Ultrastructure of the Glomerular Corpuscular Nerve Endings in the Subepithelium of Human Epiglottis

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Summary. The nerve endings in the subepithelial tissue of the surgically removed human epiglottis were observed by electron microscopy. The nerve varicosities (3–6 μm in diameter) with an accumulation of mitochondria and interconnecting nerve fibers supported by Schwann cells constituted the glomerular corpuscles (50–70 μm in diameter). The processes of the nerve varicosities, containing small clear and large dense cored synaptic-like vesicles, were in contact with basal cells of the stratified squamous epithelium. The glomerular corpuscular nerve endings are presumed to be concerned with low threshold mechanoreceptive functions, such as tactile sensation.

Previous light microscopic studies after silver staining have revealed a dense distribution of free nerve endings as well as several types of corpuscular nerve endings in the human epiglottis (KADANOFF, 1927; SASAKI, 1943; ROBERTO, 1957; KÖNIG and LEDEN, 1961; WYKE and KIRCHNER, 1976). Although the functional significance of these nerve endings has not been fully understood, noncapsulated sensory corpuscles have been suggested to be low threshold mechanoreceptors probably involved in the reflex control of deglutition (SASAKI, 1943; SETO, 1957; WYKE and KIRCHNER, 1976). Mechanoreceptive sensory modalities seem to be most intimately related to interactions between the nerve endings and their surrounding tissues, and their ultrastructural investigation is expected to reveal valuable information for this field.

As the ultrastructure of sensory nerve terminals in the epiglottis has not been clarified yet, the present study was undertaken to demonstrate the fine structure of the glomerular corpuscular nerve endings of the human epiglottis with special reference to the relationship of nerve endings to their adjacent structures.

MATERIALS AND METHODS

The epiglottis was surgically removed from two patients, a 72 year old male and a 75 year old male, suffering from laryngeal cancer. After excision, the lateral portions of the epiglottis were dissected out, cut into smaller pieces and immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hrs. The specimens were fixed by 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) for 2 hrs and were dehydrated with ethanol

Fig. 1. Low magnification survey micrograph showing a sectional plane of the glomerular corpuscle in the subepithelial region of the human epiglottis. The nerve varicosities containing many mitochondria are indicated by circles. The upper third of the figure is stratified squamous epithelium. Myelinated nerve fibers are seen in the lower left corner. $\times 3,400$

and embedded in epoxy resin. Sections of $1\ \mu\text{m}$ thickness were cut and scanned for nervous elements in the subepithelial tissues after toluidine blue staining. Ultrathin sections were cut with a Porter-Blum ultramicrotome MT-1A and examined with a JEOL 1200EX electron microscope after staining with both uranyl acetate and lead citrate.

RESULTS

Although the materials were obtained from patients with laryngeal cancer, the examined areas were confirmed to be free of pathological changes. Contralateral halves of the materials were examined by silver staining for light microscopy, and free and glomerular nerve endings were observed in the subepithelial layer. As nerve fiber bundles and terminals were most densely distributed in the lateral third of the posterior surface of the epiglottis, the present electron microscopic studies were concentrated in this particular region of the epiglottis. The stratified squamous epithelium in the region lacked papillary formation and a dense network of nerve fiber bundles was seen in the subepithelial connective tissue. Nerve fiber bundles in the submucosa were composed of a few myelinated fibers of 2 to $5\ \mu\text{m}$ diameter and several unmyelinated fibers enclosed in Schwann cells, and were covered by a few layers of cytoplasmic processes extending from connective tissue cells. The myelinated nerve fibers devoid of a connective tissue sheath became solitary and were covered by a Schwann cell sheath at the subepithelial region.

Under the electron microscope, nerve terminals with varicosities and interconnecting unmyelinated fibers were distributed as a cluster in the subepithelial region, forming glomerular corpuscular endings about $50 \times 70\ \mu\text{m}$ in diameter (Fig. 1). In order to confirm the continuity of sectional profiles of nerve fibers, serial sectioning and montage electron micrography were performed. At the subepithelial layer, nerve varicosities and intervaricose fibers became unmyelinated and invested in the Schwann cell sheath (Fig. 2).

Nerve varicosities characterized by numerous mitochondria, many glycogen particles, some synaptic-like vesicles, microtubules, microfilaments and lysosomes were observed in clusters in the subepithelial region. The Schwann cell sheath was often absent on the part of varicosities where they were in direct contact with the extracellular space. The diameter of these varicosities varied from 3 to $6\ \mu\text{m}$ (Fig. 3). Adjacent to the stratified squamous epithelium, processes of the nerve varicosities formed very close associations with its basal cells (Fig. 4, 5). The processes were devoid of a Schwann cell sheath and contained small clear vesicles, large dense cored vesicles, some glycogen particles and occasional tubular structures (Fig. 4, 5). Lysosome-like dense bodies were also found in the nerve varicosities (Fig. 3-5). Occasionally, several nerve processes in contact with the epithelial cells were arranged side by side as shown in Figure 6. The contact zone of these nerve processes was not packed with mitochondria, but with vesicular components instead (Fig. 4-6). No membrane specializations were observed between the nerve varicosities and the basal epithelial cells in the contact zone. Intraepithelial nerve fibers were rarely observed in the region examined in the present study.

Well developed caveolar systems were often seen in the cytoplasm of Schwann cells covering the nerve varicosities packed with mitochondria (Fig. 7).

Fig. 2. A nerve varicosity filled with mitochondria as well as intervaricose fibers (*N*) containing neurotubules are enclosed in a Schwann cell sheath (*S*). $\times 9,000$

Fig. 3. A group of nerve varicosities enclosed in a single Schwann cell sheath. Several of them are packed with mitochondria while others contain mainly synaptic-like vesicles. The nerve varicosities partially lack a Schwann cell sheath (at arrows). $\times 15,000$

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Fig. 4. A process (*P*) devoid of a Schwann cell sheath in contact with the basal cells (*B*). Vesicles, glycogen particles, and tubular structure occupy the process. Dense bodies are seen in the nerve varicosity in addition to many mitochondria and glycogen particles. $\times 15,000$

Fig. 5. The process (*P*) of the nerve varicosity which is in close contact with the basal cells (*B*). Small clear and large dense cored vesicles (arrow) are seen in the process. Dense bodies and mitochondria are contained in the adjacent nerve varicosity. $\times 32,000$

Fig. 6. Four nerve varicosities (*N*) side by side are seen in close contact with the basal cells (*B*). Schwann cell sheaths cover the lower part of the nerve varicosities. $\times 17,000$

Fig. 7. Numerous tubulo-vesicular components (arrows) are often seen in the Schwann cell cytoplasm covering the nerve varicosity packed by mitochondria. $\times 23,000$

DISCUSSION

The present study revealed the ultrastructure of non-capsulated corpuscular nerve endings having a possible sensory function in the human epiglottis. The nerve endings were characterized by swollen portions in their processes, nerve varicosities which were filled with mitochondria, glycogen particles, synaptic-like vesicles, dense bodies, neurofilaments and neurotubules in a variable combination. These cytological features of the nerve varicosities are similar to sensory nerve terminals in different types of epithelial tissues (MUNGER, 1971, 1977).

According to the silver impregnation studies on the sensory nerve terminals in the human epiglottis, simple and complex corpuscular nerve endings and free nerve endings were classified (KADANOFF, 1927; SASAKI, 1943; ROBERTO, 1957; KÖNIG and LEDEN, 1961). The nerve endings observed in the present study were confined to localized areas, rather than being dispersed over a wide area, subepithelially in close relation to the stratified squamous epithelium, and were considered to correspond to simple glomerular corpuscular nerve endings (SASAKI, 1943; SETO, 1957; ROBERTO, 1957; WYKE and KIRCHNER, 1976). SASAKI (1943) described special types of subepithelial nerve endings which he thought to be mechanoreceptors involved in the depressor reflex. The nerve endings designated as type I in the subepithelium were characterized by the "neurofibrilläre Endlamelle" which may correspond to the cluster of nerve varicosities seen under the electron microscope. However, in silver stained light microscopy, the "neurofibrilläre Endlamelle" of the nerve endings measured about 10–15 μm in diameter (SASAKI, 1943; SETO, 1957), while the nerve varicosities in the present study measured only 3 to 6 μm in diameter. This difference is probably due to two factors. Firstly, clusters of nerve varicosities are often enclosed in a single Schwann cell sheath which can be observed as a single "neurofibrilläre Endlamelle" with neurofilamentous continuity by light microscopy. Secondly, in the present study, the materials were obtained surgically and fixed using glutaraldehyde, while the materials for light microscopy in the previous study were obtained from cadavers after formalin fixation which might have caused unavoidable deterioration. Thus, the type I nerve endings seem to correspond to a class of subepithelial corpuscular nerve endings.

Considering sensory modalities, one of the most important morphological features of sensory nerve terminals is their relationship with their surrounding structures. In this regard, the nerve terminals are characterized by their processes being in close contact with the basal cells of the stratified squamous epithelium. Although the nerve endings observed in the present study satisfy most of the criteria for free nerve endings described by MUNGER and HALATA (1984), they differ from the free endings in that they are composed of clusters of nerve varicosities enclosed in Schwann cell sheaths forming glomerular corpuscles measuring 50–70 μm in diameter, and they have processes which are in a membrane-to-membrane contact with the basal cells of the stratified squamous epithelium. Direct membrane contact of axons with the basal cells was also seen in Meissner's corpuscles of the human palmar skin (HASHIMOTO, 1973). Synaptic-like vesicles were found in these processes which rarely penetrated deeper into the intra-epithelial spaces. Structurally similar spiny processes have been reported in Merkel cells (GARANT et al., 1980) as well as in free nerve endings contacting the epithelial cells of the oral mucosa (WATANABE and YAMADA, 1984) and have been considered to be sensory apparatuses.

The significance of the synaptic-like vesicles in the portion of the nerve endings

contacting the epithelial cells is not clear at present. Existence of substance P in nerve terminals in the laryngeal mucosa and the epiglottis has been suggested (NINOYU et al., 1983). Investigations into neurotransmitters or modulators which might be contained in the vesicles in the nerve terminals in question is a most interesting problem to be pursued with immunohistochemical techniques.

The clustered nerve varicosities observed in the present study resemble the simple corpuscles demonstrated by MUNGER and HALATA in a type of nerve terminal in the human eyelid (MUNGER and HALATA, 1984) as well as the glomerular corpuscles seen in the lingual and palatal mucosa of the rhesus monkey (MUNGER, 1973, 1975). The processes in contact with the basal cells of the epiglottis observed in the present study may react to the movement of the epithelium with respect to the underlying connective tissue, which is more stable as it is supported by the cartilage of the epiglottis. From the above mentioned morphological features, the nerve endings examined here appear to be similar to, but less differentiated than, the Meissner's corpuscles and may presumably be involved in tactile sensation. As the posterior surface of the epiglottis has been proved to be specifically sensitive to the flow of water (STOREY, 1968a, b), the nerve endings under study here may recognize this kind of stimulus and might be involved in the protective reflex for aspiration.

Although free nerve endings as well as taste buds were found in adjacent areas, no encapsulated nerve endings were observed in the region of the epiglottis examined. This is in agreement with the results of previous light microscopic studies on the human epiglottis (SASAKI, 1943, ROBERTO, 1957, KÖNIG and LEDEN, 1961).

Numerous pinocytotic vesicles observed in the Schwann cell cytoplasm have been found also in the laminar cells of Meissner's corpuscles (HASHIMOTO, 1973) and of Pacinian corpuscles (MUNGER 1971; MUNGER, 1975) as well as in the palatine free nerve endings (WATANABE and YAMADA, 1984). Those authors have postulated that they may play a role in the maintenance of the internal milieu of corpuscles as well as nerve endings stabilizing the irritability of sensory nerve terminals.

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