

IDENTIFICATION OF THE SOLITARY NEUROENDOCRINE CELLS (SNC's) AND NEUROEPITHELIAL BODIES (NEB's) IN THE BRONCHOPULMONARY TRACT OF THE NEWBORN RABBIT LUNG

Hakkı DALÇIK, Ph.D., Aysel ŞEFTALIOĞLU, Ph.D., Cannur DALÇIK, Ph.D., Ülken ÖRS, M.D.

Hacettepe University, Faculty of Medicine, Department of Histology and Embryology,
Ankara, Turkey
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SUMMARY : *Amine precursor uptake and decarboxylation (APUD) neuroendocrine cells were stained by Grimelius silver nitrate stain for paraffin sections and metilen blue and Azur 11 for araldite semithin sections. The solitary neuroendocrine cells and the neuroepithelial bodies were identified in the simple columnar and pseudostratified columnar epithelium of the newborn rabbit bronchopulmonary segments. The intense silver grain deposition were present in both of the cellular groups. Both the solitary neuroendocrine cells and the neuroepithelial bodies in most part were in contact with the luminal surface of the bronchopulmonary tract. A paracrine and / or chemoreceptor role is proposed for the solitary neuroendocrine cells and the neuroepithelial bodies in the newborn rabbit respiratory tract.*

Key Words : *Neuroendocrine Cells, Pulmonary Tract, Epithelium, Newborn Rabbit.*

INTRODUCTION

Many investigators have identified argyrophilic cells within the bronchial epithelium that displays endocrine function (17, 20, 24). Hage (1974), demonstrated argyrophilic endocrine cells in mouse, guinea-pig and fetal rabbit lung (14). The same author reported the increased number of these cells in the fetal rabbit lung compared to mouse and hamster. The respiratory epithelium of many vertebrates reveals the presence of solitary neuroendocrine cells (3, 8, 16) and groups of these cells which are referred to as neuroepithelial bodies (18). These small granulated pulmonary epithelial cells are distinctive with their morphological and histochemical features, because of this they are considered to be part of the amine precursor uptake and decarboxylation (APUD) polypeptide hormone-producing cells (21). It has been demonstrated that some of these cells are immunoreactive for bombesin (5,

6), leu-enkephalin (5), and calcitonin (1). The literature dealing with the morphology of the neuroendocrine cells in the lungs of rabbit is rather scarce. In the adult rabbit lung, the demonstration of the neuroendocrine cells was rather difficult due to its low quantity (2) in contrast to the fetal rabbit lung (12, 19).

The purpose of the present study was first : to improve methods for staining the respiratory endocrine secretory cells using silver nitrate stain, and second : to identify the neuroendocrine cells (solitary or clusters) in semithin Araldite sections using Toluidin blue and Azur 11, and third : to examine these cells in the newborn rabbit which has not been studied. With this approach, the morphology and the anatomic relationship of the neuroendocrine cells with the normal epithelium will be better understood.

MATERIALS AND METHODS

Animals

The tissue for the study comprised lungs from 15 male and female newborn (1-15 day old) white New Zealand and Vienna rabbits. The animals were housed in Hacettepe Medical Faculty animal care unit (vivarium) in cages under 24 hour light and 24 hour dark and food ad libitum.

Tissue Preparation

Animals were deeply anaesthetized by a single intraperitoneal injection of sodium pentobarbital (65 mg/kg bw). Bouin and 10 % neutral buffered formalin solution was injected intratracheally. The lungs were excised divided into lobes, cut into 1 mm cubes, and immersed in the same fixative at 4°C for 24-48 hours.

Staining

The fixed tissues were processed with paraffin and were stained for light microscopy according to Grimelius's silver nitrate stain (13), and tissues processed with Araldite were stained with Toluidin blue and Azur 11 for light microscopy according to Dorland and colleagues (7).

RESULTS

The neuroendocrine cells were easily identified as such in the light microscopic pictures possessing argyrophilic staining in the epithelium of the small bronchus and in the bronchiols, some of the pyramidal shaped solitary neuroendocrine cells were demonstrated to be located away from the lumen (Fig 1, 2) and some were demonstrated to be in contact with the lumen (Fig 3). The solitary neuroendocrine cells localized in the basal compartment in the pseudostratified columnar epithelium possessed accumulation of staining in the basal cytoplasm adjacent to the basement membrane (Fig 2). However, the solitary neuroendocrine cells in the simple columnar epithelium possessed argyrophilic staining more at the apical cytoplasm (Fig 3).

In the simple columnar epithelium (Fig 4) and in the pseudostratified columnar epithelium (Fig 5), argyrophilic cell clusters were identified. These cell clusters, the neuroepithelial body, appeared to be shaped like a cone, wide base and a thin apex (Fig 4). The cells of the neuroepithelial bodies usually remained in a direct contact with the basal membrane and the apical lumen (Fig 4, 5). Figure 4 demonstrates a neuroepithelial body composed of five neu-

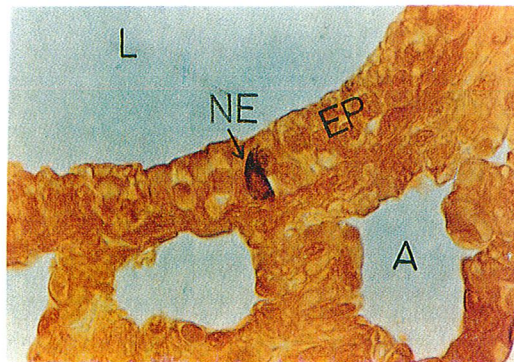


Fig - 1 : Bronchiol pulmonary tissue from a newborn rabbit lung. A solitary neuroendocrine cell (NE) is demonstrated in the epithelium (EP). L : Lumen, A : Alveolus, X1000.

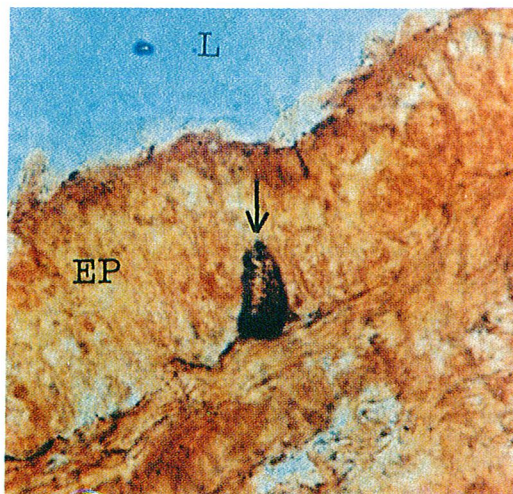


Fig - 2 : A pyramidal shaped solitary neuroendocrine cell (arrow). Note the intense black silver deposition in the basal cytoplasm. L : Lumen, EP : Epithelium, X1000.

roendocrine cells and the intense argyrophilic staining is present in the apical cytoplasm, adjacent to the lumen. Figure 5 also demonstrates a neuroepithelial body composed of argyrophilic 10-13 neuroendocrine cells, some localized in the base near the basement membrane and some localized in the apex near the lumen.

A pseudostratified columnar epithelium in a semithin section stained with Toluidin blue and Azur 11 is shown in Figure 6. In this figure the neuroepithelial body appear to be truncated, wide at the base and narrow at the apex, composed of 5-9 neuroendocrine cells. In the apical cytoplasm of the neuroendocrine cells appear to be filled with clear looking vesicles, resembling vacuoles (Fig 6).

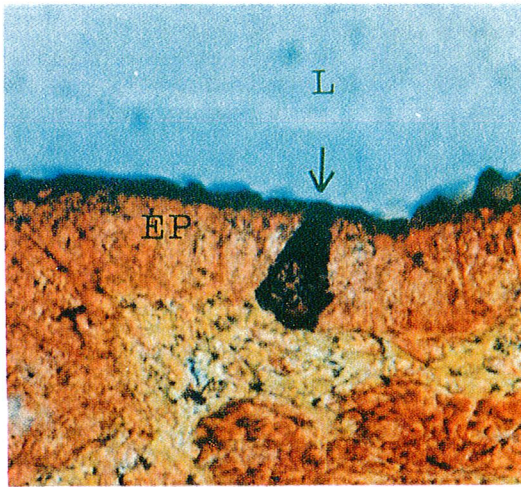


Fig - 3 : A pyramidal shaped solitary neuroendocrine cell (arrow). Note the intense black silver deposition in the apical cytoplasm. L : Lumen, EP : Epithelium, X1000.

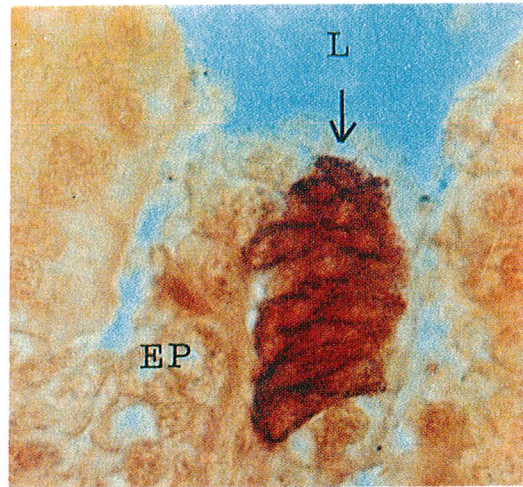


Fig - 5 : A neuroepithelial body (arrow). Note the homogenous distribution of the silver distribution in the cytoplasm of the neuroendocrine cell. L : Lumen, EP : Epithelium, X1000.

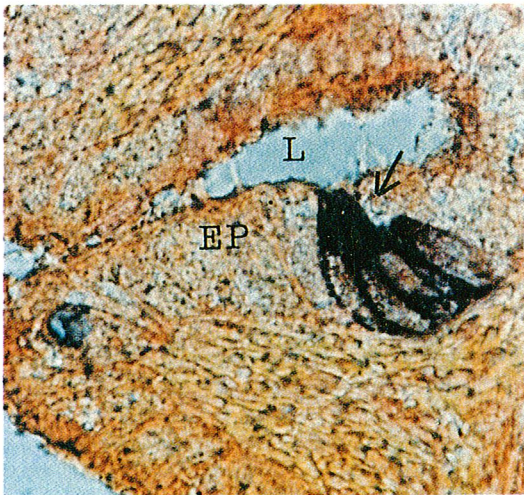


Fig - 4 : A neuroepithelial body (arrow) resembling a taste bud. Note the intense black silver deposition in the apical cytoplasm of the neuroendocrine cells. L : Lumen, EP : Epithelium, X1000.

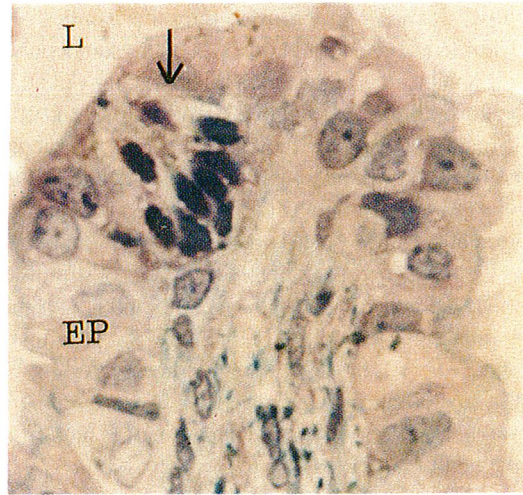


Fig - 6 : A neuroepithelial body (arrow) resembling a taste bud. Note the apically located small vacuoles. L : Lumen, EP : Epithelium, X1000.

DISCUSSION

Some investigators have identified endocrine secretory cells using silver salts (18). The present study have utilized the method of Grimelius by silver nitrate (15) and Metilen blue and Azur 11 to demonstrate the neuroendocrine cells in the newborn rabbit lung. Stahlman and colleagues (23) have localized paracrine endocrine cells in human newborn airways. Although neuroendocrine cells have been demonstrated in the neonatal rabbits (4, 14, 24), neuroendocrine cells in the newborn rabbit using silver nitrate for paraffin section and Metilen blue and Azur 11 for Araldite semithin section hasn't been studied.

The morphology of the solitary neuroendocrine cells was demonstrated to resemble a pyramid. In the present study, the argyrophilic cells in the pseudostratified columnar epithelium, were located in the base adjacent to the basement membrane, however others in the simple columnar type epithelium the argyrophilic cells were in contact with the lumen. These findings are in parallel with the work of Cutz and colleagues (4) and Lauweryns and colleagues (17). In addition, the present study have demonstrated that the silver grains have accumulated more in the basal cytoplasm in the basally located cells. However, in the apical cells the silver grains was concentrated more at the apical cytoplasm. According to Goniakowska - Witalinska (11) using

immunocytochemistry have shown the presence of serotonin, met-enkephalin and leu-enkephalin immunoreactivity in the solitary neuroendocrine cells in the lung of Tiger Salamander. Co-occurrence of met-enkephalin and leu-enkephalin was also found in the neuroendocrine cells of some Teleost fish species (27). According to the same authors these cells probably have endocrine or paracrine functions that may be affected by the environmental changes of the lumen. It is also possible that the silver positive cells demonstrated in the present study could have the same function.

As in the present study many other investigators have reported that the neuroepithelial bodies are also in contact with the air space in the bronchopulmonary epithelium (6, 14, 26). The morphology of the neuroepithelial bodies resembled a taste bud, which is in agreement with the other studies (9). The vacuoles present in the cells of the neuroepithelial bodies, may indicate a vesicle that may contain a neuropeptide. Goniakowska-Witalinska and colleagues (11) have demonstrated that the neuroepithelial bodies in the lungs Tiger Salamander, possess short microvilli and a single modified cilium. Rogers and Haller (22) hypothesized that the apical cells may serve as receptors transducing stimuli to the basal cells. Our results seem to agree with this supposition. In addition, Goniakowska-Witalinska and Cutz (10) have demonstrated nerve endings in the lungs of three Anuran species. According to the authors, this suggests a transmission of stimuli from the neuroendocrine cells to the nerve endings, thus supporting the hypothetical neuroreceptor function of the neuroepithelial bodies. It is possible that such function may also exist in the newborn rabbit lung.

In the course of ontogeny, the air tract originates from the alimentary canal and the neuroendocrine cells occurring in the lungs are a part of gastroenteric paraneurons. The role of the solitary neuroendocrine cells and the neuroepithelial bodies in the respiratory epithelium of the newborn rabbit has not been fully elucidated so far. But according to the anatomical localization and the presence of specific granules in the cytoplasm, these cells may serve as receptors sensitive to the local chemical changes (such as oxygen and carbondioxide) and could be modulated by the central nervous system. They probably also may have endocrine and / or paracrine functions.

Correspondence to : Dr.Hakkı DALÇIK
Hacettepe Üniversitesi Tıp Fakültesi
Histoloji ve Embriyoloji Anabilim Dalı
06100 ANKARA - TÜRKİYE
Phone : 312 - 311 92 08

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