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Neuroepithelial Bodies in the Lungs in Rats

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We report here our studies of neuroepithelial bodies (NEB) in the lungs in healthy Wistar rats (n = 12). Immunocytochemical reactions for synaptophysin identified NEB and similar immunopositive nerve terminals. NEB in the lungs in rats were found to be consistently present elements. In contrast to diffuse neuroendocrine elements, these structures were characterized by grouped cells. Some NEB lacked efferent innervation.

Keywords: neuroepithelial bodies, neuroendocrine cells, synaptophysin, lungs.

Neuroepithelial bodies (NEB) are specific receptor structures in the bronchial epithelium discovered at the end of the 1930s [11]. Despite a long history of investigation, the functions of NEB remain incompletely understood. It has been suggested that the main functions consist of analysis of gas composition and a role in physiological changes in lung functions in response to changes in the qualitative composition of inhaled air [1, 9]. Until recently there has still been some uncertainty regarding questions of the structural organization of NEB and the classification of the neuroendocrine elements of the lungs. Despite the suggested important role of NEB in the system providing the normal regulation of lung functions, there has been very little investigation, probably due to the fact that these structures cannot be identified by the general and specific staining methods used for studies of lung function in health and disease.

The aim of the present work was to detect NEB in the lungs in rats and identify their cellular organization.

Materials and Methods

Experiments were performed on 12 adult male Wistar rats. Animal keeping and all experimental manipulations were performed in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of August 12, 1977). The lungs of intact rats were subjected to morphological analysis. Specimens were fixed in zinc-ethanol-formaldehyde [4], dehydrated, and embedded in paraffin using standard methods. NEB were detected using a reaction for synaptophysin [2]. Sections were deparaffinated and antigens were thermally demasked before immunocytochemical reactions. Endogenous peroxidase was blocked with 3% aqueous hydrogen peroxide (10 min). Nonspecific antibody binding sites were blocked with Protein Block (Springbio, USA) blocking solution for 10 min. NEB were detected using mouse monoclonal antibodies (SY38) to synaptophysin (diluted 1:30, Dako, Denmark) and secondary antibodies conjugated with polymer and peroxidase (EnVision + System Labelled Polymer-HRP Anti-Mouse, Dako, Denmark). Immunocytochemical reaction product was visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB+, Dako, Denmark). Some sections were counterstained either with Alcian blue or hematoxylin after reactions. Specimens were dehydrated in ethanol and isopropanol, clarified in xylene, and embedded in Cytoseal 60 (Thermo Scientific, USA) [3]. The proportions of NEB containing different numbers of cells were determined.

Results

Reactions for synaptophysin detected NEB in all the animals studied. NEB were apparent on preparations as groups of cells straining an intense brown with chromogen (Fig. 1); more rarely they consisted of single cells located in the unilamellar cubic epithelium of small bronchi and res-

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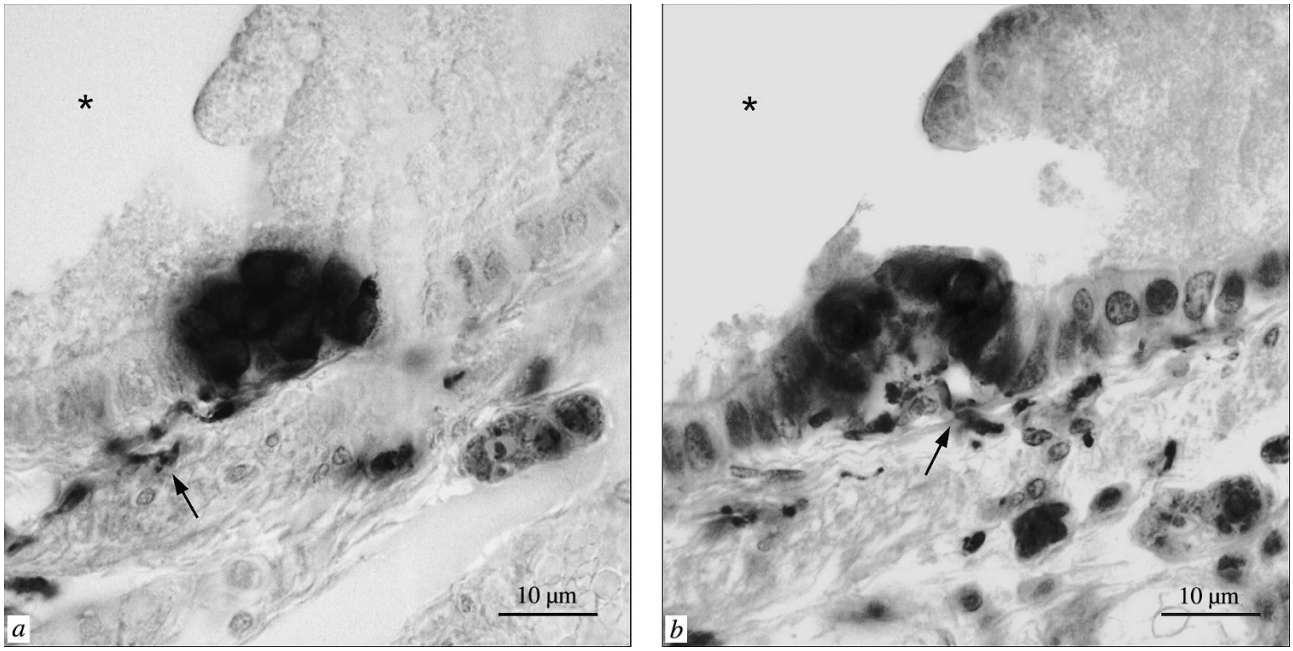

Fig. 1. Neuroepithelial bodies in the epithelium of a small bronchus in a rat. *Bronchial lumen: the arrows show synaptophysin-immunopositive terminals. Immunocytochemical reaction for synaptophysin without counterstaining (*a*) and the neighboring section with hematoxylin counterstaining (*b*).

piratory bronchioles. Cells in NEB were oval in shape and had clearly defined nuclei, around which there was no immunocytochemical reaction product. The numbers of NEB were 2–6 per lung section and were found in all lobes.

Synaptophysin-immunopositive terminals (SPIT) were found alongside a majority of NEB. NEB lacking nearby SPIT were also seen. Single synaptophysin-immunopositive cells c be found in the unilamellar epithelium of small bronchi. A number of cases showed isolated cells in the epithelial plate, which differed from NEB cells in that they were displaced towards its basal part. The number of cells in the NEB observed here varied. Many NEB (45%) consisted of 5–10 cells; 40% contained 10–20 cells, 8% 2–5 cells, and 7% had single synaptophysin-immunopositive cells. Apart from NEB, there were also SPIT, which were mainly located in the muscular sheath of the bronchi and along the blood vessels.

Discussion

The experiments reported here show that NEB are present in the unilamellar cubic ciliated bronchiolar epithelium. In contrast to other cells, the cells of these structures protrude slightly from the surface of the epithelium and their nuclei are generally not displaced towards the basal part of the epithelial plate. Single synaptophysin-immunopositive cells were displaced towards the basal part of the epithelial plate and appeared not to be receptor structures but elements of the diffuse neuroendocrine system of the lungs, as recorded by other methods [8]. Our studies used immunocytochemical detection of synaptophysin using specific monoclonal antibodies [2]. Synaptophysin is the main integral membrane protein in synaptic vesicles of nervous system neurons [12], so it is involved in the regulation and support of synaptic transmission. Its presence in cells is therefore evidence that these cells are nervous or neuroendocrine elements. This explains the reason why specimens showed simultaneous staining of NEB, isolated neuroendocrine cells, and SPIT. Published data indicate that afferent innervation of NEB consists of three type of terminals. The first type are vagus nerve fibers arising in the ganglion nodosum; the second are afferent fibers of spinal origin forming a plexus beneath the NEB, not penetrating between epithelial cells; the third type consists of nitrergic fibers [5, 9]. Reactions for synaptophysin are not selective in relation to individual transmitters and identify all terminals, promoting their wide use in investigations of the efferent innervation of various organs [6, 7].

Thus, NEB in the lungs of rats are consistently present elements. In contrast to diffuse neuroendocrine elements, they were characterized by consisting of groups of cells. The interaction of NEB and SPIT did not appear to be a consistent combination, which is evidence that some of these bodies lack efferent innervation.

REFERENCES

1. E. V. Evsyukova, " The neuroendocrine system of the human lungs," *Fiziol. Cheloveka*, **32**, No. 4, 121–130 (2006).

2. O. V. Kirik, P. A. Voronchikhin, E. I. Chumasov, and D. E. Korzhevskii, "Utilization of a reaction for synaptophysin for detection of neuroepithelial bodies in rat lungs," in: *Babukhinskie Lecture in Orel*, Retinoidy Press, No. 32, 70–71 (2011).
3. D. E. Korzhevskii, O. V. Kirik, M. N. Karpenko, et al., *Theoretical Basis and Practical Applications of Immunohistochemistry Methods*, SpetsLit, St. Petersburg (2012).
4. D. E. Korzhevskii, E. G. Sukhorukova, E. G. Gilerovich, et al., "Advantages and deficiencies of the zinc-ethanol-formaldehyde as a fixative for immunocytochemical studies and confocal laser microscopy," *Morfologiya*, **144**, No. 2, 81–85 (2013).
5. L. V. Filippova and A. D. Nozdrachev, "Sensory structures of the lungs and airways," *Byull. Sib. Med.*, **10**, No. 3, 100–104 (2011).
6. E. I. Chumasov, E. S. Petrova, and D. E. Korzhevskii, "Immunomorphological analysis of the innervation of chromaffin paraganglia of the arteries and heart in mammals," *Zh. Evolyuts. Biokhim.*, No. 4, 325–331 (2011).
7. E. I. Chumasov, E. D. Petrova, and D. E. Korzhevskii, "Distribution and structural organization of the nerve apparatus in the rat pancreas," *Morfologiya*, **139**, No. 3, 51–58 (2011).
8. I. Brouns, F. Oztay, I. Pintelon, et al., "Neurochemical pattern of the complex innervation of neuroepithelial bodies in mouse lungs," *Histochem. Cell Biol.*, **132**, No. 2, 34–42 (2008).
9. I. Brouns, J. Van Genechten, D. W. Scheuermann, et al., "Neuroepithelial bodies: a morphologic substrate for the link between neuronal nitric oxide and sensitivity to airway hypoxia," *J. Comp. Neurol.*, **449**, 343–354 (2002).
10. E. Cutz, J. Pan, H. Yegar, et al., "Recent advances and controversies on the role of pulmonary neuroepithelial bodies as airway sensors," *Semin. Cell Dev. Biol.*, **24**, 40–50 (2012).
11. F. Feyrter, "Über diffuse endokrine epithelie Organe," *Zbl. Inn. Med.*, **545**, 31–32 (1938).
12. M. J. Hannah, A. F. Schmidt, and W. B. Huttner, "Synaptic vesicle biogenesis," *Annu. Rev. Cell Dev. Biol.*, **15**, 733–798 (1999).