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Immunocytochemical evidence of a met-enkephalin-like substance in the dense-core granules of mouse Merkel cells

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Summary. The electron-microscopic immunogold method was applied to Merkel cells of adult mice to demonstrate the subcellular localization of met-enkephalin-like immunoreactivity. Post-embedding incubation with met-enkephalin antisera showed that the gold particles were associated with the dense-core granules of the Merkel cells. The majority, but not all, of the dense-core granules were strongly labelled. Osmication caused a significant reduction in the number of gold particles on these granules. The nerve terminal associated with the Merkel cell did not show met-enkephalin-like immunoreactivity. To the best of our knowledge, this is the first report of the ultrastructural localization of a positive met-enkephalin immunoreactivity in the dense-core granules of Merkel cells in mice.

Key words: Immunocytochemistry – Met-enkephalin – Merkel cells – Dense-core granules – Mouse (ICR)

"Touch dome" or Merkel cell-neurite complexes are specialized sensory mechanoreceptors located in the epidermo-dermal junction of the skin (Merkel 1875). Functionally, they belong to the slowly adapting type I or SA I (Iggo 1966) mechanoreceptors. Ultrastructurally, they contain a large number of dense-core granules which are concentrated in the region subjacent to the afferent nerve terminal supplying the cell. The function of the granules is not known, nor is their content understood. Recently, it has been reported that these granules contain met-enkephalin (Hartschuh et al. 1979; Gottschaldt and Vahle-Hinz 1982), as well as vasoactive intestinal polypeptide (VIP) (Hartschuh et al. 1983, 1984). All these reports are, however, based on light-microscopic immunocytochemical studies. Although it has been suggested that the Merkel cell dense-core granules are the most likely candidate to contain these substances, the question can only be solved at the electron-microscopic level, and with this in mind, the present study investigates whether the polypeptide, met-enkephalin, is present in these granules by using the electron-microscopic immunogold labelling method.

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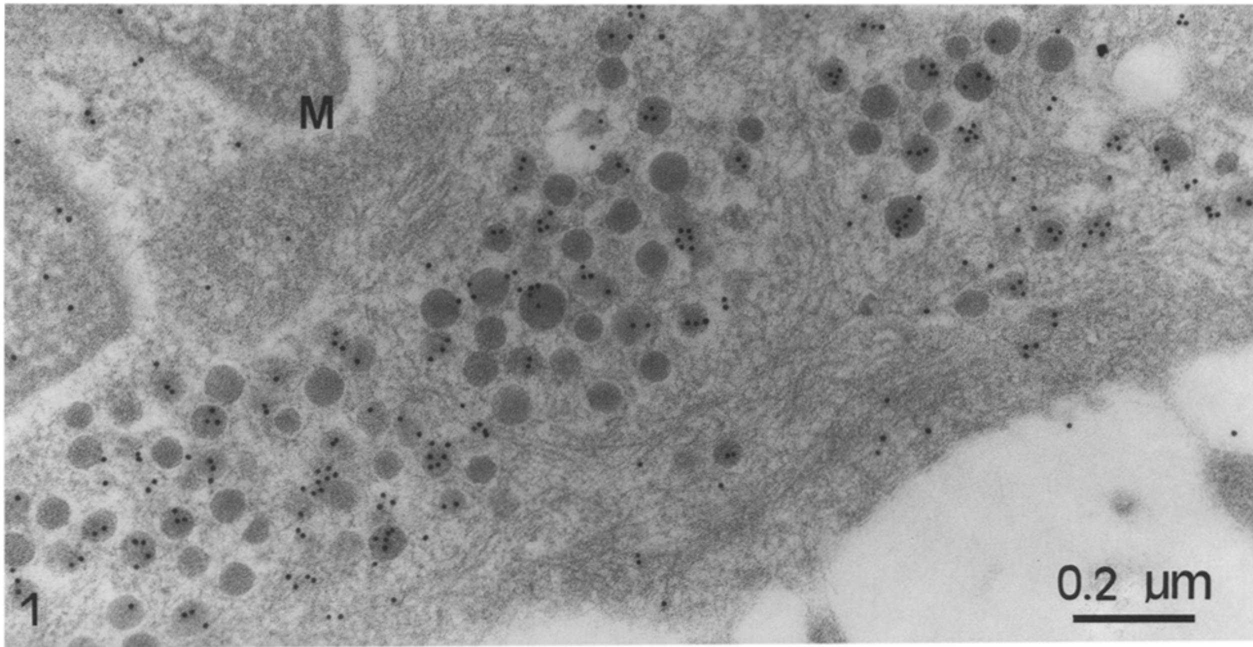
Materials and methods

Fresh samples of skin containing "touch dome" complexes, were removed from the hindlimb of anaesthetized (sodium pentobarbitone, 70 mg/kg) adult ICR mice, and immediately immersed in an ice-cold solution of 1% paraformaldehyde plus 2% glutaraldehyde for electron-microscopic studies. Three ICR mice (1 male, 2 female) were used, and 2 "touch domes" were taken from each mouse. After 2 h fixation, half of the specimens were rinsed in buffer and postfixed in 1% sodium tetroxide (osmicated) in Millonig's buffer, pH 7.2, for a further 1 h at 4° C. The remaining 3 specimens were rinsed in buffer (non-osmicated) and, after conventional processing, were finally embedded in Spurr's medium (Polysciences).

Thick sections (1.0–2.0 μm) were cut using a glass knife, and were stained with toluidine blue to locate Merkel cells. Then silver to silver-grey sections were cut using a diamond knife and collected on nickel grids.

Electron-microscopic immunocytochemistry. Methionine enkephalin-like immunoreactivity was detected by using a modified on-grid immunogold staining method (De Mey et al. 1981; Gu et al. 1981), which involved the use of a second layer of goat anti-rabbit antiserum conjugated directly to colloidal gold (Romano et al. 1974). The procedure was as follows: (1) Grid-mounted sections of osmicated and non-osmicated tissue were dried overnight, then etched in saturated aqueous sodium metaperiodate for 30 min. Before etching, grids were rinsed with drops of double distilled water for 2 min. (2) Sections were incubated in 5% normal goat serum (NGS) in TRIS-bovine serum albumin (TBS) for 60 min at room temperature. (3) Excess NGS was drained off, then the sections were incubated in primary antiserum (rabbit anti-methionine enkephalin, Amersham) diluted in TBS (1:1000) for 20–24 h at 4° C. (4) After washing briefly with TBS and a further changes, each of 15 min; the grids were transferred to the gold-labelled (10 nm) secondary antiserum (dilution 1:8) for 60 min at room temperature. (5) Sections were washed thoroughly in TBS and finally rinsed in doubled distilled water.

Positive controls, subjected to the same procedures, had sectioned cat adrenal medullary tissue as a replacement for the sectioned touch dome complexes (Varndell et al. 1982). Negative con-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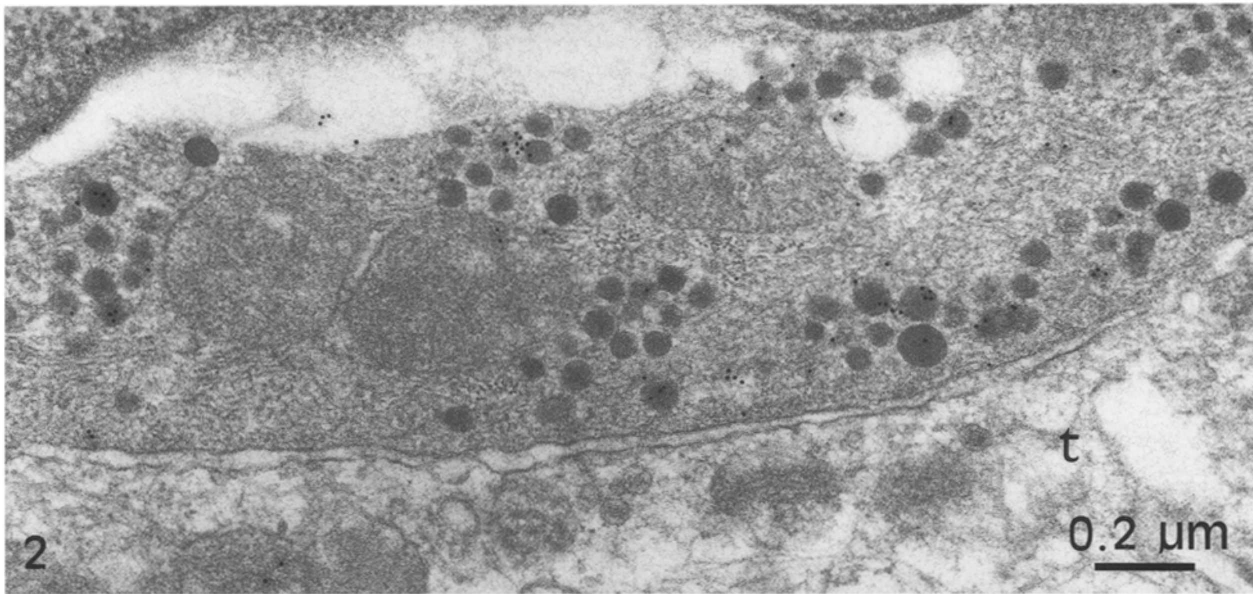
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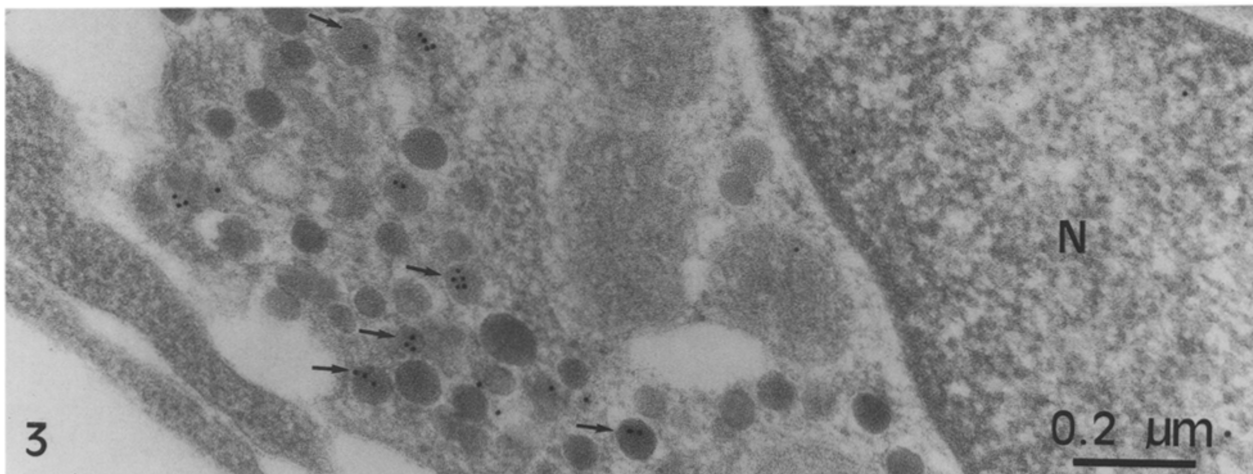
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trols were obtained by omitting the use of the primary antisera, or by preabsorption of the primary antiserum (1:100) with electrophoretically purified met-enkephalin (Peninsula, Calif.).

Finally, all sections were double-stained in 2% uranyl acetate and lead citrate, and examined under a Jeol 100 CX 2 electron microscope operating at 80 kV.

Results and discussion

The met-enkephalin antiserum produced immunocytochemical reactivity in the mouse Merkel cell dense-core granules. The immunogold particles were concentrated on the sectioned surface of these granules (Figs. 1–3). Although not all the dense-core granules were labelled, the majority were, especially those located in the basal region of the cells. Both osmicated and non-osmicated tissues showed positive results, but the number of gold particles on dense-core granules in the former, was greatly reduced (Fig. 2). Some sections were buffered with Tween 20 and NaCl. This reduced the non-specific background staining but did not abolish the labelling of dense-core granules (Fig. 3). Nerve terminals supplying Merkel cell did not show met-enkephalin immunoreactivity (Fig. 2).

No gold particles were seen in the negative controls, but were evenly distributed in the secretory granules of the cat adrenal medullary tissue used as a positive control for this study.

Earlier immunocytochemical studies at the light-microscopic level had revealed the presence of met-enkephalin-like immunoreactive substance in the Merkel cells of rat touch dome complexes (Hartschuh et al. 1979), and the present ultrastructural study shows that, in the mouse, this met-enkephalin-like immunoreactivity is located in the dense-core granules of these cells. These results represent the first demonstration of met-enkephalin-like immunoreactivity in the mouse Merkel cell dense-core granules, and support the previous light-microscopic observations of Hartschuh et al. (1979, 1983) on rat Merkel cells. Surprisingly, a pilot study by the present authors on 6 touch dome complexes removed from 3 rats, failed to demonstrate a positive met-enkephalin immunoreactivity in Merkel cell dense-core granules using similar staining conditions as used in the present mouse study (unpublished results). This, however, does not exclude the possibility of the presence of met-enkephalin in rat Merkel cell granules. The absence of a positive result may have been due to a low concentration of met-enkephalin in these cells, and further trials with different antibody dilutions must be carried out to verify whether there is any species specific difference in the chemical content of these granules in various rodent species.

Since, in post-embedding immunogold staining, the tissue is first embedded, sectioned, and then immunolabelled, only the antigen exposed on the cut surface of the section is available for immunolabelling, and material located elsewhere in the granules may not react. This may explain the negative staining of some granules in obviously positive cells. The greater frequency of gold labelling in the basal region of the cells may simply be due to the accumulation of large numbers of dense-core granules in this region. Osmication partially cross-links the amino acid sequence of met-enkephalin, thus causing a marked reduction in the number of gold particles on the dense-cored granules of the cell. Non-osmicated tissue is, therefore, better suited for the demonstration of met-enkephalin immunoreactivity. Also, since the addition of 0.05% Tween 20 plus 0.5 M NaCl in the buffer further reduces non-specific background staining without abolishing the immunoreactivity of the dense-cored granules, it further indicates the high specificity of the immunoreactivity in the latter.

The functional role of met-enkephalin-positive dense-core granules of mouse Merkel cell is not known, but a modulatory role in Merkel cell function is a possibility, and needs to be investigated further.

Figs. 1–3. Immuno-electron micrographs of Merkel cells showing different degrees of met-enkephalin immunoreactivity with different processing conditions. Immunogold staining procedure with 10-nm gold particles, counterstained with uranyl acetate and lead citrate

Fig. 1. Non-osmicated mouse Merkel cell (*M*) showing part of the basal region of the cell. The dense-cored granules are heavily labelled with gold particles. ×78900

Fig. 2. Osmicated Merkel cell-neurite complex shows a significant reduction of labelled gold particles on the dense-cored granules. The associated nerve terminal (*t*) is without any met-enkephalin immunoreactivity. ×64000

Fig. 3. 0.05% Tween 20 plus 0.5 M NaCl in the buffer solution has reduced background staining, but positive labelling (*arrows*) on the dense-cored granules is unaffected. *N* Nucleus. ×79500

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