The Occurrence and Fine Structure of Merkel Cells in the Lingual Epithelium of the Turtle, *Clemmys japonica*

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Summary: Electron microscopic examination of the lingual mucosa of the turtle, *Clemmys japonica*, revealed the occurrence of Merkel cells that shared many morphological features with Merkel cells in other vertebrates. Merkel cells were located exclusively in the basal portion of the epithelium near the taste buds. We also found occasional Merkel cells devoid of nerve contact. Nerve terminals approaching these non-innervated Merkel cells were occasionally found in the connective tissue just beneath these cells.

It has been reported that Merkel cells appear in the skin and oral mucosa of all vertebrates (Halata, 1975; Saxod, 1978; Hartschuh *et al.*, 1986; Whitear, 1989; Toyoshima and Shimamura, 1991). However, there are few morphological studies of Merkel cells in reptiles compared with the large number of studies on these cells in other vertebrate classes.

In her study on the fine structure of the skin of *Caiman crocodilus*, Düring (1975) found Merkel cell-neurite complexes in the dermis of this animal. Landmann and Halata (1980), on the other hand, reported the presence and ultrastructural details of Merkel cells in the labial epidermis of a lizard, *Lacerta sicula*. Recently, Watanabe (1988) reported the occurrence of Merkel cells in the gingival epithelium of the tegu lizard, *Tupinambis teguixin*. Thus the location of Merkel cells, dermis or epidermis, varies among reptiles.

Despite the fact that Merkel cells appear in large numbers in the oral mucosa, studies of these cells in the lingual mucosa have been relatively few among vertebrates (Toyoshima *et al.*, 1987). While investigating the morphology of the turtle tongue, we found numerous Merkel cells, which are located exclusively in the epithelium of this organ. The present paper briefly describes the fine structure of these cells in the turtle lingual epithelium.

Materials and Methods

Two turtles, *Clemmys japonica*, were used in this study. After anesthetizing the animals deeply with sodium pentobarbital (50 mg/kg), the tongues were

dissected free and placed in a trialdehyde-DMSO mixture (Kalt and Tandler, 1971) buffered with phosphate, then the specimens were cut into small pieces and immersed in the same fixative for 3 hours. These specimens were postfixed for 2 hours in phosphate-buffered 2% osmium tetroxide, dehydrated in a series of ethanols, passed through propylene oxide, and embedded in Epon-Maraglas (Tandler and Walter, 1977). Semithin sections were stained with toluidine blue and examined by light microscopy. Thin sections for electron microscopy were stained with uranyl acetate followed by lead citrate, and examined in a JEM-1200EX electron microscope.

Results

The dorsal surface of the tongue consists of folds or wrinkles and is covered by nonkeratinized stratified squamous epithelium. Merkel cells appear in the basal portion of the epithelium lining the folds and can be identified easily as less intensely stained cells than the surrounding keratinocytes by both light and electron microscopy. Most Merkel cells are ovoid in shape, ranging from 8–10 μ m in diameter, and possess highly lobulated nuclei.

At the electron microscopic level, the most conspicuous feature of Merkel cells is the presence of numerous dense-cored granules, ranging from 80–100 nm in diameter, in the cytoplasm. The density of the contents of granules is variable. These granules are distributed preferentially in the basal cytoplasm. The Golgi complexes are always located in the supranuclear cytoplasm opposite the major

aggregation of dense-cored granules. A few dense-cored granules appear sometimes in the Golgi area. Centrioles are observed occasionally near the Golgi region. Mitochondria, lysosomes and cytoplasmic filaments in varying numbers are also components of Merkel cells.

Another characteristic feature of Merkel cells is the presence of long microvilli, about 3–4 μm in length, extending into the intercellular spaces between the neighboring keratinocytes. These microvilli contain a parallel pattern of numerous filaments. Merkel cells are connected with adjacent keratinocytes by small desmosomes.

Nerve terminals closely associated with the basal plasma membrane of the Merkel cell are often observed. These terminals contain synaptic-type vesicles, small mitochondria and abundant glycogen particles. Junctional specializations are sometimes observed between Merkel cells and the associated nerve terminals. These are characterized by symmetrical thickenings of plasma membranes of the Merkel cell and apposed axon.

Occasionally, Merkel cells devoid of nerve contact and adjoining directly to the basal lamina are observed. In these cases, the dense-cored granules of these Merkel cells are relatively small in size, ranging from 60 to 80 nm in diameter, are less electron-dense and tend to accumulate in the cytoplasm toward the basal lamina, where numerous mitochondria are located. Microvillar projection is rare in this type of Merkel cell. Nerve terminals occasionally appear in the subepithelial connective tissue just below these Merkel cells.

Discussion

The present study examined the occurrence and fine structure of Merkel cells in the lingual epithelium of the turtle. Merkel cells were located exclusively near the basal portion within the epithelium. This is in accord with observations in the lizard by Landmann and Halata (1980). The morphology of Merkel cells in the turtle fulfilled all the criteria described by other authors (reviews, see Whitear,

1989): 1) localization restricted to the epithelial basal layer, 2) presence of characteristic dense-cored granules in the cytoplasm, 3) microvillar projections extending into the intercellular space of the neighboring epithelial cells, and 4) desmosomes on the cell surface.

Since Merkel first described a distinct type of cells as "Tastzellen" in 1875, the Merkel cell has been considered to be a mechanoreceptor on the basis of its morphology and physiology. According to this concept, the Merkel cell senses mechanical deformation by its microvillar projections and releases transmitters to the associated nerve terminals. Several transmitter candidates have been reported from immunohistochemical studies (Hartschuh *et al.*, 1989). It has been speculated, further, that these bioactive substances are stored in the dense-cored granules and coexist with chromogranin A (Hartschuh *et al.*, 1989).

Recently, evidence has accumulated suggesting that Merkel cells may have a trophic effect on the mechanosensory nerve fibers (Diamond *et al.*, 1988). Electrophysiological studies have also provided evidence that not the Merkel cell but the nerve terminals juxtaposed to these cells are the mechanoelectric transducer elements (Gottschaldt and Vahle-Hinz, 1982). Merkel cells may act as target sites for mechanosensory axons (Diamond *et al.*, 1988).

In the present study, we found occasional Merkel cells devoid of nerve contact. These non-innervated Merkel cells usually lacked microvillar projections. Further, we occasionally found nerve terminals approaching non-innervated Merkel cells in the connective tissue just below these cells. Although circumstantial, these findings seem to favor the view that Merkel cells may act as target sites for nerve fibers. It is of interest to note the finding obtained by Morohunfola *et al.* (1992) that the initial differentiation of Merkel cells appeared to occur before the arrival of nerve terminals in the epidermis. Recently, Vos *et al.* (1991) demonstrated nerve growth factor-immunoreactivity in Merkel cells. This study strongly supports a trophic role for Merkel cells.

Explanation of Figures

Plate I

Fig. 1. Light micrograph showing lingual epithelium of the turtle. Merkel cells (arrows) are seen in the basal portion of the epithelium. (asterisk: taste bud) $\times 280$.

Fig. 2. Electron micrograph of a Merkel cell (M) and associated nerve terminal (N). Numerous dense-cored granules are accumulated in the infranuclear cytoplasm, while Golgi complexes are located in the supranuclear cytoplasm opposite the aggregation of dense-cored granules. Microvillar projections extend to the surrounding epithelial cells (arrows). $\times 11,000$.

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Plate II

Fig. 3. Higher-magnification electron micrograph showing a microvillar projection extending from the Merkel cell. $\times 16,000$.

Fig. 4. A portion of a Golgi complex in the Merkel cell. Immature dense-cored granules are seen in the Golgi region. $\times 21,000$.

Fig. 5. Electron micrograph showing a Merkel cell devoid of nerve contact and adjoining directly to the basal lamina. Note that a nerve terminal approaching the non-innervated Merkel cell is seen in the subepithelial connective tissue (arrow). $\times 12,000$.