ENDOCRINE-LIKE CELLS IN THE LARYNGEAL MUCOSA OF ADULT RABBITS DEMONSTRATED BY ELECTRON MICROSCOPY AND BY THE GRIMELIUS SILVER-IMPREGNATION METHOD

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ABSTRACT

Endocrine-like cells in the laryngeal mucosa of adult rabbits were demonstrated by electron microscopy and by the Grimelius silver-impregnation method. Endocrine-like cells were found in both the ciliated pseudostratified and non-ciliated stratified epithelium. Some of the cells reached the airway lumen with a tuft of microvilli. The cells contained small round granules of uniform size and with a core of variable electron density. Close apposition of nerve fibers to endocrine-like cells was occasionally seen. Endocrine-like cells were also seen in the epithelium of laryngeal submucosal glands. The epiglottic taste buds, similar in structure to the taste bud in the oral cavity, were occasionally seen in the vicinity of endocrine-like cells.

The airway endocrine-like cell system has been known partly as solitary endocrine-like cells and partly as clusters of innervated endocrine-like cells: neuroepithelial bodies (9, 27). These endocrine-like cells have been characterized by the presence of numerous cored granules, the capacity to synthesize and store dopamine or 5-hydroxytryptamine after the administration of their precursor amino acids, and an argyrophilic nature. Bombesin- and calcitonin-like immunoreactivities of these cells have been shown in the tracheal epithelium of the cat (8), golden hamster (20) and rat (19). Therefore, it has been considered that the airway endocrine-like cell is a member of the paraneuron (7) and APUD series (23). Numerous electron microscopic studies on the neuroepithelial body have been reported, but there have been few electron microscopic studies on the solitary endocrine-like cells in the extrapulmonary airway epithelium. Moreover, electron microscopic studies have previously been performed mostly on the airway endocrine-like cells of 'fetal' or 'young' animals, and relatively few studies have been done on these cells in 'adult' animals (2-4, 6, 15, 25, 30, 34). Fine struc-

tures and cytochemical properties of solitary endocrine-like cells in the laryngotracheal mucosa, especially in adult animals, have not been well established.

In our previous study, relatively many endocrine-like cells have been recognized in the laryngeal and upper tracheal mucosa of mice (30). The present study demonstrates the presence of endocrine-like cells in the airway epithelial lining as well as small submucosal glands of the larynx and upper trachea of adult rabbits.

MATERIALS AND METHODS

Ten adult male rabbits, weighing about 2-5 kg, were used in this study. The animals were killed by exsanguination, and the larynx and upper trachea were disclosed immediately. The fixative, 2% glutaraldehyde and 2% paraformaldehyde dissolved in 0.12 M phosphate buffer (pH 7.5) or 2% glutaraldehyde, 2% paraformaldehyde and 2% acrolein dissolved in 0.12 M cacodylate buffer (pH 7.5), was injected into the tracheal lumen for about 5 min. Subsequently the larynx and upper trachea were dissected, and placed in a

fresh fixative for about 3 h at 4°C. During rinsing in a buffer solution containing 7.5% sucrose for about 12 h, the airway mucosa was peeled off from the wall of the larynx and trachea with syringe needles under a dissecting microscope. The peeled tissues were dissected into small pieces and postfixed in 1% osmium tetroxide dissolved in phosphate buffer (pH 7.5) containing 0.5% glucose for 3 h at 4°C. According to the routine tissue-processing method, the tissues were dehydrated with a graded series of ethanol and embedded in Epoxy resin. The sections, stained with uranyl acetate and lead hydroxide, were examined with a JEOL JEM-100U or JEM-100C electron microscope.

Silver-Impregnation Method

After the intratracheal injection of a phosphate-buffered fixative solution, the larynx and trachea were immersed in a modified Bouin's fixative (5 parts of freshly prepared 4% paraformaldehyde, 15 parts of saturated picric acid and 1 part of acetic acid) for 4 days at 4°C. Paraffin sections, cut in 5 μ m thickness, were impregnated according to the Grimelius silver-impregnation method (10).

RESULTS

Silver-Impregnation Method

Argyrophilic cells in the ciliated pseudostratified epithelium of the laryngotracheal mucosa were pyramidal or fusiform, and most of them were located perpendicular to the epithelial surface with the base resting on the basement membrane (Fig. 1). Occasionally some of these cells seemed to be located near the epithelial surface apart from the base. Most of these cells reached the airway lumen with their apical processes. They occurred singly, but some were gathered in groups (Fig. 1). Argyrophilic cells were also seen in the acini or ducts of sero-mucous glands in submucosal tissues of the larynx (Fig. 2). Some of these cells projected a slender cytoplasm among acinar cells. Argyrophilic cells were also seen in the region of the non-ciliated stratified epithelium of the upper half of the larynx (Fig. 2), and some of them appeared to reach directly the airway lumen with an apical slender cytoplasm.

Electron Microscopy

Endocrine-like cells were seen in both the non-

Fig. 1 Columnar argyrophilic cells (arrows) in the airway epithelial lining of rabbit trachea (a) and larynx (b). A group of argyrophilic cells in the stratified epithelium (c). a: $\times 430$, b: $\times 430$, c: $\times 430$

Fig. 2 Argyrophilic cells (arrows) in the submucosal gland and the airway epithelial lining (a). Two argyrophilic cells (arrows) in the stratified epithelia are seen near the epithelial surface (b). a: $\times 430$, b: $\times 1,100$

ciliated stratified and ciliated pseudostratified regions of the airway epithelium, and some of them reached the airway lumen with slender cytoplasm provided with a tuft of microvilli (Figs. 3–5). Profiles of non-myelinated nerve fibers were occasionally seen in close apposition to these endocrine-like cells (Fig. 6). The microvilli measured about 150 nm in thickness, twice as the dimension of the microvilli of surrounding ciliated cells. The microvillar cores were composed of bundles of microfilaments which extended deep into the cytoplasm (Fig. 7). Profiles resembling endocytotic vesicles were frequently seen between the bases of these microvilli. A round or oval nucleus was seen in the basal part of the cell. The cytoplasm was filled with cell organelles: small mitochondria, Golgi complex, fairly-developed rough endoplasmic reticulum, multivesicular bodies and dense bodies. Bundles of microfilaments were usually seen in the cytoplasm (Figs. 4 and 5). Occasionally the nucleus of some endocrine-like cell was entirely surrounded by a large number of microfilaments. The lateral cell surface adjacent to the neighbouring epithelial cells was equipped with some interdigitations and junctional com-

plexes. A marked characteristic of endocrine-like cells was the presence of cored granules with variable electron density (Fig. 8). Most of these granules were located in the perinuclear or basal part of the cell. However, occasional granules were found in the apical regions between bundles of microfilaments (Fig. 5). The Golgi complex occasionally contained small dense granules, which were spherical and about 130 nm in their average diameter.

Brush cells with a well-defined tuft of microvilli were seen in both the ciliated pseudostratified and non-ciliated stratified regions of the airway epithelium. The microvilli of brush cells closely resembled those of endocrine-like cells. Brush cells contained tubulo-vesicles and microfilaments, but cored granules resembling those of endocrine-like cells were scarcely detected. Cell organelles were poor in the basal cytoplasm of brush cells.

Taste buds were occasionally seen in the anterior surface epithelium of the epiglottis (Fig. 9). They appeared as slightly pale, oval bodies among the dark epithelial lining cells and were found in both the stratified and ciliated part of the epiglottic epithelium. Myelinated nerve

Fig. 3 Electron micrograph of an endocrine-like cell (ELC) in the stratified epithelium. $\times 2,800$

Fig. 4 A higher magnification of the endocrine-like cell shown in Fig. 3. The apical part of the endocrine-like cell reaches the airway lumen (L) with a tuft of microvilli. Cored granules (arrows) with variable electron density are seen in the cytoplasm. $\times 7,700$

fibers were seen in the connective tissue underlying these bodies (Fig. 10). Granule containing type III (gustatory) cells of the taste bud (21) were also seen in this body and had dense-cored granules. Their apical microvilli also closely resembled in morphological characteristics those of airway endocrine-like cells. Unmyelinated nerve fibers were also identified in the epithelial linings surrounding the taste buds.

In the laryngeal submucosal gland, granule-containing endocrine-like cells were occasionally seen among the acinar cells (Fig. 11). The apical part of the cells reached the acinar lumen in occasional cells. These glandular endocrine-like cells also contained bundles of microfilaments, cored granules, dense bodies and small mitochondria. Nerve fibers containing both clear and dense-cored vesicles of uniform size were sometimes seen in close apposition with glandular endocrine-like cells.

DISCUSSION

Morphological characteristics of laryngeal endocrine-like cells essentially corresponded to those of a similar type of cells in pulmonary neuroepithelial bodies. Laryngeal endocrine-like cells were located with the base resting on or apart from the basement membrane, and some of these cells reached the airway lumen with a tuft of microvilli. The perinuclear and basal parts of the cells contained a large number of cored granules. Images were obtained which suggest granule formation in the Golgi complex of the endocrine-like cell. Presence of serotonin in endocrine-like cells of the rabbit tracheal epithelium has been shown by a microspectrofluorometric technique (3). In addition, presence of serotonin in cored granules in these cells has been demonstrated by the ferric ferricyanide technique (4). Immunohistochemical studies

Fig. 5 An endocrine-like cell (ELC) in the ciliated epithelium. Its cytoplasm appears clearer than that in the neighbouring ciliated cell (Cc). Many cored granules (arrows) are seen in the cell. G, Golgi complex; L, lumen. $\times 6,300$
Fig. 6 A part of an endocrine-like cell and a nerve fiber (N) containing mitochondria and small granules. Note the presence of an invagination of the cell membrane of an endocrine-like cell (an arrowhead). A lysosome-like body in an endocrine-like cell (an arrow) contains cored granules. $\times 18,500$
Fig. 7 An apical part of an endocrine-like cell showing bundles of microfilaments, apical tubulo-vesicles (arrowheads) and a tuft of microvilli which are twice as thick as the microvilli of the adjacent ciliated cell (Cc). An arrow points an invagination of the apical cell membrane. $\times 22,000$
Fig. 8 An area of the Golgi complex in an endocrine-like cell containing bundles of microfilaments (arrowheads) and many cored granules with variable electron density. Nu, nucleus. $\times 22,000$

Figs. 9-11

have revealed bombesin- and calcitonin-like immunoreactive cells in the tracheal epithelium of the cat (8), golden hamster (20) and rat (19). Based on these findings, it is very probable that serotonin and the above-mentioned peptides are also contained in the cored granules of laryngeal endocrine-like cells. Some cored granules were seen close to the basal cell membrane, but features indicative of exocytotic granule release were not demonstrated in the present study. In an earlier study, exocytotic granule release from endocrine-like cells of neuroepithelial bodies has been reported in the newborn rabbit lung during hypoxia and after the administration of nicotine (17). In the tracheal epithelium of young rabbits, it has been suggested that endocrine-like cells might respond to tissue hypoxia (5). It seems likely that the inspired air was monitored by endocrine-like cells, and then the cell releases cored granules containing amines and peptides within the epithelium, into the lumen or into the nearby vasculature via the underlying connective tissue. Therefore, these cells are thought to exert a recepto-secretory function. The laryngeal endocrine-like cell may have a regulatory role in the airway mucosa, *i.e.*, mucous secretion, ciliary movement, laryngo- and vaso-constriction.

A close contact between nerve fibers and endocrine-like cells was occasionally seen in the present study. In the neuroepithelial bodies, sensory nerves have been identified (18) and synaptic contacts between nerve fibers and endocrine-like cells have been demonstrated (31, 32). It seems likely that the secretion from an endocrine-like cell stimulates nerve fibers located in close vicinity. Endocrine-like cells are thought to exert, in addition to a recepto-secretory function, a neuroreceptor function.

Endocrine-like cells have been shown in laryngeal submucosal gland of adult guinea pigs by the silver-impregnation method (16), and the present study also demonstrated these cells by electron microscopy. The morphological characteristics of cored granules in these endocrine-like

cells closely resembled those of cells in the surface epithelium. A close contact between nerve fibers and glandular endocrine-like cells was occasionally seen. It has been shown that both adrenergic and noradrenergic nerve fibers are distributed in the submucosal gland of the canine larynx (11, 12). Bombesin- and calcitonin-like immunoreactive cells have been recognized in the tracheal gland of adult cats and golden hamsters (8, 20). In the ferret tracheal gland, glandular secretion is increased by adrenergic and cholinergic agonists (1), and also by vasoactive intestinal polypeptide (24). These findings suggest a possibility that secretion from the submucosal gland might be mediated by glandular endocrine-like cells and nerve fibers.

Taste bud or chemosensory corpuscles have been identified by electron microscopy in the laryngeal mucosa of the rhesus monkey (13) and mouse (28). The present study disclosed some taste buds in the laryngeal mucosa of adult rabbits. The morphology of the laryngeal taste bud, with its rich innervation, closely resembled that of the taste bud in the lingual papillae (21, 22, 29). Cored granules in the laryngeal taste bud cells were similar to those of the airway endocrine-like cells. Both gustatory cells and airway endocrine-like cells have been included in the member of the paraneuron family (7). This view seems supported by the probable chemoreceptive function, possession of neuropeptides and amines and close relation to nerves shared by these cells.

Brush cells, characterized by the presence of a well-defined tuft of microvilli, have been detected in the epithelial lining of the airway and gastrointestinal tract and have been called variously: tuft cells (14), fibrillovesicular cells (33) and multivesicular cells (26). The morphological profiles of endocrine-like cells closely resembled those of brush cells except for the presence of numerous cored granules. In the present study, a few cored granules resembling those of endocrine-like cells were seen in occasional brush cells. Therefore, it seems likely that these two cell types are, at least in the airway mucosa, identical with each other representing two different stages of the cell function. A similar suggestion has been made in previous reports on the nature of the brush cells in the tracheal epithelium of rats (34) and mice (30). However, further studies are required for the confirmation of the suggestion.

In order to elucidate the similarities and differences among the intraepithelial, sensory and endocrine-like cell types, light, fluorescence and electron microscopy together with immunohis-

Fig. 9 A laryngeal taste bud reading a hollowed epithelial surface (L) with microvilli. Nerve fibers (N) are seen among the taste bud cells and in the underlying connective tissue. $\times 2,200$

Fig. 10 Close apposition between nerve fibers (N) and a granule containing taste bud cell. The arrow indicates a bundle of microfilaments in the taste bud cell. Nu, nucleus. $\times 11,300$

Fig. 11 An endocrine-like cell (ELC) containing cored granules with variable electron density in the laryngeal submucosal gland. L, lumen. $\times 7,400$

tochemistry would be necessary. The laryngeal mucosa appears to be useful in studying solitary endocrine-like cells in the extrapulmonary endocrine-like cell system.

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