ORGANIZATION OF THE APUD SYSTEM IN MAMMALIAN LUNGS AT DIFFERENT STAGES OF ONTOGENY

S. A. Blinova

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Endocrine cells of the APUD system in the respiratory system have been studied in most detail in the human lungs. There have been far fewer studies of these regulatory structures in mammalian lungs. However, our ideas on the role of the APUD system in the lungs may be widened as a result of studies of its organization and properties in animals under normal and experimental conditions.

The aim of the present investigation was to compare the endocrine apparatus in the lungs of laboratory animals at different stages of ontogeny.

EXPERIMENTAL METHOD

The lungs of 69 rabbits, 95 rats, and 25 guinea pigs were studied in the fetal period of development, 1-30 days after birth, and in the adult state. Material was fixed in Bouin's fluid for 18-24 days. Endocrine cells were detected in paraffin sections by double impregnation with silver nitrate by Grimelius' method and also by the methods of Pascual, and of Sevier and Munger, argentaffin and the argentaffin reaction of Masson and Hamperl also was used. A fluorescence study of the lungs also was undertaken on freshly frozen cryostat sections 25 μ thick, after treatment with a 2% solution of glyoxylic acid [1]; sections in these experiments were studied in the LYUMAN-I2 luminescence microscope, with FS-1 filter 4 mm thick. To analyze luminescent structures, the FMÉL-1 photometric attachment was used, with output voltage of 1000 V and probe 0.5; serotonin was determined with a No. 8 filter (515 nm) and catecholamines with a No. 6 filter (481 nm). The intensity of luminescence was measured in scale units of the instrument, with different transmission factors. The values obtained were compared after reduction for strength of the current. The ratio of serotonin to catecholamines was obtained after division of the corresponding conventional units.

EXPERIMENTAL RESULTS

In the lungs of the rabbits and rats, an endocrine apparatus consisting of single cells of the APUD system (apudocytes) and neuroepithelial bodies (NEB) was discovered by argyrophilic methods. The argyrophilic cells were triangular, cylindrical, or rocket-shaped. The number of apudocytes and NEB was smaller in the lungs of the rabbits during growth of the animals compared with fetuses and newborn animals. However, even in adult animals argyrophilic cells were detected in bronchi of varied diameter, terminal bronchioles, and in the respiratory division. In the rat lungs apudocytes and NEB were identified in large numbers in the fetal period and in the early times of observation. Apudocytes and NEB were very rare 21 and 30 days after birth and also in the adult animals. Single apudocytes and NEB were found in the lungs of fetal and newborn guinea pigs. The Masson—Hamperl reaction was weakly positive in a small number of apudocytes and NEB in rabbit lungs but negative in the lungs of rats and guinea pigs.

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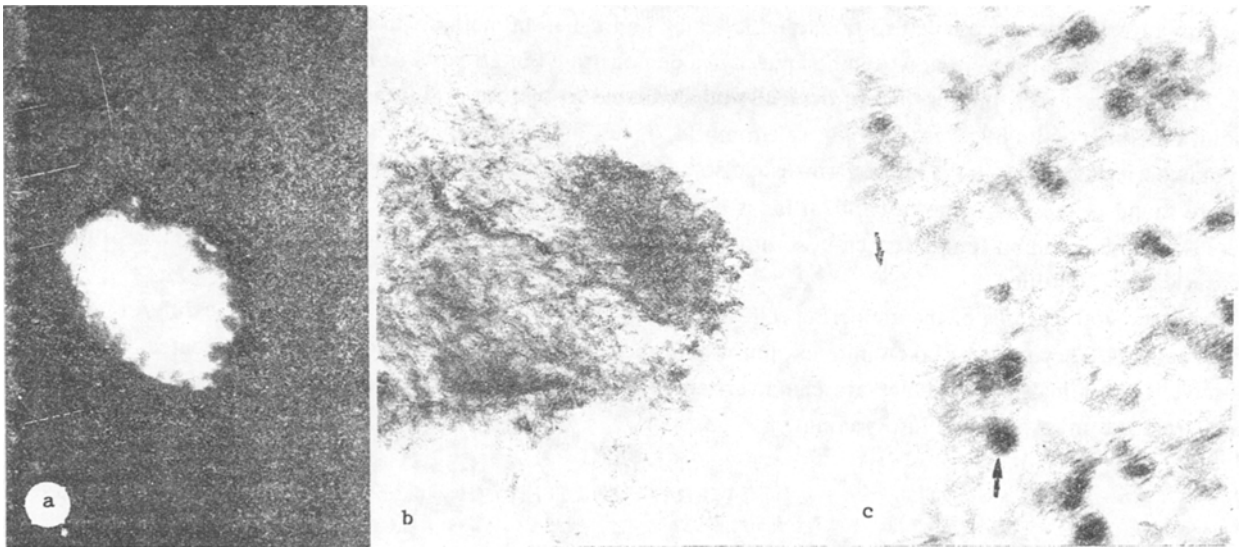

Fig. 1. NEB in bronchial epithelium of rabbit lung. 120×. a) Fluorescent NEB cells. Incubation in 2% glyoxylic acid; b) the same preparation after incubation by Grimelius' method; c) degranulation of endocrine granules of an apudocyte (arrows) in lung of rat fetus. 20,000 times.

Fluorogenic amines were determined by the luminescence method in endocrine structures of the rabbit lungs. Apudocytes and NEB gave yellow fluorescence, but changed to yellowish-green if the intensity of the reaction was low. Microfluorometric analysis revealed the presence of serotonin and catecholamines in them. NEB were richer in monoamines than apudocytes. The ratio of serotonin to catecholamines varied in the apudocytes and in NEB from 5.0 to 11.1. The number of apudocytes and NEB containing fluorogenic amines fell gradually with an increase in age of the rabbits. Subsequent impregnation of sections incubated in glyoxylic acid by Grimelius' method showed that all luminescent cells in NEB are argyrophilic (Fig. 1a, b). No endocrine cells with fluorogenic amines were found in the lungs of rats and guinea pigs.

Electron-microscopic investigation of the rabbit lungs revealed apudocytes and NEB. Apudocytes were located in the bronchial epithelium. Oval mitochondria, cisterns of the rough endoplasmic reticulum, and endocrine granules were distributed in the cell cytoplasm. The granules were oval in shape. The short diameter of the granules was 120-190 nm, the long diameter 170-200 nm. The core of the granules was electron-dense, and it was surrounded by a narrow pale border and a single membrane. NEB consisted of cylindrical cells, located on a basement membrane. On reaching the lumen of the respiratory passages, the cells possessed microvilli. They contained large mitochondria, with short profiles of the smooth endoplasmic reticulum. Endocrine granules with a dense core and narrow border were located mainly in the basal part of the cytoplasm. The granules were oval in shape and measured 110 × 130 nm.

In the fetal period, apudocytes in the rat lungs had a definitive ultrastructure. They contained a round nucleus with large masses of chromatin, long mitochondria with an electron-dense matrix, bundles of microfibrils, and endocrine granules. The granules were circular, and most had a dense core and border between the core and the membrane, which varied in width. The granules measured 120-140 nm. Signs of degranulation were frequently observed in the apudocytes (Fig. 1c). NEB in rats were composed of cylindrical cells. A few cells reached the lumen of the bronchi, and contained microvilli. The endocrine granules were small and round, with a dense core and narrow border. They varied in size from 120 to 140 nm. The apical surface of NEB was covered by polygonal cells without granules. No endocrine cells could be seen in adult guinea pigs by electron-microscopic investigation.

By the use of identical conditions of fixation and staining, it could be clearly shown that the endocrine apparatus in lungs of different animals possessed species-specific features of organization. In the rabbit lungs apudocytes and NEB were identified in the fetuses and throughout the period of postnatal ontogeny. The endocrine apparatus in the rabbit lungs was characterized by a high monoamine content. In the lungs of 21-day and more adult rats, the number of apudocytes and NEB was significantly smaller than in fetuses and newborn animals. No monoamines were found in the endocrine structures of the rat lungs with the aid of glyoxylic acid. The ultrastructure of the endocrine cells of rabbits and rats was similar. In the guinea pig lungs, argyrophilic apudocytes and NEB were just as rare in the newborn animals as in the adult guinea pigs.

Many investigators have failed to find apudocytes by impregnation methods in the respiratory passages of guinea pigs. Only in one investigation [6] were argyrophilic apudocytes demonstrated in all parts of the respiratory passages in adult guinea pigs. The difference between the results can evidently be attributed to the use of different lines of animals. The authors themselves point out this possibility. Serotonin [5], calcitonin [4, 7, 11], and a peptide dependent on the calcitonin gene [10], were found in apudocytes and NEB in rat lungs. Bombesin also has been identified by radioimmunoassay and electron microscopy [8]. Peptides are found in the endocrine cells of rat lungs both in the early period of postnatal development and in adult animals. Endocrine structures in rabbit lungs are rich in serotonin [3]. They probably also contain polypeptide substances [9], which have not previously been identified.

The structural features of the endocrine cells mentioned above evidently reflect species-specific differences in the lungs of different animals. They must be taken into account when experimental models of various states are created. The possibility of creating models in animals whose lungs are characterized by quantitative predominance of monoamines or peptides in their endocrine structures must be taken into account.

LITERATURE CITED

1. V. N. Shvalev and N. I. Zhuchkova, Arkh. Anat., **76**, No. 6, 114 (1979).
2. V. V. Yaglov and Yu. A. Loshchilov, Abstracts of Proceedings of the Tenth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Poltava (1986), p. 406.
3. T. Cho, W. Chan, and E. Cutz, Cell Tissue Res., **255**, No. 2, 353 (1989).
4. G. R. Gosney and M. C. J. Sissons, Thorax, **40**, 194 (1985).
5. J. Keith, R. Day, S. Lemaire, and I. Lemaire, Exp. Lung Res., **13**, 311 (1987).
6. A. M. Lauweryns and M. Liebens, Experientia, **99**, No. 11, 1510 (1977).
7. J. M. Lauweryns and L. Van Ranst, Thorax, **42**, 183 (1987).
8. A. Marchevsky and J. Kleinesmann, Arch. Path. Lab. Med., **106**, No. 8, 400 (1982).
9. A. Marchevsky, W. L. Carrol, J. Jacobs, et al., Lung, **161**, 245 (1983).
10. T. W. Moody, The Endocrine Lung in Health and Disease, Ed. by K. L. Baeker et al., Philadelphia (1984), pp. 328-335.
11. M. C. J. Sissons and G. R. Gosney, Thorax, **40**, 862 (1985).