Stereo Architecture of the Lamina Propria in the Mouse Laryngopharynx in Scanning Electron Microscopy

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Summary: In the ventral wall of the mouse laryngopharynx, a fairly large number of the epithelial papillae containing taste bud (provisionally denominated the pharyngeal papillae) were observed. The NaOH cell-maceration method was applied in order to demonstrate the stereo architecture of the connective tissue papillae (CTP) of the pharyngeal papillae. The CTP appeared as a cylindrical wall surrounding a round depression, and consisted of a delicate meshwork of collagen fibrils. It is suggested that the CTP constitute the skeletal framework of the pharyngeal papillae and that the round depression corresponds to the site of taste bud. Furthermore, the collagen fibrillar architecture in the extrapapillary region appeared to be arranged to meet specific functional needs. That is, in the rostral end of the laryngopharynx, the collagen fibrils ran solitarily to form a coarse meshwork and seemed to allow the epithelium a certain degree of freedom of motion in swallowing. On the other hand, in the caudal part the fibrils concentrated into the thick bundles of the fibers running side by side along the long axis of the laryngopharynx and, therefore, appeared to play an important role in resisting the excessive stretching force.

Recently, as the result of the direct observation on the connective tissue papillae (CTP) of the lingual papillae by SEM, it has been pointed out that the architecture of the CTP is various according to the types of the lingual papillae (Toyoshima and Shimamura, 1982; Hull and Warfel, 1986; Kobayashi et al., 1987, 1988, 1989a, 1989b, 1989c; Suzuki and Takeda, 1987; Kobayashi and Iwasaki, 1989, Nagato et al., 1989; Kobayashi, 1990; Ohshima et al., 1990). On the other hand, the present author (Nakano and Muto, 1986a) reported that the epithelial papillae containing taste bud (provisionally denominated the “pharyngeal papillae” in this study) were observed in the mouse laryngoharynx. The pharyngeal papillae are morphologically differed from any types of the lingual papillae, i.e., dome-like in form. Therefore, it is assumed that the CTP of the pharyngeal papillae take a characteristic appearance.

Furthermore, the laryngopharynx including the arytenoid region is involved in the intricate mechanism of the swallowing reflex (Nakano and Muto, 1985, 1986b, 1987). It is suggested that the collagen fibers of the lamina propria in the laryngopharynx are arranged to meet the functional needs, the swallowing reflex. In this study, the NaOH cell-maceration method (Ohtani, 1987) was applied to the mouse laryngopharynx in order to demonstrate the stereo architecture of the lamina propria including the CTP of the pharyngeal papillae.

Materials and Methods

20 SMA mice (10 male and 10 female) aged from 6 to 12 months were killed by chloroform anesthesia and their pharyngeal regions were removed. The specimens were fixed with 2.5% glutaraldehyde in phosphate buffer for 2 hours and rinsed in distilled water. For studies of the epithelial surface, the specimens were postfixed with 2% osmium tetroxide for 2 hours, immersed in 2% tannic acid for 2 hours, and stained with 2% osmium tetroxide for 1 hour. They were dehydrated in graded ethanol and dried with t-butyl alcohol freeze-drying method.
(Inoue and Osatake, 1988). All specimens mentioned above were coated with gold and observed under a Jeol-U3 scanning electron microscope.

Results

In the ventral wall of the laryngopharynx, from the caudal border of the interarytenoid notch to the rostral fourth of the plate of the cricoid cartilage, there are 13–22 epithelial papillae (provisionally denominated the “pharyngeal papillae”) containing taste bud (Nakano and Muto, 1986a). The surface of the pharyngeal papillae is about 80–130 μm in diameter, dome-like in form and slightly protruding from the general level of the epithelial surface (Figs. 1, 2; Nakano and Muto, 1986a).

After NaOH treatment, the cellular elements as well as the basement membrane are completely dissolved. The most conspicuous structures in the surface of the lamina propria are the CTP of the pharyngeal papillae in the ventral wall. They appear as a cylindrical wall surrounding a round depression (Figs. 3, 4). Although they are often opened one end (Figs. 4, 5), no distinct polarity is seen. The diameter of the CTP is 15–40 μm. At higher magnifications, the cylindrical CTP consist of rather smoother sheet of interwoven collagen fibrils. There is no specific pattern of arrangement of the fibrils (Fig. 5). The bottom of the round depression surrounded by the cylindrical CTP is covered with delicate meshwork of collagen fibrils, which come from the inner surface of the CTP and are arranged in two opposing spirals (Fig. 6). The meshwork of the collagen fibrils showed many round fenestrations of 1–1.5 μm in diameter. At the periphery of the fenestrations, some collagen fibers run circularly along the rims (Fig. 6).

In the extrapapillary region, the arrangement of the collagen fibers is various according to the region of location. In the rostral end of the laryngopharynx, including the arytenoid region, the collagen fibrils run solitarily in random directions in the same plane as well as in different planes to form a coarse meshwork. There are many switchback fibrils (Fig. 7). When the surface of the lamina propria is traced caudally, the collagen fibrils gradually fuse into the fibers lying parallel with the surface (approximately 0.3–0.7 μm in diameter), which run along the long axis of the laryngopharynx (Fig. 8). In the caudal part of the laryngopharynx, some collagen fibers fuse each other to concentrate into the thick bundles of the fibers, about 2–4 μm in diameter. Although the bundles are densely packed, some of the fibers deviate from the bundle and wind around the same bundle (Fig. 9). In between the bundles, there are many collagen fibers with frequent branchings and anastomoses, which come from the bundles and fuse into the neighboring bundle. They also frequently reverse their course and then enter the adjacent bundle (Fig. 9).

Discussion

Connective tissue papilla of pharyngeal papilla

It must be of interest to study the stereo architecture of the connective tissue papillae (CTP), because the CTP constitute the skeletal framework of the epithelium and act as an adaptive structure which enlarges the epithelial-connective tissue interface in order to achieve a broader anchorage for the epithelium (Horstmann, 1954; Kobayashi et al., 1987). Furthermore, transplantation experiments have suggested that the features of the epithelium result from the action of unknown connective tissue inducers (Plagman et al., 1974; Karring et al., 1975). Recently, direct observation of the CTP by SEM, after removal of the epithelium, has been developed. Some investigators treated the specimens with sodium bromide (Hull and Warfel, 1986; Suzuki and Takeda, 1987) or EDTA (Toyoshima and Shimamura, 1982). Nagato et al. (1989) applied HCl treatment, however, it resulted in the destruction of the connective tissue. Kobayashi and his co-workers reported that long term HCl treatment could successfully remove the epithelial layer from the lamina propria which was then preserved in its integrity (Kobayashi et al., 1987, 1988, 1989a, 1989b, 1989c; Kobayashi and Iwasaki, 1990; Kobayashi, 1990). Further, Ohtani introduced the cell-maceration method with NaOH solution, and reported that the method was able to remove cellular elements much more effectively and consistently than any other methods (Ohtani, 1987; Ohtani et al., 1988). However, these studies on the CTP have been restricted within the lingual papillae.

It has been reported that taste buds are observed in the laryngopharynx and/or the entrance of the esophagus of some mammals such as man (Grossmann, 1921; Patzelt, 1921), Cercopithecidae (Rodrigo et al., 1980), the mouse (Suzuki and Takeda, 1983) and Suncus murinus (Nokubi, 1988). Furthermore, Nakano and Muto (1986a) reported at first the surface structure of the epithelial papillae containing taste bud (provisionally denominated the “pharyngeal papillae” in this study) in the mouse laryngopharynx. In this study, the NaOH cell-maceration method (Ohtani, 1987) was applied to the mouse laryngopharynx in order to demonstrate the stereo architecture of the lamina propria, especially the CTP of the pharyngeal papillae.

According to the studies on the CTP of the lingual
papillae, the architecture of the CTP is various owing to the types of the papillae (Toyoshima and Shimamura, 1982; Hull and Warfel, 1986; Kobayashi et al., 1987, 1988, 1989a, 1989b, 1989c; Suzuki and Takeda, 1987; Kobayashi and Iwasaki, 1989; Nagato et al., 1989; Ohshima et al., 1990). Generally speaking, however, the CTP of the lingual papillae appear as conical or board-like projections. The CTP of the mouse pharyngeal papillae extremely differed from those of any types of the lingual papillae, i.e., they appeared as cylindrical wall surrounding a round depression. They are, if anything, somewhat similar to the horseshoe-shaped CTP of the filiform papillae in the posterior marginal region of the anterior tongue of Suncus murinus (Kobayashi et al., 1989b), which opened rostrally to show a distinct polarity. Although the CTP of the mouse pharyngeal papillae were often opened one end, no distinct polarity was seen. Further, the cylindrical CTP of the mouse pharyngeal papillae are more and more taller than the horseshoe-shaped CTP of the Suncus filiform papillae.

The round depression (15–40 μm in diameter) surrounded by the cylindrical CTP appear to correspond to the site of the taste bud situating on the free surface of the pharyngeal papillae. In other words, the CTP of the pharyngeal papillae are regarded as the structure maintaining the taste bud. The depression corresponding to the taste bud was also observed in the lingual fungiform papillae (Kobayashi et al., 1987, 1989b, 1989c; Nagato et al. 1989) and the palatal papillae (Nakano, 1991) where the taste bud was situated on the free surface. In a teleologic sense, the taste bud on the free surface of the papillae is subjected to considerable mechanical stress by food during swallowing or mastication and, therefore, it is fit in the depression.

The configuration of the collagen fibers constituting the CTP has been little studied with a few exceptions. In the CTP of the dog filiform papillae and of the mouse fungiform ones. Kobayashi et al. (1987, 1989c) reported that a number of collagen fibers ran side by side along the outer curvature of the CTP, and that the fibers and the furrows between them seemed to provide a stronger attachment between the epithelial layer and the CTP by interdigitating with the basal surface of the epithelium. In this study, however, the collagen fibrils did not concentrate into fibers but ran independently to form a delicate meshwork. Considered from these findings, it is suggested in the mouse pharyngeal papillae that the connection between the epithelium and the CTP is weaker and, therefore, the epithelium is allowed a certain degree of freedom of motion in swallowing.

At the bottom of the round depression surrounded by the cylindrical CTP, there were many fenestrations rimmed by the interlacing collagen fibrils. Suzuki and Takeda (1987) observed in the mouse lingual circumvallate papillae that the basement membrane underlying the taste bud was perforated by many fenestrations, and suggested that the fenestrations were the passageway of nerve fibers across the basement membrane. The fenestrations in this study appear to correspond to the pathway of the nerve fibers, which continue to the fenestrations in the basement membrane and enter the taste bud.

**Extrapapillary region**

In the extrapapillary region, the architecture of the collagen fibrils was more and more various according to the region of location. In the rostral end of the laryngopharynx, the collagen fibrils ran solitarily in various directions to form a coarse meshwork. When the surface of the lamina propria was traced caudally, the collagen fibrils gradually concentrate into the fibers running longitudinally. In the caudal part of the laryngopharynx, the fibers tended to collect into thick bundles which ran along the long axis of the laryngopharynx. In between the bundles, there were many branchings and anastomoses of the collagen fibers.

These findings suggest that the collagen fibrillar content and architecture are arranged to meet specific functional needs. The rostral end of the ventral wall, including the arytenoid region, is maintained by the arytenoid cartilage and appears to be pulled ventrally in swallowing (Nakano, 1986; Nakano and Muto, 1986b, 1987). Further, the rostral end of the dorsal wall which is maintained by the cranial base takes part in the closure of the “nasopharyngeal hiatus” (Wood Jones, 1940)” in swallowing (Nakano and Muto, 1985). That is, in the rostral end of the laryngopharynx, the coarse meshwork of the collagen fibrils allows the epithelium a certain degree of freedom of motion in swallowing. In the caudal part of the laryngopharynx, on the other hand, the bony and cartilaginous structures are absent and, therefore, the collagen fibers concentrate into the thick bundle to play an important role in resisting the excessive stretching force along the long axis of the laryngopharynx. The branchings and anastomoses of the collagen fibers between the longitudinal bundles may contribute to the mechanical reinforcement of the bundles and prevent an irreversible change in its basic structural state in swallowing.

**References**


Explanation of Figures

Plate I

Fig. 1. The epithelial surface in the ventral wall of the laryngopharynx. A fairly large number of pharyngeal papillae are seen (arrowheads). \( \times 100 \).

Fig. 2. Higher magnification of figure 1. The pharyngeal papillae are dome-like in form and slightly protrude from the surface (arrowheads). \( \times 300 \).

Fig. 3. The surface of the lamina propria in the ventral wall of the laryngopharynx. Many connective tissue papillae (CTP) of the palatal papillae are seen (arrowheads). \( \times 100 \).

Fig. 4. Higher magnification of figure 3. The CTP appear as cylindrical wall surrounding a round depression (arrowheads). \( \times 300 \).
Plate II

Fig. 5. Close examination of the CTP. It opens one end (arrow) and consists of a delicate meshwork of the collagen fibrils. ×1,000.

Fig. 6. Close examination of the bottom of the round depression surrounded by the CTP. The collagen fibrils are arranged in two opposed spirals. Many fenestrations are seen (arrowheads). ×10,000.
Fig. 7. The surface of the lamina propria in the rostral end of the laryngopharynx. The collagen fibrils run solitarily in random directions to form a coarse meshwork. ×3,000.

Fig. 8. The surface of the lamina propria in the rostral part of the laryngopharynx. The collagen fibrils (F) gradually fuse into the fibers which run along the long axis of the laryngopharynx (arrowheads). ×3,000.

Fig. 9. The surface of the lamina propria in the caudal part of the laryngopharynx. The collagen fibers (arrowheads) concentrate into thick bundles (B). In between the bundles, there are many fibers with branchings and anastomoses (arrows). ×3,000.