Neuroepithelial Bodies in the Lung of the Tree Frog, Hyla arborea L.

A Scanning and Transmission Electron Microscopic Study*

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Summary. The endocrine-like cells (ELC), which together with nerve endings form the neuroepithelial bodies, are located on primary and secondary septa in the non-ciliated epithelium of the lung of Hyla arborea. ELC protrude markedly toward the lumen of the lung and are surrounded by pneumocytes, which separate ELC from the lumen by thin cytoplasmic processes. ELC possess a light cytoplasm containing two types of granules: (i) numerous small granules, 50–110 nm in diameter, and (ii) single large granules, 290–860 nm in diameter. Numerous nerve fibers, often forming synaptic junctions, can be observed in contact with ELC.

Key words: Lung – Amphibia – Anura – Neuroepithelial bodies

Specific granulated cells with the morphological and histochemical features of endocrine elements have been described in the tracheal and bronchial ciliated epithelium of the lungs of birds and mammals. These so-called endocrine-like cells (ELC) can be either single and dispersed (Cook and King 1969; Ericson et al. 1972; Walsh and McLelland 1974; Jeffery and Reid 1975) or form clusters, which together with intraepithelial nerve endings constitute the neuroepithelial bodies (NEB), the latter occurring only in the bronchial epithelium (Lauweryns et al. 1972, 1977; Hung et al. 1973, 1979; Cutz et al. 1978). Scarce, dispersed ELC were found in the ciliated pulmonary epithelium of Urodela (Goniakowska-Witalińska 1980), whereas in Anura the presence of NEB was described (Rogers and Haller 1978; Wasano and Yamamoto 1978). In Hyla arborea, the subject of the present study, ELC form clusters resembling in structure the mammalian NEB rather than those of Anura.

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Materials and Methods

Transmission Electron Microscopy (TEM). Two adult animals, male and female of *Hyla arborea* L. were anesthetized with MS222. The air-filled lungs were fixed, washed and postfixed according to conventional TEM methods (2% glutaraldehyde in 0.12 M cacodylate buffer, pH 7.4, 2% OsO₄, at room temperature). The material was then rapidly dehydrated in acetone and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope at 60 kV.

Scanning Electron Microscopy. The lungs of one male and one female tree frog were isolated, opened, rinsed several times in cacodylate buffer to remove the mucus, then fixed, washed, and dehydrated by conventional TEM methods. The dehydrated material was dried by the critical point method, coated with carbon and gold and examined in a JSM 35 scanning electron microscope at 15 kV.

Results

The lungs of *Hyla arborea* L. possess numerous primary, secondary and tertiary septa, similar to the lungs of *Rana temporaria* (Dierichs 1975). The primary and secondary septa display small and irregularly distributed patches of ciliated epithelium. The remaining surface is lined with only one type of pneumocytes. Dome-shaped protrusions covered completely by pneumocytes are located in the vicinity of the ciliated epithelium, particularly in the anterior part of the lung (Figs. 1, 2); they measure 45–90 μm in diameter and 27–60 μm in height. In contrast to the surface of the adjacent pneumocytes, their laminal surface is characterized by longer microvilli, sometimes forming microridges at the cell apex (Fig. 3). Light- and electron-microscopic observations reveal that the dome-shaped protrusions are composed of numerous specific granulated cells, the so-called endocrine-like cells (ELC), which form clusters and are separated from the lumen of the lung by both flattened pneumocytes bodies and their thin cytoplasmic processes (Figs. 4, 5). The granulated cells rest on the basal lamina, beneath which a capillary vessel is usually located (Figs. 4, 5). The cytoplasm of ELC is lighter than that of the surrounding pneumocytes; the nucleus is large and possesses numerous invaginations of the nuclear envelope and a well defined nucleolus. Heterochromatin occurs exclusively as a thin layer adjacent to the nuclear envelope. The cytoplasm contains a prominent Golgi apparatus, numerous microfilaments, mitochondria, free ribosomes, rough endoplasmic reticulum and, occasionally, multivesicular bodies. In the entire cytoplasm, particularly in its basal region, two types of granules occur (Figs. 5, 6): (i) numerous small granules, 50–110 nm in diameter, completely filled with a substance of moderate electron density or, more frequently, possessing an electron-dense core separated from the limiting membrane by a clear space, 25–35 nm wide; and (ii) single large granules 290–860 nm in diameter, round or oval in shape, containing a dark, homogenous interior surrounded by a clear space, 25–28 nm wide. Granules of the latter type are situated mainly in the basal region of the cell.

ELC are interconnected with each other or with the adjacent pneumocytes by desmosomes or occasional interdigitations of the cell membrane. They are innervated by numerous intraepithelial nerve endings characterized by lighter cytoplasm, numerous mitochondria, microtubules and two kinds of vesicles