

Microscopic Anatomy of the Pulmonary Neuroepithelial Bodies in Spontaneously Hypertensive Rats

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Neuroepithelial bodies (NEBs) are clusters of highly specialized cells spread in the epithelium of intrapulmonary airways. The present study aimed at the identification and morphological description of the pulmonary NEBs in spontaneously hypertensive rats (SHRs). Tissue slices from the lungs of 1-month-old male SHRs were stained routinely with hematoxylin and eosin (H&E) or with the vital dye neutral red. The H&E staining revealed the neuroendocrine cells as visible clusters of clear cells seen in the airway epithelium. Neutral red staining visualized the NEBs as reddish cell clusters protruding in the airway lumen. Our results support the general morphological structure of sensory receptors in SHRs. Their role and significance in the development of essential hypertension remains to be clarified.

Key words: neuroepithelial bodies, hematoxylin and eosin, neutral red, lung, spontaneously hypertensive rat

Introduction

Neuroendocrine cells are specialized epithelial cells that can be found throughout the tracheobronchial epithelium as solitary or grouped cells [1]. When grouped into discrete cell clusters within intrapulmonary airways they are called pulmonary neuroepithelial bodies (NEBs) [1, 2]. As previously revealed, in the NEBs the neuroendocrine cells are supported by Clara-like cells, which surround them and leave only the apical domains of the neuroendocrine cells in contact with the pulmonary airway's lumen [9]. The neuroendocrine cells of the NEBs and the Clara-like cells comprise the so-called NEB microenvironment. The NEBs are composed of up to 25 neuroendocrine cells with lucid cytoplasm and they are protruding into the lumen of the pulmonary airways. Their cytoplasm is rich in vesicles storing a wide variety of bioactive substances such as acetylcholine, serotonin, calcitonin and others [2, 3, 5].

Our prior research has demonstrated the structural features of the NEBs in normotensive Wistar rats [7]. Therefore, the aim of the present study was to visualize and describe the morphology of the NEBs in spontaneously hypertensive rats (SHRs), the commonly used model of essential hypertension.

Material and Methods

In this study, we used 1-month old male SHRs weighing approximately 120 g. The animals were bred and housed at 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 transcidentally perfused with cold 4% paraformaldehyde. Subsequently, intratracheal infusion with 4% paraformaldehyde was performed. The lungs were quickly removed, put into a cuvette with 4% paraformaldehyde and then proceeded to mild deaeration using a vacuum aspiration pump. Afterwards, we prepared 6 μm thick paraffin sections and routinely stained them with hematoxylin and eosin (H&E) following a protocol that included dewaxing and rehydration in decreasing concentrations of ethanol, staining with hematoxylin, differentiation with 0.3% acid alcohol, rinsing in water and blueing, staining with eosin, dehydration in ascending ethanol solutions, clearing in xylene and coverslipping in Entellan (Merck, Darmstadt, Germany). For the staining with neutral red, the deparaffinized sections of the lungs were rehydrated and then stained with neutral red dye for 3-4 min until the desired intensity was obtained. Finally, they were dehydrated, cleared in xylene and coverslipped.

Results and Discussion

Using the H&E staining we were able to observe NEBs as clusters of oval cells with lucid cytoplasm, protruding into the lumen of the terminal bronchioles (**Fig. 1A**) and alveoli (**Fig. 1C**). Using neutral red as a vital stain, we observed on adjacent sections the NEBs in terminal bronchioles (**Fig. 1B**) and alveoli (**Fig. 1D**). They were seen as intensely-stained red clusters of cells protruding into the lumen of intrapulmonary airways.

The visualization of the NEBs with routine staining procedures using H&E and neutral red stain is possible yet hard to achieve, due to the low count of NEBs in the lungs and the lack of distinctive feature of the neuroendocrine cells. The present results show for the first time the microscopic structure of NEBs in SHRs. Our observations coincide with the general pattern of the NEBs as clusters of neuroendocrine cells with lucid cytoplasm spread throughout the intrapulmonary airways, including the terminal bronchioles and the alveoli. A striking feature is the protrusion of the apical domain of their cells into the lumen of the intrapulmonary airways. Such a localization implies an oxygen sensing role; yet more recent studies ascribe such a function mainly to the neonatal lungs [5]. Indeed, in adults NEBs are more often associated with mechanical and chemical reception, and sensing changes in the local extracellular matrix [6].

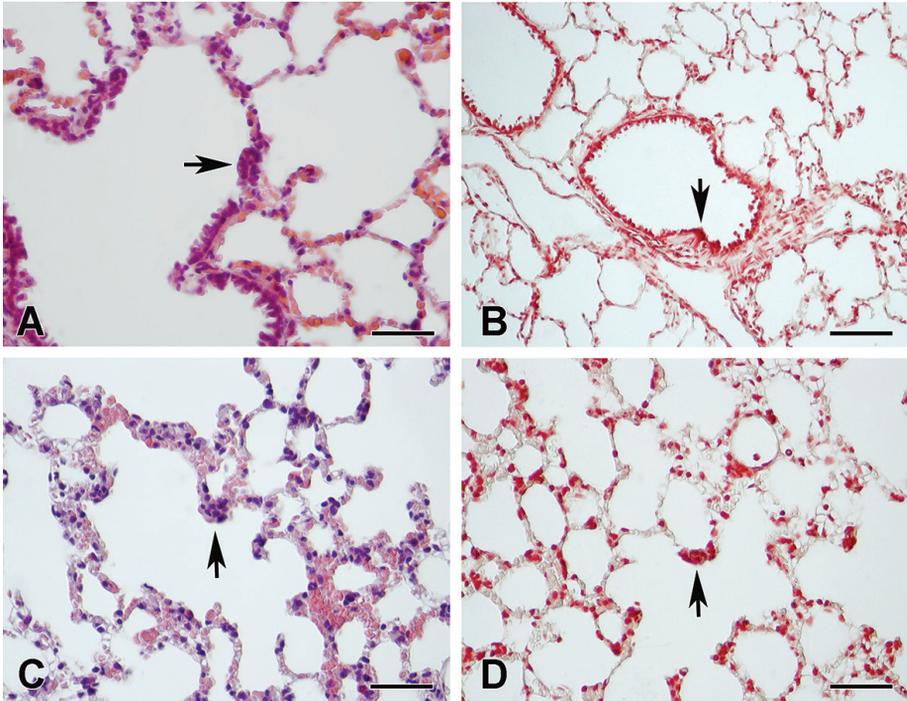


Fig.1. Staining of the NEBs in the intrapulmonary airways. **(A)** Higher magnification of a H&E-stained section through a terminal bronchiole revealing a cluster of neuroendocrine cells in the epithelium (arrow). **(B)** Neutral red staining of a terminal bronchiole showing an intensely-stained cluster of cells making up the NEB (arrow). **(C, D)** Adjacent sections of the alveoli displaying a group of oval cells with lucid cytoplasm (arrow), protruding into the lumen, **(C)**, H&E staining and **(D)**, Neutral red staining. Scale bars = 50 μm .

The SHR is the most commonly used animal model of human essential hypertension [4]. Literature data suggest that NEBs play a role in the pathogenesis of pulmonary hypertension through an increased serotonin expression [8, 10]. Based on these findings future studies will focus on analysing the potential role of NEBs in essential hypertension and involvement of certain bioactive substances in the mechanisms of high blood pressure.

In conclusion, the visualisation of the NEBs in SHR using routine histological methods is possible and the obtained results are on a par with the literature data. Their unique localization and the broad spectrum of bioactive substances they utilize make them an interesting target for future immunohistochemical research in an attempt to find a connection between the NEBs and pathological conditions of the lungs.

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