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ULTRASTRUCTURAL LOCALIZATION OF CHOLINESTERASE ACTIVITY IN HUMAN HAARSCHIEBEN

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Cholinesterase activity (ChA) in human Haarscheiben was ultrastructurally examined with Karnovsky's method and following results were obtained:

- 1) No ChA was observed on the membrane of myelinated nerve fibers.
- 2) Axons of non-myelinated nerves in the corium showed strikingly positive ChA on its membrane.
- 3) The membrane of Tastmeniscus revealed sometimes also positive ChA, but the part of it attached to Merkel cells in the epidermis showed frequently no ChA.
- 4) On the membrane of the prickly-like cytoplasmic protrusions of the Merkel cell positive, but not so typical ChA was also observed.

The oval shaped, very small, slightly elevated papular structure of the skin with a glossy appearance, which is usually observed in the vicinity of a lanugo hair, was first described by Pinkus (7) in 1902, and because of its characteristic feature and localization named "Haarscheibe" (7, 8).

Not only from the anatomical, but also physiological point of view, this structure is thought to be a kind of sensory, especially highly sensitive, modality-specific, slowly adapting touch receptor (11). Recent electron microscopic investigations

of the Haarscheiben (4, 11) have made its morphological details more clear.

The purpose of the present studies are to confirm the localization of the cholinesterase activity in the human Haarscheiben on the ultrastructural level and to consider the role played by it in the sensory activity of this organ.

MATERIALS AND METHODS

1) Several Haarscheiben were taken from the dorsal surface of the vorarm of a 37-year-old healthy man under the minimal local anaesthesia and by punch biopsy. The specimens were immediately fixed in the 4% formaldehyde solution prepared by dissolving paraformaldehyde, just before use, in 0.075 M phosphate buffer solution (pH 7.6) containing 0.22 M sucrose, for 2 or 3 hours in a refrigerator. After complete washing with 0.22 M sucrose solution, the specimens were left overnight in it. Then they were trimmed with scalpel under a stereoscope or cut at 150 μ thickness with a tissue sectioner, and incubated in Karnovsky's medium (3) containing acetylthiocholin iodide or in a substrate-free medium for 45 to 60 minutes at 0° to 4°C. After washing with 0.05 M phosphate buffer solution (pH 7.4) the tissues were fixed in 1% OsO₄ buffered at pH 7.4 with phosphate.

2) Some Haarscheiben which were immediately fixed in 1% OsO₄ buffered with 0.1 M cacodylate (pH 7.4) were used for morphological observation. They were obtained from Thiersch's section (4) of the normal human skin areas under the general anaesthesia.

All materials were dehydrated with ethanol series and embedded in Epon. Then they were cut on a Porter-Blum (M2) microtome. The ultrathin sections were stained with ulanylacetate and/or lead citrate, and examined with the Hitachi HU-11B electron microscope.

RESULTS

The Merkel cells (Fig. 1), which are situated in the basal layer of the epidermis on the arched giant papilla of Haarscheibe, are easily recognized by the numerous osmiophilic granules surrounded with a limiting membrane in their cytoplasm. They are obviously attached to keratinocytes by small desmosomes and project many prickly-like cytoplasmic protrusions, indenting the neighboring keratinocytes, Schwann cells, basement membrane bordering on the corium and even axons. Numerous pinocytotic vesicles are sometimes revealed on such areas of the cell membranes of keratinocytes that border on the indenting protrusion of Merkel cells (Fig. 5a). Several number of discoid shaped neural terminals which are covered with the Schwann cell cytoplasm from below, are attached to the bottom of a Merkel cell in the epidermis. There are considerable amount of glycogen and numerous mitochondria in this terminal. Just under the epidermis in this region many irregularly arrayed axons wrapped with the cytoplasm of Schwann cells are recognized.

In the sections of the material incubated in the medium with substrate, cholinesterase activity (ChA) is revealed as small electron dense granules or precipitates (Fig. 2, 2a, 3, 4, 4a, 5). The most striking deposits of these granules are seen on the membrane of the axons wrapped with Schwann cell cytoplasm and situated in the corium just beneath the epidermis (Fig. 2, 3). On conspicuous observation,

these granules seem to be located mainly in the narrow space between the axon and Schwann cell (Fig. 2, 2a, 3, 4a), but sometimes rather only on the surface membrane of the former (Fig. 2). Most axons in this area show quite distinct ChA, occasionaly, however, there are some axons without ChA (Fig. 2a). No distinct morphological difference is not discernible between the axons with ChA and those without. The deposits showing ChA were also seen in the narrow spaces between the paired membranes (they are probably mesaxons or mesaxon-like endoplasmic reticulum) of Schwann cells (Fig. 2, 3, 4a). They seem to be more conspicuous near the axons than areas distant from the latter (Fig. 2, 3). The similar deposits are occasionaly found on the cell membrane of Schwann cells, especially in some caveolae intracellulares (Fig. 3). The membrane of subepidermal meniscoid neural terminal usually show fairly striking ChA (Fig. 4a), which seems to be, however, not always uniform (Fig. 4a). ChA is usually undiscernible on its area adhering to the Merkel cell (Fig. 3). In rare instances, however, fairly distinct ChA (Fig. 4) is recognized on the area of the cell membrane of Tastmeniscus, which ChA seems to be obscure in the area of the Merkel cell (Fig. 4). The characteristic prickly-like protrusions of Merkel cells are often revealed to have considerably distinct ChA (Fig. 5).

In Haarscheiben incubated in substrate-free medium no electron dense material compatible to that in the cholinesterase-active site was found on the plasma membrane of axons in the epidermis, as well as in the corium.

FIG. 1. Horizontal section of three Merkel cells (M1, M2, M3) in a human Haarscheibe. Tm: Tastmeniscus, Pc: cytoplasmic protrusions and D: desmosome. Fixation (Fix): OsO_4 . Staining (St): uranyl acetate (Ua) and lead citrate (Lc).

DISCUSSION

Recent advances of electron microscopical histochemical technics brought on the success of demonstration of cholinesterase activity (ChA) on the ultrastructural level (1, 2, 3, 9, 10).

In our experiment, the attempt of confirmatory differentiation between the acetylcholinesterase (AChE) and non-specific cholinesterase (non-ChE) was not performed. The specificity of this cholin esters as substrate had been generally accepted (5). However, confirmatory procedures, use of inhibitors for instance, were found to be necessary for locating the specific AChE activity (1, 9). Thus, further study for differentiating between AChE activity playing a significant role in nerve conduction and non-ChE activity might be necessary. Since precipitates suggesting ChA were not revealed in control specimens incubated in the substrate-free medium, the differentiation between ChE active and ChE inactive sites was established except for a certain case later to be mentioned.

Ultrastructural details of Haarscheibe were first published by Smith (11), who found variously shaped Merkel cells at the epidermal basal layer. These cells contain many electron dense peculiar granules (cored vesicles) in the cytoplasm.

FIG. 2. A branched unmyelinated axon (Ap) in the corium, cholinesterase activity (ChA).

Incubation in Karnovsky's medium with substrate for 60 minutes. Characteristic electron dense granular deposits are seen in the axon-Schwann interspace, on the plasma membrane of axon (short arrows) and in the narrow space between the paired membrane (Rm) of Schwann cell. Fix: Formaldehyde (For) and OsO_4 . St: Ua and Lc.

FIG. 2a. An unmyelinated nerve bundle in the corium, ChA. One type of axons (An) reveals no ChA, while the other (Ap) distinctly. Vc: cored vesicles in the axon. Fix: For and OsO_4 . St: Ua and Lc.

FIG. 3. The area of the dermo-epidermal junction, ChA. The axon (Ap) situated in the corium just beneath the epidermis shows typical granular deposits in the axon-Schwann interspace and narrow space of mesaxon (Ma). On the contrary, the Tastmeniscus (Tm) attached to the Merkel cell (M) in the epidermis no ChA. Caveolae intracellulares (Ci) show also fairly distinct ChA. Sch: Schwann cell. Fix: Form and OsO_4 . St: Ua and Lc.

FIG. 4. The area of the dermo-epidermal junction, ChA. A certain Tastmeniscus (Tm) reveals a fairly distinct ChA in the epidermis. M: Merkel cell. Fix: Form and OsO_4 . St: Ua and Lc.

FIG. 4a. A meniscoid neural terminal, ChA. Typical granular deposits are seen on a certain part (left-top) of its surface membrane, as well as on the paired membrane (Rm). On the other part, however, ChA seems to be rather obscure. Fix: Form and OsO_4 . St: Ua and Lc.

FIG. 5. A Merkel cell in the epidermis, ChA. A fairly distinct ChA is observed on the surface of a prickel-like cytoplasmic protrusion (Pc) of the Merkel cell. A pinocytotic sack (short arrow) reveals no ChA. K: keratinocyte. Fix: Form and OsO_4 . St: Ua.

FIG. 5a. A prickle-like cytoplasmic protrusion (Pc). Many pinocytotic vesicles are seen on the membrane of keratinocyte in the opposite side. Fix: OsO_4 . St: Ua and Lc.

FIG. 6. The area of the dermo-epidermal junction, ChA. Incubation in the substrate-free medium for 60 minutes. No ChA is seen on the membrane of Tastmeniscus (Tm) in the epidermis. M: Merkel cell. Fix: For and OsO_4 . St: Ua and Lc.

FIG. 6a. Axons in the corium just beneath the epidermis, ChA. Incubation in the substrate-free medium. No ChA is recognized on the surface membrane of the axons (An). Fix: Form and OsO_4 . St: Ua and Lc.

Fig. 2 Fig. 2a

Fig. 3

Fig. 4 Fig. 4a

Fig. 5 Fig. 5a

Fig. 6 Fig. 6a

The nature of these granules is not yet unknown, even if they might be related to conduction of stimuli (6). In our materials fixed with OsO_4 , however, any structure suggesting intimate relationships between these granules and sensory conduction was not found. There was no evidence to show the ChA in these granules, i.e. their distinct increase in electron density by incubation with the substrate. Therefore it might be open for further study to decide whether these granules play such an immediate participation in stimulus conduction, as in case of the synaptic vesicles.

The characteristic prickly-like cytoplasmic protrusion of the Merkel cell, which has been by its structure considered to be a kind of receptor organella (4), seems to serve for receiving some stimuli, probably minute changes of tension or mechanical distortions caused by neighboring cells, as the cilium in other mechanoreceptor cells. In our experiment the surface membrane of these prickles of Merkel cells often reveal remarkable, but not so typical, positive reaction of ChE. However, whether the ChE do play an essential role in conduction mechanism occurring between the keratinocytes and these prickles might not be decided merely by our findings.

In the Tastmeniscus, there were often some vesicular structures which had no uniform size and such property as to be revealed in synaptic vesicles. They show no definite ChA. Hence it might be concluded that vesicles containing acetylcholin do not participate in the conduction of stimulus from the Merkel cell to the axon. Faintly positive ChA were, however, sometimes revealed on the membrane of Tastmeniscus attached to the Merkel cells. It seems to be not so easily to decide whether this finding reveals true ChA, only a ghost phenomenon due to diffusion or certain technical artifact.

The most distinct and typical findings of ChA was revealed in the majority of unmyelinated axons enclosed with Schwann cells in the subepidermal areas of the Haarscheibe, even at the part of knobs. In some axons, however, characteristic precipitates were not seen. The similar distinct deposits of precipitates are also found at the mesaxons, as well as mesaxon-like structures of Schwann cells. The trial to differentiate nerve elements in the Haarscheibe by the difference of ChA was not achieved completely, in our experiments, because of the lack of confirmatory differentiation between AcE and non-specific ChE activities, though the ChE active sites were distinguished from not active ones.

Summerizing the above mentioned findings, the most distinct activity of ChE was revealed not at the contact areas of Merkel cells and neural endings in the epidermis, but rather on the surfaces of axons and in the spaces between the paired epidermis, but rather on the surfaces of axons and in the spaces between the paired membranes of mesaxons, as well as mesaxon-like structures in the corium. The role of the ChE found in various strength of their activities in the function of human Haarscheibe might deserve further study.

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REFERENCES

1. Davis, R. and Koelle, G. B.: Electron microscopic localization of acetylcholinesterase and non-specific cholinesterase at the neuromuscular junction by the gold-thiocholine and gold-thiolacetic acid methods. *J. Cell Biol.*, 34; 157, 1967.
2. Hirano, H. and Ogawa, K.: Ultrastructural localization of cholinesterase activity in nerve endings in the guinea pig heart. *J. Electron Microsc.*, 16; 313, 1967.
3. Karnovsky, M. J.: The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. *J. Cell Biol.*, 23; 217, 1964.
4. Kawamura, T., Ishibashi, Y. and Mori, S.: Haarscheibe, with special reference to its Merkel cells and perineurale Pigmenthülle (periaxonale Pigmenthülle) *Jap. J. Derm (Series A)*., 81; 814, 1971.
5. Koelle, G. B.: Handbuch der Experimentellen Pharmakologie (Koelle, G. B., editor), Berlin, Springer-Verlag, 15; 191, 1963.
6. Kurozumi, K. and Suzuki, H.: Fine structure of the dendritic cells and Merkel cells in the epidermis of various mammals. *Jap. J. Derm (Series A)*., 81; 793, 1971.
7. Pinkus, F.: Über einen bisher unbekannten Nebenapparat am Haarsystem des Menschen: Haarscheiben. *Derm. Z.*, 9; 465, 1902.
8. Pinkus, F.: Die Haarscheiben, Jadassohn's Handbuch der Haut- u. Geschlechtskrht., Berlin, Springer-Verlag, 1; 351, 1927.
9. Robinson, P. M.: A cholinergic component in the innervation of the longitudinal smooth muscle of the guinea pig vas deferens. The fine structural localization of acetylcholinesterase. *J. Cell Biol.*, 41; 642, 1969.
10. Robinson, P. M. and Bell C.: The localization of acetylcholinesterase at the autonomic neuromuscular junction. *J. Cell Biol.*, 33; 93, 1967.
11. Smith, K. R.: The ultrastructure of the human Haarscheibe and Merkel cell. *J. Invest. Derm.*, 54; 150, 1970.