extrusion of cytophospholiposomes was observed in four of 30 cells (Fig. 2d).

Activation of secretion of alveolar surfactant in the lungs of mice after partial "chemical sympathectomy" was superposed on intensive synthesis of phospholipids and on energetically interrelated processes in the type II alveolocytes. A tendency was noted for the nuclei of these cells to undergo hypertrophy, and the nucleo-cytoplasmic ratio was significantly increased. Structures of the Golgi complex and smooth endoplasmic reticulum were more highly developed in the lung cells of the experimental mice than in the control (Fig. 3). The number of mitochondria and cytophospholiposomes per section through the cell was similar in the experiment and control (Table 1). Consequently, active secretion of alveolar surfactant in the lungs of partially "desympathized" animals was not accompanied by degeneration and massive death of phospholipid-synthesizing cells. Consequently, partial "chemical sympathectomy" induced by guanethidine can be regarded as a near-physiological state in which metabolic processes connected with intensification of synthesis and secretion of alveolar surfactant are simultaneously activated.

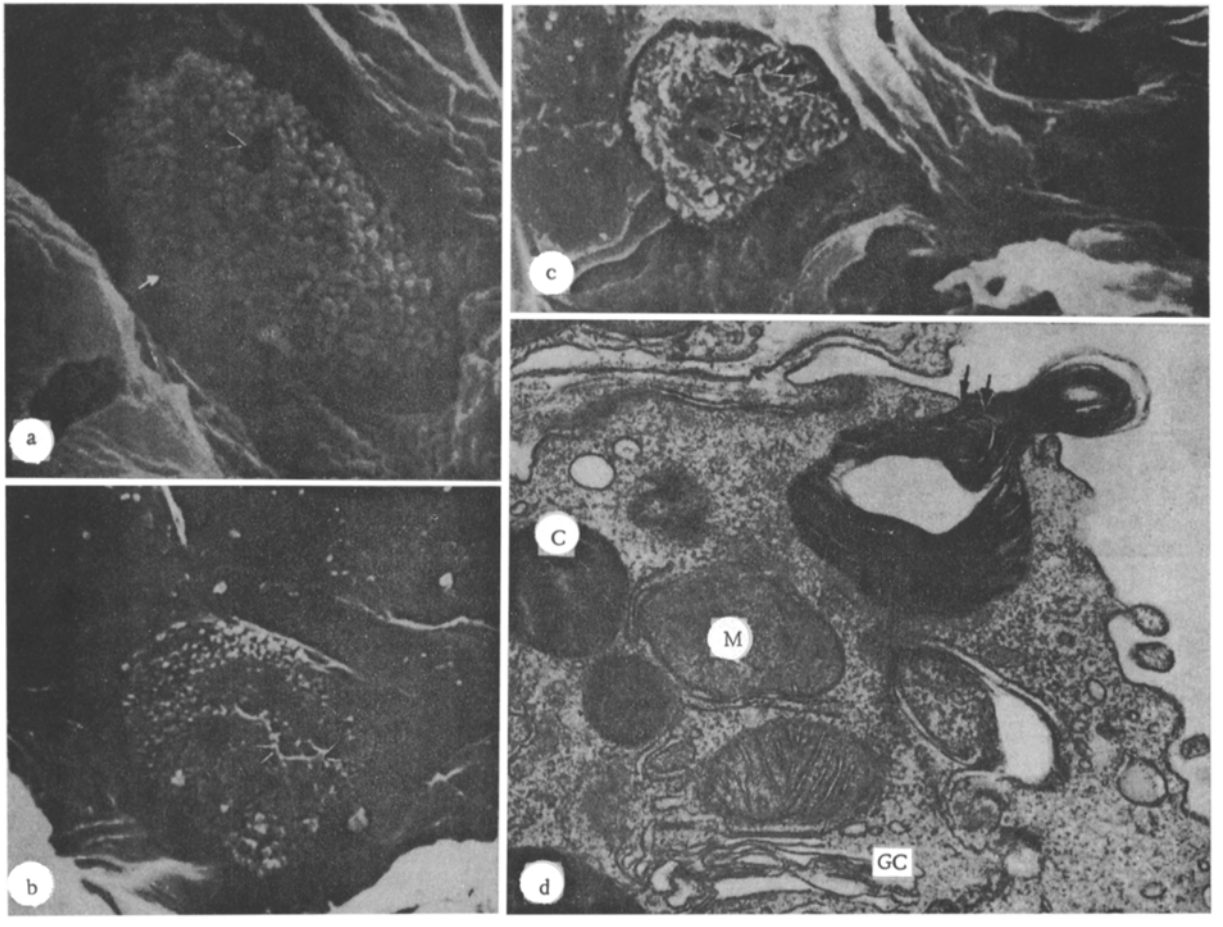

Fig. 2. Type II alveolocytes in lung of mouse receiving guanethidine. a, b, c) Postsecretory pores visible on apical surfaces of cells (SEM, arrow). Magnification: a) 22,000, b) 12,400, c) 11,500, d) type II alveolocyte during secretion of surfactant (two arrows). TEM. Magnification 40,000. M) Mitochondria, C) cytophospholiposomes, GC) Golgi complex.

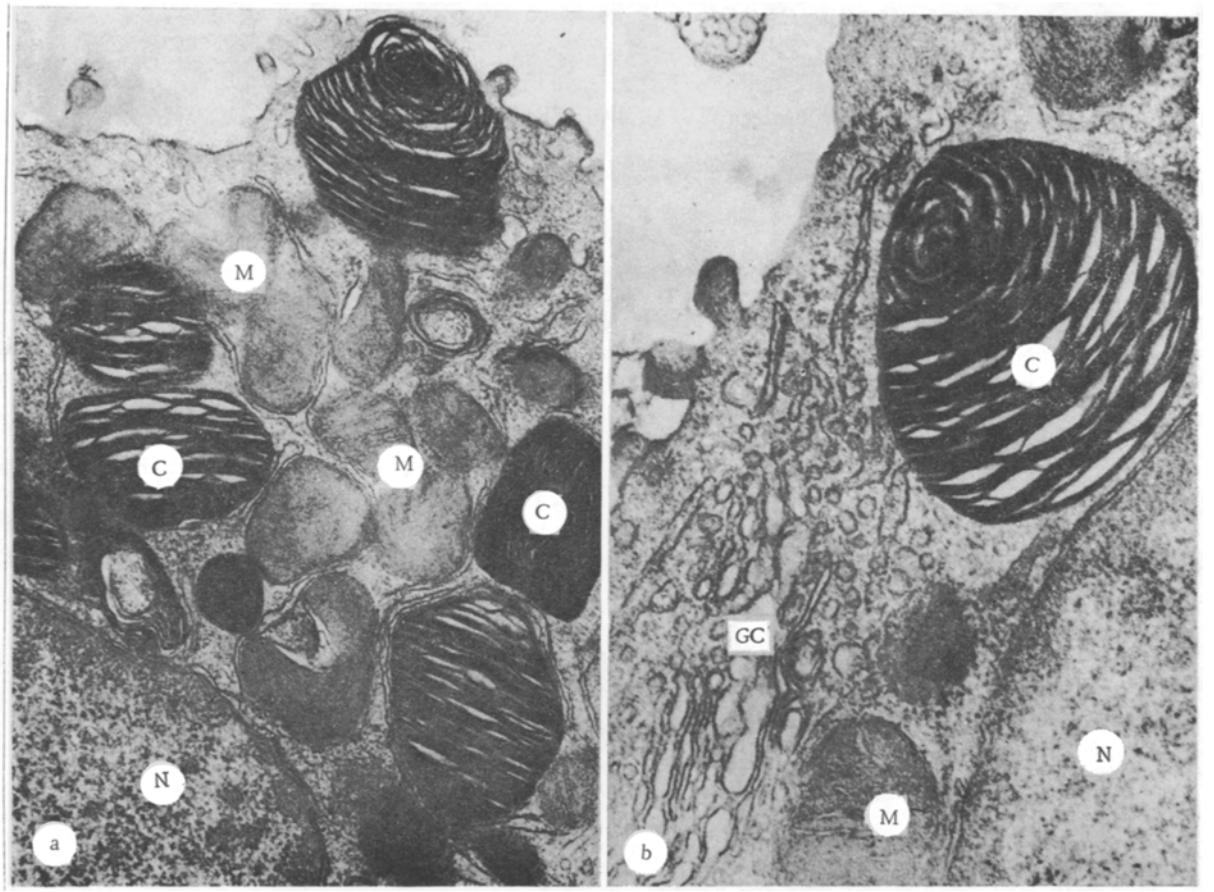

Fig. 3. Type II alveolocytes from lung of mice receiving guanethidine. Cells rich in cytophospholiposomes and mitochondria with electron-dense matrix. Number of structures of Golgi complex increased. N) Nucleus, M) mitochondria, C) cytophospholiposomes, GC) Golgi complex. TEM. Magnification: a) 34,000, b) 50,000.

The sympathetic nervous system thus participates in the regulation and secretion of alveolar surfactant. Partial "chemical sympathectomy" activates secretion of alveolar surfactant, and long-term administration of the sympatholytic guanethidine to growing animals does not prevent the development and growth of their lungs.

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