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THE HISTOCHEMISTRY OF THE MERKEL CELL NEUROEPITHELIAL ENDINGS IN CAT AND RABBIT SKIN AND ORAL MUCOSA

R. K. Winkelmann

Department of Dermatology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905, USA

Abstract. The tactile hair disks in the oronasal area of the rabbit registered positive for specific and nonspecific cholinesterase. In the cat, the tactile hair disks were positive for alkaline phosphatase in the same region. The hederiform Merkel-Ranvier ending in the epidermis of the palate was positive for cholinesterase in the rabbit and positive for alkaline phosphatase in the cat. In the rabbit, 4-methoxyleucine aminopeptidase activity was also demonstrated in the focal palate epithelium associated with nerves of the Merkel-Ranvier ending. These histochemical reactions of the Merkel neuroepithelial complexes are associated with the nerve ending and may be a specific indicator of the species' innervation pattern.

Key words: Cat; Merkel cell; Mucosa; Rabbit; Skin

The Merkel cell has been recognized as a unique intra-epithelial cell, ususally closely related to a sensory nerve ending. The cytoplasm of the Merkel cell contains small membrane-bound granules similar to those in sympathetic nerve endings and APUD cells. While no amine has been found in the Merkel cell, metenkephalin activity shows that a polypeptide hormone may be present in the Merkel cell (2). The APUD cell also contains esterases, even cholinesterase (11), as do sensory end-organs in mammalian skin. Although no histochemical studies on Merkel cell endings have reported positive findings, the tactile hair disk containing Merkel cells has been demonstrated to contain cholinesterase (17, 20). In this study, the enzyme activity in the Merkel cell-associated neuro-epithelial endings in cat and rabbit skin and mucosa is illustrated.

MATERIALS AND METHODS

Skin was biopsied from anesthetized albino rabbits or cats and used immediately or frozen at −70°C until use within 1 week. The histochemical procedures are the modified acetylthiocholinesterase of Koelle (3), the amylophosphorylase method of Takeuchi & Kuriaki (12), the nucleoside triphosphatase (ATPase) method of Wachstein & Meisel (13), the 4-methoxyleucine aminopeptidase method of Nachlas et al. (10), the Winkelmann & Schmit (19) frozen-section silver method, the diazo alkaline phosphatase technique, and the succinic dehydrogenase method (1).

RESULTS

Rabbit. The tactile hair disks of the rabbit were positive with specific and non-specific cholinesterase in the transitional glabrous skin between the haired skin of the nose and the mucous membrane. The cholinesterase-positive hair disks were clustered just within the rim of hairy skin about the nares. Hair disks were not observed by this technique in the generally hairy skin selected sections of back, chest, abdomen, scalp, legs, dorsum of feet, and tail. The intra-epithelial masses of cholinesterase were normally present on the side toward which the follicle was inclined in its passage through the epithelium (Fig. 1 A).

The palate showed clusters of cholinesterase-positive reactivity at the base of some rete ridges. These foci were observed in epidermis of the palatal rugae, but they were also distributed less densely in the epidermis between rugae. The cholinesterase demonstrated the expanded nerve endings of the Ranvier hederiform endings, which are related to the Merkel cells and, in some instances, demonstrated the associated nerve fibers. With reduced illumination, the cholinesterase-positive nerve ending as well as the adjacent Merkel cell could be seen (Fig. 1 B).

The ATPase method demonstrated nerve fibers in the dermis, as well as in capillary vessels. ATPase-positive nerves were observed in the dermis (Fig. 1 C) and in the epidermal nerve portion of the hederiform ending. The Merkel cell was seen as a sharp, clear cell closely apposed to and situated above the ATPase-positive terminal.

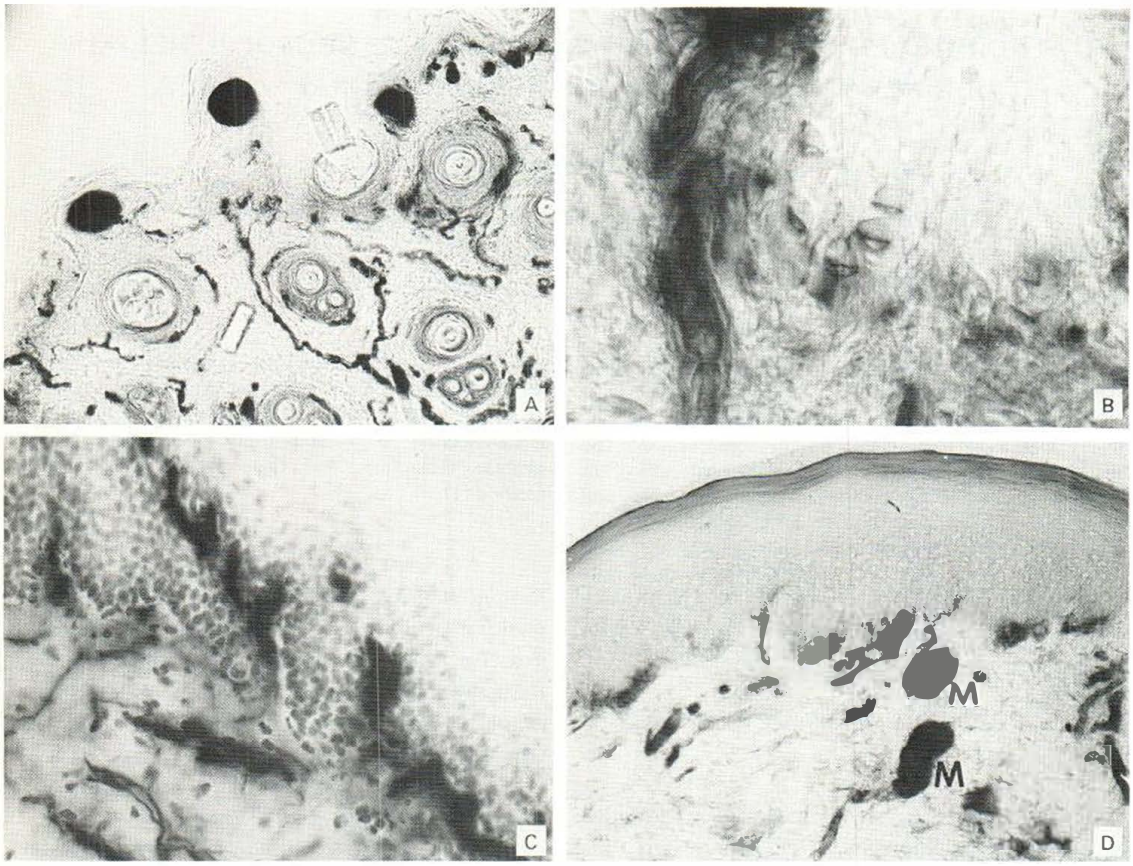

Fig. 1. Rabbit. (*A*) Cholinesterase-positive hair tactile disk in epithelial masses and dermal nerve of perioral skin. (Koelle cholinesterase; ×150) (*B*) Cholinesterase-positive nerve disk in epithelium of palate. Distinct, rounded Merkel cell above and apposed to each disk may be discerned, even though the cell is not enzyme-positive. One intraepithelial nerve that is cholinesterase-positive is seen. (Koelle cholinesterase; ×1 000.) (*C*) Dermal nerves and dermal and papillary vessels are demonstrated by nucleoside triphosphatase (ATPase). Sections of dermal nerve course near the epidermis. Clear cell in center is Merkel cell associated with nerve disk that is ATPase-positive. (Wachstein and Meisel ATPase; ×400). (*D*) Leucine aminopeptidase activity in dermal nerve blood vessel, mammalian nerve end-organ (*M*), and focal activity in basal rete ridge of palate. The focal areas of epithelial activity indicate Merkel cell nerve endings. (Nachlas 4-methoxyleucine aminopeptidase; ×250).

The Merkel cell masses at the base of some epidermal rete ridges could be selectively outlined by the product deposition from the phosphorylase method. This activity appeared to be present in the cytoplasm of the Merkel cell and was not morphologically similar to the nerve fiber or ending.

The 4-methoxyleucine aminopeptidase technique demonstrated the hederiform rete ridge-Merkel cell complexes (Ranvier hederiform endings) in some epidermal rete ridges of the rabbit palate. This also appeared to be a membrane- or cytoplasmic-bound enzyme of the Merkel cell. The enzyme activity was seen only at the tip of the rete ridge. The nerve was not visualized, though the membranes of the dermal mammalian end-organs were positive (Fig. 1 D).

Cat. The cat mammalian end-organs were positive for alkaline phosphatase as well as for cholinesterase in the glabrous skin and mucous membrane, but study of haired skin of the cat from the back, flank, belly, neck, leg, ear, tail, top of head, hair, cheek, nose, and eyelid did not reveal Merkel cell endings, particularly tactile hair disks, by this method.

As in the rabbit, the epidermal endings of the Merkel cell of the cat were concentrated in the

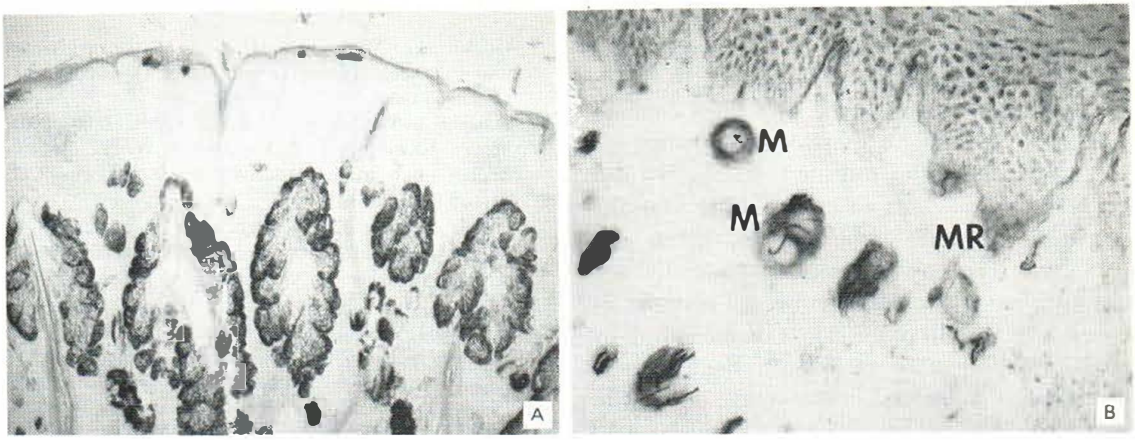

Fig. 2. Cat. (*A*) Epithelial tactile hair disks about ostia of hair follicles of nasal skin. (Diazo alkaline phosphatase; ×64.) (*B*) Neuroepithelial-Merkel cell complex (*MR*) with faint gray basal zone of peptidase and silver-positive nerve fibrils. Mammalian end-organ (*M*) in dermis shows silver-stained axial nerve and peptidase-positive peripheral membranes. (Nachlas 4-methoxyleucine aminopeptidase plus Winkelmann silver method; ×300.)

mucous membranes and glabrous skin of the muzzle area. Tactile hair disks demonstrated by alkaline phosphatase were observed just within the haired skin surrounding glabrous skin of nose and lips (Fig. 2 A). The only exception was the tip of the chin, where tactile hair disks were also observed by this method. In the whisker area, the sensory hair has alkaline phosphatase-positive end-organ structures in the external root sheath and about the infundibulum, possibly representing Merkel cell endings. Alkaline phosphatase was seen in the tips of selected epithelial rete ridges of the hard palate and gingiva, which may represent Ranvier endings. General non-selective phosphatase reaction in the base of the epidermis was observed in biopsy specimens of the dorsal and ventral surfaces of the tongue and the mucosa of the pharynx and larynx.

The frozen-section silver technique from our laboratory may be added to tissue already stained by histochemical procedures. The addition of silver stain for axoplasm to the phosphatase and the peptidase methods used in cat and rabbit tissue confirmed the presence of hederiform nerve endings in epithelial rete ridges that were selectively stained at their tips by the histochemical methods and demonstrated the central axial nerve in the mammalian nerve end-organ (Fig. 2 B).

DISCUSSION

The variability of the histochemical reactions of the Merkel cell–epithelial organs is consistent with experience reported in the literature referable to the comparative sensory nerve end-organ histochemistry in many species. Previous studies by Winkelmann (15) and by Montagna & Ellis (5, 6) indicated the unique specificity of the end-organ histochemistry in primates, other mammals, and marsupials. Hair follicle end-organ, for example, has been found to be positive for cholinesterase in most animals studied, including primates, with the exception of the lemur skin, which in all species is negative for specific and non-specific cholinesterase (8, 21). The hair follicle nerve end-organ is positive for alkaline phosphatase in the red kangaroo (18) and the lesser anteater (*Tamandua*) (4) and for 4-methoxyleucine aminopeptidase in galago (*Galago grassicaudatus*) (Winkelmann, unpublished data). The Meissner corpuscle is positive for cholinesterase in most primates, although the North Ameri-

Table 1. *Species with Merkel cell-related histochemistry*

Species	End-organ	Enzyme activity
Rabbit	Ranvier endings	Cholinesterase
		4-Methoxyleucine aminopeptidase
	Hair disk	Cholinesterase
Cat	Ranvier endings	Alkaline phosphatase
	Hair disk	Alkaline phosphatase
Guinea pig	Hair disk	Cholinesterase
		Alkaline phosphatase

can opossum (*Didelphis virginiana*) has no cholinesterase—only alkaline phosphatase—in its Meissner corpuscles (16). The gibbon (*Hylobates lar*) has alkaline phosphatase as well as cholinesterase in its Meissner corpuscles, a distinctive sensory chemistry in this primate (14). The red kangaroo (*Megaleia rufa*) also has alkaline phosphatase in its Meissner corpuscles, as well as cholinesterase (18). This summary delineates the special distribution of cholinesterase and phosphatase in nerve end-organs of the skin. Combined with the histochemical pattern of vessels, the end-organ histochemistry can lend a comparative histochemical signature to the skin (7, 17). The histochemistry of the Merkel cell endings extends this area of knowledge. Three mammals have a distinctive Merkel cell histochemistry (Table I), and it may be assumed that others will be observed. The Merkel cell itself may share species histochemical specificity, as metenkephalin activity is positive in guinea pig Merkel cells, slightly positive in the rat, and negative in other species (2).

The localization of the epidermal enzyme reaction in the tactile hair disk of Pinkus is reasonable evidence of its chemical and morphologic specificity. It is not possible to explain why tactile disks are positive in one area and negative in another. Although similar regional histochemical variations have been observed in other skin structures and in skin nerve structures (7), the presence of the sensory hair in these regions may be correlated with the more sophisticated chemistry of the hair disks. For the Merkel-Ranvier hederiform endings of the palate (the Merkel-rete endings) (9), no other satisfactory explanation can be offered. The presence of nerve in relation to the histochemically positive structure, as demonstrated by silver techniques, is confirmatory of the relationship of the chemistry to nerve endings. Less satisfying is the realization that esterases and phosphatases may involve the base of the epidermis of the tongue, pharynx, and larynx. This implies that, despite focal enzyme activity in the basal epithelium where nerves are found in the palate, one should be cautious in interpreting the location and the significance of the enzymes. Electron histochemical methods exist for the techniques used in this work, and their application to the Merkel cell structures will confirm the chemistry and localization of the enzymes in relation to the Merkel cell, the nerve ending, and the epidermal cells.

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R. K. Winkelmann, M. D.
Mayo Clinic
Rochester, MN 55905, USA