An Experimental Study of the Influence of Sensory Nerve Fibers on Merkel Cell Differentiation in the Labial Mucosa of the Rabbits

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Summary. The influence of the sensory nerve fibers on the differentiation of the Merkel cell was examined in the denervated labial mucosa of adult rabbits. Part of the lower labial mucosa was excised following mental nerve resection. Twenty-one and 50 days later, the regenerated mucosa was examined by electron microscopy with special reference to the distribution and maturation of Merkel cells, and compared with the normal and the denervated intact mucosa.

A number of immature Merkel cells appeared in the denervated and regenerated epithelium by 21st day after the operation. The distribution density of the Merkel cells in a unit area of the mucosa did not change much but the percentage of the mature Merkel cells increased significantly until 50 days after the operation. The mature Merkel cells in the denervated and regenerated mucosa showed a normal ultrastructure, though their orientation and location were not uniform, shifting more superficially than the cells in the normal mucosa. In the controlateral intact epithelium of the denervated labial mucosa, no substantial decrease in the population density of Merkel cells was recognized, though the desquamation of Merkel cells was observed. It was conspicuous that the percentage of immature Merkel cells to the total number of the Merkel cells was significantly increased after the denervation in the intact labial mucosa.

This study suggests that Merkel cells differentiate independently of sensory nerve fibers, but the latter are requisite to maintain the former in a uniform, basal disposition in the epithelium.

The origin, development and function of the Merkel cell, which is widely distributed in the oral mucosa and skin of vertebrates, have not yet been established.

Recent investigators have shown that mammalian and amphibian Merkel cells differentiate morphologically within the epidermis (and in the oral epithelium) via cells which closely resemble the germinal epidermal cells (ENGLISH, 1974; OCHIAI, 1977; OCHIAI et al., 1979; TACHIBANA, 1979; ENGLISH et al., 1980; TACHIBANA and NAWA, 1980; TACHIBANA et al., 1980; OCHIAI and SUZUKI, 1981; TACHIBANA and ISHIZEKI, 1981). However, the mechanism of differentiation of the Merkel cell is unknown.

It has been widely postulated that the sensory nerve terminals, which are usually in apposition to Merkel cells, might be involved in their differentiation, although no
definite evidence for this has ever been presented to date. On the contrary, there is much circumstantial evidence that a very immature Merkel cell has no connection with an intraepithelial nerve element (Lyne and Hollis, 1971; Ochiai et al., 1979; Tachibana, 1979; English et al., 1980; Tachibana and Nawa, 1980; Tachibana et al., 1980; Ochiai and Suzuki, 1981; Tachibana and Ishizeki, 1981). Furthermore, in amphibians, Merkel cells were found in the skin of the aneurogenic embryo and in the regenerated skin of the denervated adult animal (Tweedle, 1978; Scott et al., 1981).

Thus, the previous data strongly suggest that Merkel cells differentiate independently of sensory nerve fibers. However, a more direct and precise proof seems necessary to conclude this. The present study inquires about the significance of sensory nerve fibers in the differentiation of the Merkel cell, using the regenerating mucosa of the adult mammalian denervated labium.

MATERIALS AND METHODS

Mental nerves of nine male adult rabbits were bilaterally resected in a 5–10 mm length outside of the mental foramina under Nembutal anesthesia. To prevent regeneration of the nerves, the mental foramina were sealed with Aron-alpha, an instant adhesive resin. Two spots with a 2–3 mm interval were tattooed on the center of the left side of the lower lip after suturing. A small patch of mucosa, including the superficial muscular layer, was excised from the tattooed area. The excision was immediately fixed in 2.5% glutaraldehyde, followed by 1% OsO4, and embedded in Epon 812 for control observations.

Eight animals, excluding one which died during the course of the experiment, were sacrificed 21 (4 animals) and 50 (4 animals) days later by intravascular perfusion, using a modified Karnovsky’s fixative. The regenerated mucosa within the tattoo marks on the left side of the lower lip and the corresponding area of the controlateral intact mucosa of the right side of the lower lip were fixed in 2.5% glutaraldehyde at 4°C overnight. The tissue blocks were then postfixed in 1% OsO4 for 2 hrs and embedded in Epon 812 after dehydration with graded ethanols. Fixatives were all buffered in sodium cacodylate to pH 7.2.

Specimens were trimmed as large as possible, then several series of 0.1 μm ultrathin sections were cut at 10 μm intervals in the sagittal plane. Ultrathin sections were mounted on formvar-covered single slot grids, stained with uranyl acetate and lead citrate, and observed with an electron microscope.

Serial electron micrographs were taken and all nucleated Merkel cells, including immature Merkel cells (transitional cells, Tachibana and Nawa, 1980), were counted. Then the distribution density of Merkel cells per unit area of mucosa and the proportion of transitional cells to total Merkel cells were calculated. Intervals of 10 μm for the ultrathin sections ensured a minimum counting error, because the diameter of the nuclei of Merkel cells ranged from 9.5 to 10.0 μm.

Morphometry for the numerical density of Merkel cell granules was performed with mature Merkel cells which were presumed to be sectioned at their center, using an MOP-AM01 quantitative image analyzer. Statistical analyses were carried out by the t-test.
RESULTS

1. Control observations (normal labial mucosa)

Microscopic and electron microscopic structure of normal labial mucosa and Merkel cells of the rabbit have been previously reported (TACHIBANA and NAWA, 1980; TACHIBANA and ISHIZEKI, 1981). It is important to note that Merkel cells in the normal lip are usually located in the basal epithelial layer in a uniform orientation. The distribution density of Merkel cells is shown in Table 1. It is noteworthy that only a small number of transitional cells is contained in the normal labial mucosa of the adult rabbit. The numerical density of the specific granules in mature Merkel cells is shown in Table 2.

2. Experimental observations

Denervated intact mucosa: Though the mental foramina were sealed with adhesive resin, a number of unmyelinated nerve fibers were detected in the degenerative nerve trunks in the connective tissue layer underlying the muscular layer in four cases. These fibers seemed to indicate the autonomic nerve fibers which had joined the branches of the mental nerves distal to the point of the lesion. Because no myelinated nerve fibers were found in the specimens, these cases were treated as denervated materials.

In the epithelial layer of the denervated intact mucosa, as well as in the normal mucosa, many Merkel cells were identified. All Merkel cells were nerve-free, and many were located in the suprabasal layer (22-26% at 21 days, 33-55% at 50 days; Fig. 2). As reported elsewhere (TACHIBANA et al., 1982), some Merkel cells existed in

Table 1. Distribution density of the Merkel cells and the proportion of the immature Merkel cells to total Merkel cells in the normal and experimental labial mucosa of adult rabbits

| Experimental condition | Day after operation | Density of Merkel cells (/mm²) | Proportion of immature Merkel cells (%) |
|------------------------|---------------------|--------------------------------|----------------------------------------|
| Normal                 |                     | Mean ± S.D. (min.-max.)        | Mean ± S.D. (min.-max.)                 |
| Denervated-intact      | 21                  | 790 ± 90 (700-880)             | 27.5 ± 9.4 (18.2-36.9)                 |
|                        | 50                  | 934 ± 238 (708-1,264)          | 27.0 ± 5.1 (19.9-30.9)                 |
| Denervated-regenerated | 21                  | 154 ± 77 (62-247)              | 88.3 ± 9.8 (75.0-100.0)                |
|                        | 50                  | 169 ± 11 (156-182)             | 49.9 ± 15.1 (30.0-66.7)                |

Table 2. Numerical density of the specific granules in the mature Merkel cells of normal and experimental labial mucosa of an adult rabbit (/μm²)

| Experimental condition | Mean ± S.D. | Minimum | Maximum |
|------------------------|-------------|---------|---------|
| Normal                 | 3.18 ± 1.05 | 1.37    | 4.39    |
| Denervated intact (50 days after) | 3.95 ± 1.33 | 2.01    | 6.06    |
| Denervated and regenerated (50 days after) | 4.02 ± 0.96 | 1.97    | 5.40    |
the spinous or more superficial cell layers, indicating surface migration. The ultrastructure of the migrating Merkel cells was normal until they reached the granular cell layer, though the orientation of the cells was often disorganized.

The density of Merkel cells in a unit area of mucous membrane and the proportion of transitional cells to the total number of Merkel cells are listed in Table 1. Compared with a normal lip, the distribution density of Merkel cells did not change much, but the proportion of transitional cells to the total number of Merkel cells significantly increased after the denervation. The numerical density of the specific granules in the 50 day denervated Merkel cells was statistically comparable to that of the normal Merkel cells (p < 0.05; Table 2).

**Fig. 1.** Light micrographs of the intact (A) and regenerated (B) labial mucosa of the rabbit 50 days after the denervation. *lp* Lamina propria, *nt* degenerative nerve bundle. × 230

**Denervated and regenerated mucosa:** The regenerative mucosa in a healing wound of the denervated lip was composed of the keratinized squamous epithelium and the underlying dense connective tissue (Fig. 1B). The regenerated epithelium in most of the animals seemed to be normal, but in some cases reduced cell layers and hyperkeratinization were noticed. The epithelial ridges, which were characteristic of the normal labial mucosa, were not completely reconstructed by 50 days after the operation. The regenerated connective tissue was characteristic of the late phase of the granulation tissue containing tightly piled fibrous stroma and fibroblasts (Fig. 1B), differing from the loose irregular appearance of the normal lamina propria (Fig. 1A). This nature of granulation tissue of the lamina propria persisted in the regenerative mucosa until 50 days after the operation.

No nerve fiber was found in the regenerative lamina propria. On the other hand, a small number of immature Merkel cells in various developmental stages were identified in the regenerated epithelium 21 days later (Fig. 3). The distribution density of Merkel cells was considerably lower than in the normal and the denervated intact
mucosa (Table 1). The density of Merkel cells in the denervated and regenerated epithelium did not increase much until 50 days after the operation (Table 1), but the proportion of transitional cells to the total number of Merkel cells reduced substantially.

Mature Merkel cells in the denervated and regenerated epithelium showed a normal ultrastructure, except for having no contact with nerve fibers. However, they sometimes showed disorganization in orientation and location (Fig. 4, 5) differing from the Merkel cells in the normal labial mucosa, where most Merkel cells were located in the basal layer and shifted their specific granules toward the lamina propria. The numerical density of the granules in the mature Merkel cells in the denervated and regenerated epithelium of 50 days after the operation was statistically equivalent to those of the normal and simply denervated Merkel cells (p<0.05; Table 2).

DISCUSSION

It has previously been shown that new Merkel cells developed in the regenerative epithelium of innervated labial mucosa in adult rabbits (TACHIBANA and ISHIZEKI, 1981). In the present study, Merkel cells were identified in the regenerated epithelium of denervated labial mucosa in adult rabbits. Furthermore, the Merkel cells in denervated and regenerated mucosa showed a tendency to mature with time. Because no sensory nerve fibers were found in the denervated and regenerated mucosa, it is
presumed that the Merkel cells appearing in the regenerated epithelium are differentiated without any effect of the sensory nerve fibers. This is in agreement with Scott et al. (1981), who showed that Merkel cells appeared in the regenerated epidermis of the denervated salamander skin. From the results, it is conceived that sensory nerve fibers exert no essential influence to the cytodifferentiation of the Merkel cell. The previous data showing that very immature Merkel cells in normally developing skin and oral mucosa have no contact with nerve terminals (Lyne and Hollis, 1971; Ochiai et al., 1979; Tachibana, 1979; English et al., 1980; Tachibana and Nawa, 1980; Tachibana et al., 1980; Ochiai and Suzuki, 1981; Tachibana and Ishizeki, 1981) strongly support this view.

According to Tachibana and Ishizeki (1981), the full-thick wound in the innervated labial mucosa of the rabbits healed completely with no scar tissue remaining in the lamina propria within 30 days, and the distribution density of Merkel cells in the regenerative epithelium increased with time. The density of the Merkel cells in the regenerative epithelium of the denervated mucosa, however, increased only slightly until 50 days later. Up to 50 days later, granulation tissue remained in the lamina propria of the denervated and regenerated mucosa. Furthermore, the proportion of the immature Merkel cells (transitional cells) in the denervated and regenerated epithelium was larger than in the regenerated epithelium of the innervated mucosa, which we studied previously (Tachibana and Ishizeki, 1981). Therefore, it may be said that an inhibition or delay in the regeneration procedure of the wound might occur after denervation. Besides, desquamation of the Merkel cells from the regenerative epithelium may be considered, as well as in the simply denervated intact mucosa (Tachibana

Fig. 3. Immature Merkel cells (M) in the denervated and regenerated epithelium of the labial mucosa of a rabbit 21 days after the operation. A: x 6,600, B: x 7,500
et al., 1982). Actually, the Merkel cells located in the suprabasal and more superficial cell layers have sometimes been observed in the denervated and regenerated mucosa.

Reports on the effect of denervation to Merkel cells have been inconsistent. Some authors have shown that very little change occurred in the number and ultrastructure of Merkel cells after denervation (SMITH, 1966, 1967; HARTSCHUH and WEIHE, 1977; BENKENSTEIN, 1979; TACHIBANA et al., 1982). On the other hand, other authors (PALMER, 1965; BURGESS et al., 1974; ENGLISH, 1977; KUROSUMI et al., 1979) have shown or suggested a marked decrease in the number of Merkel cells and the specific granules in Merkel cell following denervation, and elicited a supposition that some neurotrophic influence exists between the Merkel cell and the nerve terminal. The present study shows that the distribution density of Merkel cells in the intact mucous epithelium does not change much after denervation, in spite of the fact that many cells desquamate from the epithelium after migration to the surface layer. A remarkable increase in the number of immature Merkel cells (transitional cells) was noted in the denervated intact epithelium. This strongly suggests that the reproduction of Merkel cells is induced or activated in the denervated labial mucosa of the rabbits. If we accept the hypothesis that such phenomenon is common to all kinds of vertebrate, the discrepancy on the influence of denervation to the Merkel cell may be explained as an effect of balance between the desquamation speed and the reproduction speed of Merkel cells in the denervated tissues of the different species.

Fig. 4. Mature Merkel cell (M) in the denervated and regenerated epithelium of a rabbit 50 days after the operation. Specific granules in this cell are located in the lateral cytoplasm. \( \times 6,900 \)
The Merkel cells appearing in both the intact and the regenerated epithelium of the denervated mucosa in the present study often showed abnormal location and orientation, though their ultrastructure had not been changed. It is therefore thought that an important influence of the axon terminal to the Merkel cell might put the Merkel cell in a uniform basal position in the epithelium with a uniform orientation.

It is of interest that the newly developed mature Merkel cells in the regenerated epithelium of the denervated labial mucosa of the rabbit contained a statistically equivalent numerical density of the specific granules to the normal Merkel cells. Functional independency of the Merkel cell from nerve fiber is supposed from the result. The mechano-reception and transduction function of Merkel cell, which has been a long-standing conception for the function of Merkel cell, was recently questioned by Gottschaldt and Vahle-Hinz (1981, 1982) on the basis of electrophysiological studies. The other possible functions, such as a target function for nerve fibers proposed by Scott et al. (1981) or an endocrine function, should be inquired about the Merkel cells in the future.

REFERENCES

Benkenstein, M.: Veränderungen der Ultrastruktur der Merkelschen Nervenendigungen an Sinus-
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haaren von Ratten nach Denervation. Acta anat. 105: 409–422 (1979).

Burgess, P. R., K. B. English, K. W. Horch and L. J. Stensaas: Patterning in the regeneration of type I cutaneous receptors. J. Physiol. 236: 57–82 (1974).

English, K. B.: Cell types in cutaneous type I mechanoreceptors (Haarscheiben) and their alterations with injury. Amer. J. Anat. 141: 105–126 (1974).

———: The ultrastructure of cutaneous type I mechanoreceptors (Haarscheiben) in cats following denervation. J. comp. Neurol. 194: 138–164 (1977).

English, K. B., P. R. Burgess and D. K. Norman: Development of rat Merkel cells. J. comp. Neurol. 194: 475–496 (1980).

Gottschaldt, K. M. and C. Vahe-Hinz: Merkel cell receptors; Structure and transducer function. Science 214: 183–186 (1981).

Hartschuh, W. and E. Weihe: The effect of denervation on Merkel cells in cats. Neurosci. Lett. 5: 327–332 (1977).

Kurosumi, K., U. Kurosumi and K. Inoue: Morphological and morphometric studies with the electron microscope on the Merkel cells and associated nerve terminals of normal and denervated skin. Arch. histol. jap. 42: 243–261 (1979).

Lyne, A. G. and D. E. Hollis: Merkel cells in sheep epidermis during fetal development. J. Ultrastr. Res. 34: 464–472 (1971).

Ochiai, T.: The ultrastructural and situational changes of Merkel cells according to aging in the newborn rat (In Japanese). Jap. J. Dermatol. 87: 911–920 (1977).

Ochiai, T., S. Baba and H. Suzuki: Ultrastructural studies of the Merkel cells in the newborn rat (In Japanese). Jap. J. Dermatol. 89: 777–782 (1979).

Ochiai, T. and H. Suzuki: Fine structural and morphometric studies of the Merkel cell during fetal and postnatal development. J. Invest. Dermatol., 77: 437–443 (1981).

Palmer, P.: Ultrastructural alterations of Merkel cells following denervation. Anat. Rec. 151: 396–397 (1965).

Scott, S. A., E. Cooper and J. Diamond: Merkel cells as targets of the mechanosensory nerves in salamander skin. Proc. Roy. Soc. Lond. B 211: 455–470 (1981).

Smith, K. R.: The effect of denervation on Merkel cells in Haarscheiben of rats. Physiologist 9: 288–289 (1966).

———: Fine structure and function of the Haarscheibe. J. comp Neurol. 131: 459–474 (1967).

Tachibana, T.: The Merkel cell in the labial ridge epidermis of the anuran tadpole. II. Electron microscope observation on the appearance and differentiation of the Merkel cell. Arch. histol. jap. 42: 129–140 (1979).

Tachibana, T. and K. Ishizeki: Merkel cell development in the wound healing in the labial mucosa of adult rabbits. Arch. histol. jap. 44: 151–165 (1981).

Tachibana, T. and T. Nawa: Merkel cell differentiation in the labial mucous epithelium of the rabbit. J. Anat. 131: 145–155 (1980).

Tachibana, T., Y. Sakakura and T. Nawa: Merkel cell differentiation in the developing tentacles of Xenopus laevis (Japanese text with English abstract). Acta anat. nippon. 55: 588–599 (1980).

Tachibana, T., Y. Sakakura, K. Ishizeki, S. Iida and T. Nawa: Migration of Merkel cells in the labial mucous epithelium of adult rabbits following mental nerve resection. Cell Tiss. Res. 223: 659–664 (1982).

Tweedle, C. D.: Ultrastructure of Merkel cell development in aneurogenic and control amphibian larvae (Ambystoma). Neurosci. 3: 481–486 (1978).

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