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Structure and Innervation of the Pulmonary Neuroepithelial Bodies in Rats

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Neuroepithelial bodies (NEBs) are highly specialized clusters of cells spread in the epithelium of intrapulmonary airways. The present study aimed at the visualization and morphological description of the pulmonary NEBs. For this purpose, we used tissue slices from the lungs of 1-month-old Wistar rats stained either routinely with hematoxylin and eosin (H&E) or with the vital dye neutral red. In addition, we processed them by the histochemical NADPH-diaphorase (NADPH-d) technique to get insight on their complex innervation. The H&E staining revealed the neuroendocrine cells as visible clusters of clear cells with lucid cytoplasm. Neutral red staining visualized the NEBs as pinkish red cell clusters protruding in the airway lumen. The varicose nerve fibers innervating the neuroendocrine cells were NADPH-d-reactive. Our results show that NEBs possess distinct morphological characteristics of sensory structures which have to be further investigated neurochemically with specific markers.

Key words: neuroepithelial bodies, H&E stain, neutral red, NADPH-diaphorase, lungs, rat

Introduction

Neuroepithelial bodies (NEBs) are polymodal sensors dispersed throughout the epithelium of the intrapulmonary airways [4]. They are formed by up to 25 neuroendocrine cells with elongated shape and a cytoplasm with an abundant number of vesicles containing a variety of bioactive substances [1, 4, 5]. They include typical neurotransmitters, like acetylcholine and serotonin, and also local regulatory peptides like bombesin, calcitonin, somatostatin, etc. [4]. NEBs are dually innervated by vagal and spinal primary afferents and by postganglionic fibers from sympathetic and parasympathetic ganglia [2, 3].

Materials and Methods

For the present study we used three 1-month-old Wistar rats. The animals were bred and provided by the vivarium of the Medical University of Sofia. The experiments were performed in agreement with the European Communities Council Directive 2010/63/EU for the protection of animals used for scientific purposes and approved by the

Research Ethics Commission of the Medical University of Sofia. The rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (70 mg/kg) and then transcardially perfused with cold 4% paraformaldehyde. After lung removal, we prepared 6 µm thick paraffin sections and routinely stained them with H&E following a basic protocol that included dewaxing, dehydration in increasing concentrations of ethanols, staining with hematoxylin, differentiation with 0.3% acid alcohol, rinsing, staining with eosin, dehydration in ascending ethanol solutions, clearing in xylene and coverslipping in Entellan (Merck). For the staining with neutral red, the deparaffinized sections of the lungs were rehydrated and then were stained with neutral red dye for 3-4 min until desired intensity was obtained. Thereafter, they were dehydratated, cleared in xylene and coverslipped.

In addition, we prepared 20 µm thick frozen sections from the lungs with freezing microtome and processed them with the NADPH-d technique according to Scherer-Singler et al. (1983). Briefly, the sections were incubated for 30-60 min at 37ºC in a staining solution consisting of 1 mg/ml β-NADPH, 0.25 mg/ml nitroblue tetrazolium (both from Sigma), and 0.3% Triton X-100 dissolved in Tris-buffered saline (TBS), pH 7.4. After incubation, the sections were rinsed in TBS, washed in distilled water (3 × 15 min) and coverslipped in glycerol jelly. For control purposes, sections were treated in the same way with omission of the substrate from the incubation medium. No specific reactivity was observed in any of the control sections under these conditions.

Results

The H&E staining revealed the NEBs as clearly visible clusters of oval cells with lucid cytoplasm protruding into the lumen of the intrapulmonary airways (**Fig. 1**). With the neutral red staining we were able to detect the NEBs in the mucosa of the terminal bronchioles and in the alveoli as well. The NEBs were observed as intensively stained red clusters of cells (**Fig. 2**) and the apical compartments of the neuroendocrine cells protruded into the airways lumen (**Fig. 3**). After applying the NADPH-diaphorase technique we observed the presence of varicose neve fibers in the NEBs reaching and terminating on the neuroendocrine cells of the NEBs (**Fig. 4**).

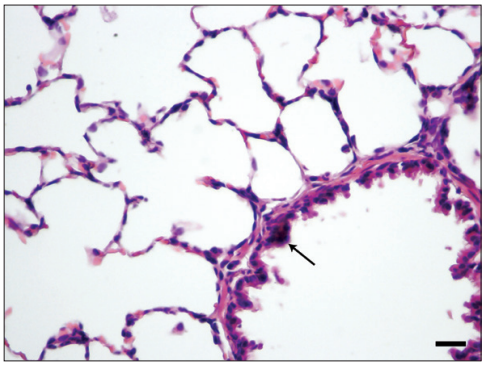

Fig. 1. Cross-section of a terminal bronchiole depicting a group of oval cells with abundant eosinophilic cytoplasm that are protruding to the airway lumen (arrow). Scale bar = 50 µm.

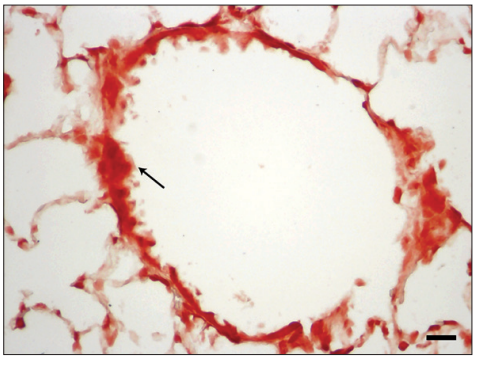

Fig. 2. A cross-sectional view of a terminal bronchiole showing a few red clusters of neuroendocrine cells forming the NEB (arrow). Scale bar = 50 µm.

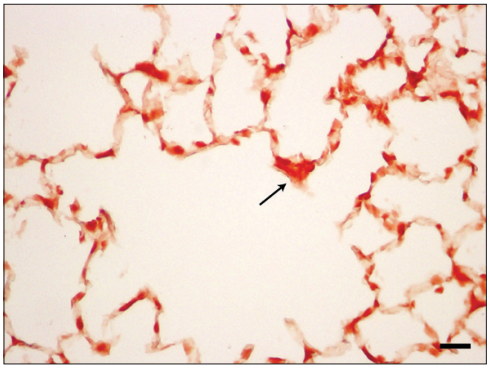

Fig. 3. A group of intensely stained neuroendocrine cells (arrow) protruding into the alveolar cavity. Scale bar = 50 μm.

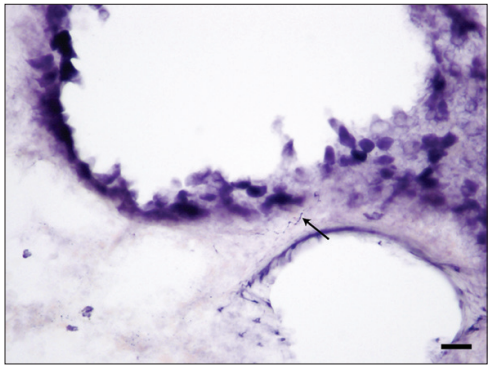

Fig. 4. NADPH-diaphorase staining demonstrating a varicose nerve fiber (arrow) innervating a protruding-like structure of weakly-stained neuroendocrine cells resembling the NEB. Scale bar = 50 μm.

Discussion

Our results support the general structural patterns of the typical sensory receptors such as the NEBs. In particular, following routine histological stainings we were able to observe clusters of neuroepithelial cells. With the NADPH-d staining we found that these cells are contacted by terminals of afferent nerve fibers. Such a location, morphology and complex innervation of the NEBs support their role as peripheral sensory receptors [3]. The peculiar positioning of NEBs within the lumen of the intrapulmonary airways implies their oxygen sensing role, which is mainly registred at the neonatal stages of the development of the lungs [5]. In adults, NEBs are considered to be important mechanical and chemical receptors which detect changes in the local chemical composition of the extracellular fluid [6].

In conclusion, NEBs possess an intricate internal structure and distinct morphological characteristics of typical sensory receptors in the lungs. Their visualization with routine histological methods is possible yet limited due to their small number and size. Moreover, NEBs could be visualized by NADPH-diaphorase technique, that makes it possible to observe the nerve fibers contacting the basal pole of neuroendocrine cells. Nonetheless, because of the lack of studies on the neurochemical patterns of NEBs under hypertensive conditions, further immunohistochemical experiments with specific markers are needed to clarify their chemical nature.

References

1. **Adriaensen, D., I. Brouns, J. Van Genechten, JP. Timmermans.** Functional morphology of pulmonary neuroepithelial bodies: extremely complex airway receptors. – *Anat. Rec. A Discov. Mol. Cell Evol. Biol.,* **270**, 2003, 25-40.
2. **Adriaensen, D., I. Brouns, I. Pintelon, I. De Proost, J. P. Timmermans.** Evidence for a role of neuroepithelial bodies as complex airway sensors: comparison with smooth muscle-associated airway receptors. – *J. Appl. Physiol. (1985),* **101**, 2006, 960-970.

3. **Brouns, I., J. Van Genechten, H. Hayashi, M. Gajda, T. Gomi, G. Burnstock, J. P. Timmermans, D. Adriaensen.** Dual sensory innervation of pulmonary neuroepithelial bodies. – *Am. J. Respir. Cell Mol. Biol.,* **28**, 2003, 275-285.
4. **Cutz, E., J. Pan, H. Yeger, N. J. Domnik, J. T. Fisher.** Recent advances and contraversies on the role of pulmonary neuroepithelial bodies as airway sensors. – *Semin. Cell. Dev. Biol.,* **24**, 2013, 40-50.
5. **Linnoila, R. I.** Functional facets of the pulmonary neuroendocrine system. – *Lab. Invest.,* **86**, 2006, 425-444.
6. **Scherer-Singler, U., S. R. Vincent , H. Kimura , E. G. McGeer.** Demonstration of a unique population of neurons with NADPH-diaphorase histochemistry. – *J. Neurosci. Methods*, **9**, 1983, 229-234.
7. **Verckist, L., R. Lembrechts, S. Thys, I. Pintelon, J. P. Timmermans, I. Brouns, D. Adriaensen.** Selective gene expression analysis of the neuroepithelial body microenvironment in postnatal lungs with special interest for potential stem cell characteristics. – *Respir. Res.*, **18**, 2017, 87.