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Immunocytochemical analysis of calcitonin gene-related peptide and vasoactive intestinal polypeptide in Merkel cells and cutaneous free nerve endings of cats

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Summary. Calcitonin gene-related peptide (CGRP)- and vasoactive intestinal polypeptide (VIP)-immunoreactivity were observed to coexist in Merkel cells of cats. No differences in peptide content were found between Merkel cells located in epithelia of the hard palate, in hairy and glabrous skin of the upper lip, and in vibrissae follicles. CGRP- and VIP-immunoreactive nerve fibres were also found near CGRP/VIP-immunoreactive Merkel cells. In the vibrissae follicles some CGRP- and VIP-immunoreactive nerve terminals end abutting on the glassy membrane. Other CGRP-immunoreactive nerve fibres penetrate the epithelium of the skin and end within it. Electron microscopy of vibrissae follicles revealed that Merkel cell neurites are not immunostained and that immunostained nerve fibres form unmyelinated bundles before ending freely. Thus, CGRP- and VIP-immunoreactive nerve fibres in cat skin do not end as Merkel cell neurites but as different kinds of free nerve endings.

Key words: Calcitonin gene-related peptide (CGRP) – Vasoactive intestinal polypeptide (VIP) – Merkel cells – Free nerve endings – Cat

Merkel cells are specialized cells located in the epidermal basal layer of vertebrates (Merkel 1875). They are present in both glabrous and hairy mammalian skin in a variety of anatomical settings. In glabrous skin, Merkel cells are contained preferentially in the bottom of epithelial rete pegs (Hashimoto 1972; Munger 1975). In hairy skin they have been found in clusters under elevated specializations of the skin named "touch domes" (Iggo and Muir 1969) and often they are in close relation to the exits of hairs (Smith 1967). They are also present in the external root sheath of vibrissae follicles (Patrizi and Munger 1966).

Ultrastructural studies have revealed that Merkel cells contain dense-core vesicles concentrated in the cytoplasm adjoining a closely associated nerve ending (Cauna 1962). Electrophysiological studies indicate that the Merkel cell-neurite complex may act as a slowly adapting type I mechanoreceptor (Iggo and Muir 1969). Accordingly, it has been proposed that neurochemicals released from Merkel cells may function as neurotransmitters, neuromodulators or trophic factors over the neurite (Horch et al. 1974; Hartschuh et al. 1979; Hartschuh and Weihe 1980; Gottschaldt and Vahle-Hinz 1981, 1982). In addition, some authors consider Merkel cells may contain endocrine or paracrine substances capable of long distance actions (Fujita 1977; Hartschuh and Grube 1979; Gu et al. 1981; Hartschuh et al. 1984).

However, the details of Merkel cell actions over the mechanoreceptive neurite and/or adjacent cells and tissues are unclear. Thus a first step for understanding Merkel cell function is the identification of the "hormones" and/or "neurotransmitters" they use.

Vasoactive intestinal polypeptide (VIP), met-enkephalin and serotonin have been previously found in Merkel cells, although many species differences have been reported (Hartschuh et al. 1979, 1983, 1984; Zaccone 1986). ATP has also been proposed as a Merkel cell neurotransmitter (Crowe and Whitear 1978). In this study, we describe immunoreactivity to calcitonin gene-related peptide (CGRP) in cat Merkel cells, and compare it to VIP immunoreactivity in single and double immunolabelling experiments. Different cutaneous areas have been examined in order to establish if Merkel cells in different locations share the same peptidergic content. In addition, numerous CGRP- and VIP-immunoreactive fibres were found near Merkel cells. Further studies were carried out to establish how these CGRP/VIP-immunoreactive nerve fibres finish in the skin epithelia and in the vibrissae follicles of the cat.

Materials and methods

Seven adult cats were used. All were anesthetised by intraperitoneal injection of sodium pentobarbital (35 mg/kg body weight) and perfused via the aorta with 300 ml of vascular rinse (0.8% NaCl, 0.025% KCl and 0.05% $NaHCO_3$ in 0.01 M phosphate buffer, pH 7.4; saturated with 95% O_2 and CO_2) followed by 1.5 litres of fixative.

Fixation

For light-microscopic studies two animals were perfused with a fresh solution of 0.4% parabenzoquinone in 0.01 M phosphate buffered saline (PBS) pH 7.2 at room temperature. The areas containing the vibrissae, upper lip and the hard palate were removed and immersed in the same solu-

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tion for 60 min. Samples were immersed in 15% sucrose in PBS at 4° C overnight. Tissue blocks were frozen, cryostat sections cut (60 μm) and collected free floating. An additional cat was perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The vibrissae-containing area was removed and placed in 15% sucrose in PBS at 4° C overnight. Cryostat sections (10 μm) were obtained and collected on glass slides for double immunolabelling purposes.

For electron microscopy four animals were perfused with a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in PB. The vibrissae and hard palate were removed and placed in 4% paraformaldehyde in PB for 3 h. Small blocks were cut and after a 30 min incubation with 0.01% sodium cyanoborohydride (Merck) in PB, were cryoprotected by immersion in 30% sucrose in PB at 4° C overnight. Tissue blocks were rapidly frozen in liquid nitrogen, cryostat sections were obtained (40 μm) and collected free floating.

Immunocytochemical procedures

Sections from hard palate, hairy and glabrous skin of the upper lip, oral mucosa, and vibrissae follicles were immunostained for light and electron microscopy with antisera against CGRP and VIP.

Light microscopy. The sections were stained with the PAP method. Sections were soaked in PBS containing 0.3% H_2O_2 to block endogenous peroxidase, washed in PBS and incubated with non-immune goat serum 1:10 for 4 h. They were then placed in primary antiserum and incubated for three days at 4° C. Two rabbit anti-CGRP sera diluted 1:1000 (ref. n°: 1072 and 1105) and one rabbit anti-VIP serum diluted 1:2000 (ref. n°: 652) in PBS were used. The sections were then washed overnight in PBS, incubated for 5–6 h with goat anti-rabbit IgG serum (Miles Lab.) diluted 1:50 in PBS, washed again overnight in PBS, and finally incubated for 5–6 h with rabbit PAP complex (Miles Lab.) diluted 1:100 in PBS and washed overnight again. The peroxidase marker was revealed with a solution of 0.06% diaminobenzidine tetrahydrochloride (Sigma) and 0.03% H_2O_2 in PBS. The sections were mounted on polylysine subbed slides, dehydrated through graded concentrations of ethanols, cleared in xylene and covered with Dammar resin (Merck).

Electron microscopy. Free floating sections were rinsed in PBS, incubated in non-immune goat serum 1:10 in PBS, and placed in rabbit anti-CGRP serum diluted 1:1000 or rabbit anti-VIP serum diluted 1:2000 in PBS for 12 hours at 4° C. The same developing reagents used for LM were used for EM. Briefly, sections were incubated in the antibodies for 2–3 hours and washed between incubations with several rinses of PBS for 30 min. The same peroxidase developing reagents were used but the concentration of H_2O_2 was lowered to 0.003%. Sections were washed in PBS and then immersed in 1% OsO_4 in PB for 45 min at room temperature. The sections were dehydrated through ethanol, contrasted with 1% uranyl acetate in 70% ethanol and flat-embedded in Durcupan. Areas containing immunoreactive Merkel cells and nerve fibres were selected by light microscopy, trimmed, and reembedded in Durcupan. Ultrathin sections were cut with an OMU3 Reichert Jung ultramicrotome and collected on 100 mesh copper grids. The ultrathin sections were then stained with lead citrate and examined with a Jeol 100B or a Philips 300 electron microscope. Adjacent semithin sections (1–2 μm) were counterstained with toluidine/borax and analysed by light microscopy. The immunostaining was heaviest in ultrathin sections cut near the surface of the 40 μm cryostat section. However, at this level the ultrastructural preservation is poor. Therefore, Merkel cell ultrastructure was studied in deeper sections while immunoreactive Merkel cells were only observed in superficial sections.

Double immunostaining

Cryostat sections (10 μm) mounted on glass slides were immunostained with rabbit anti-CGRP serum (1:200) followed by goat anti-rabbit IgG serum conjugated to FITC (1:50). Immunopositive sections were photographed by use of a Leitz epi-fluorescence microscope fitted with an I2 dichroic filter block. Antibody complexes were eluted with Glycine-hydrochloric acid buffer (pH 2.2) (Nakane et al. 1968). A PAP technique against VIP was carried out on the same sections after removal of the antisera from the first immunoreaction. No cross-reactivity between the developing reagents of the second immunostaining and the antibodies of the first was observed in control sections.

Specifity of the immunostaining

All antisera were raised in rabbits. Their characteristics and specificity have been reported previously (i.e.: Roberts et al. 1980; Rodrigo et al. 1985; Cadieux et al. 1986). The specificity of the immunostaining was verified by preabsorption of the antisera overnight at 4° C with a range of peptide concentrations (10–0.0001 nmols per ml of diluted antiserum). Tests were carried out in both aldehyde- and paraben-zoquinone-fixed sections. Immunostaining with CGRP antiserum could be prevented by absorption with 0.01 nmol of synthetic rat CGRP (Peninsula) per ml of diluted serum and staining with VIP antiserum was blocked by preabsorption with 10 nmol of VIP per ml of diluted antiserum. No cross reactivity was observed between CGRP and VIP antiserum in paraformaldehyde sections. 10 nmol/ml CGRP or VIP did not abolish the staining with VIP or CGRP antisera respectively. Specifity of the method was established by incubating some sections with PBS or non-immune rabbit serum instead of primary antibodies. Neither of these gave positive staining.

Results

CGRP-immunoreactivity

CGRP-immunoreactivity (CGRP-IR) was found in Merkel cells present in all the epithelia under study.

In areas of glabrous skin such as hard palate and lip oral mucosa, CGRP-IR Merkel cells were common at the bottom of epithelial rete pegs. The greatest density of immunoreactive Merkel cells was found under the palatal ridges. In this location Merkel cells formed two broad laminae, one anterior and the other posterior to the palatal ridge, each containing numerous Merkel cells (Fig. 1 A). The palatal epithelial rete pegs were surrounded by an extensive plexus of CGRP-IR peripapillar nerve fibres. Most of these

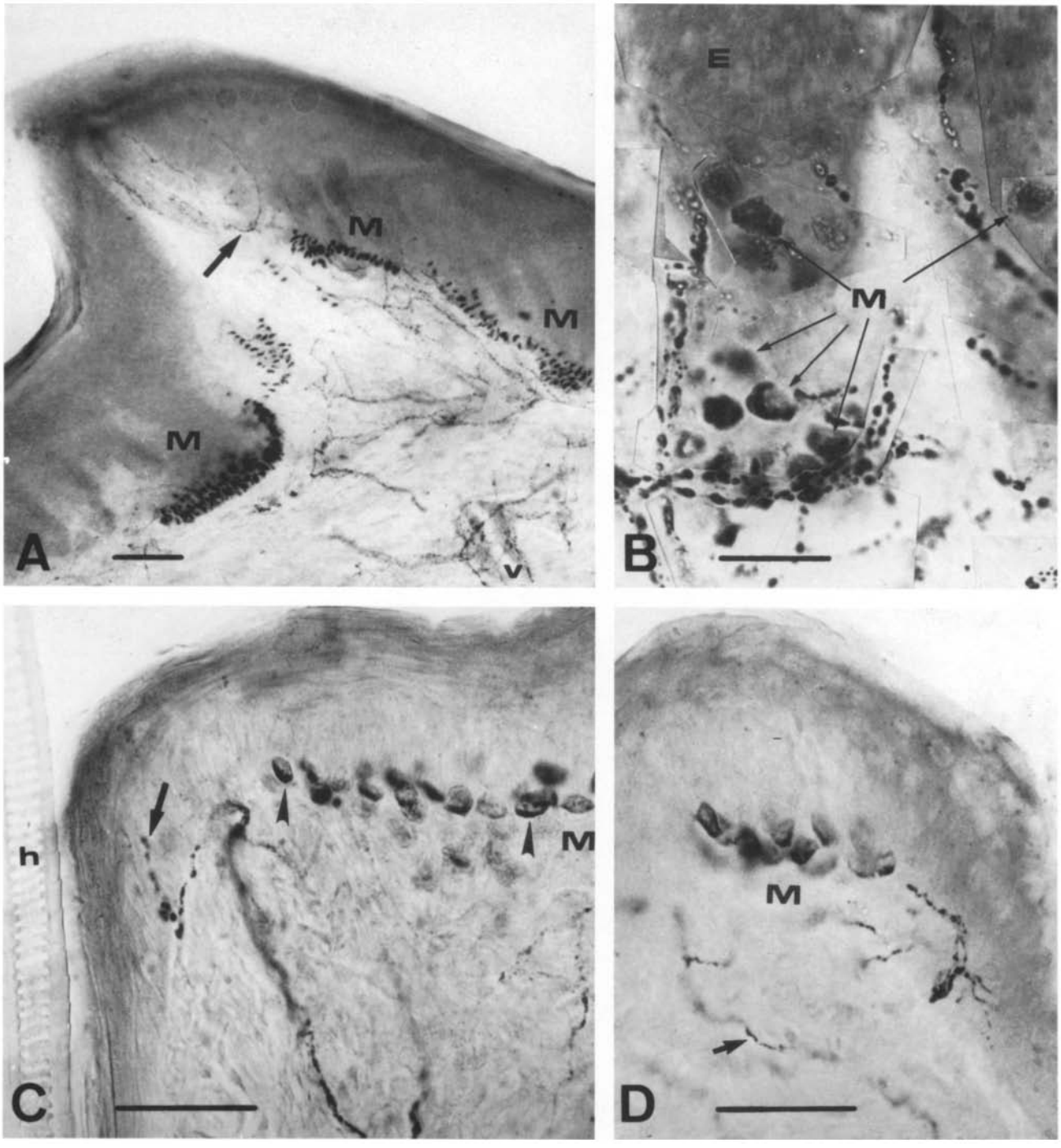


Fig. 1. CGRP-IR Merkel cells (*M*) in different epithelia of cats. **A** CGRP-IR Merkel cells and nerve fibres under a palatal ridge. CGRP-IR nerve fibres form a dense subepithelial plexus, some nerve fibres are related to blood vessels (*v*) while others penetrate the epithelium mainly at the tips of the palatal ridge (*arrow*). **B** Photomontage of various focal planes of a 60 µm section. CGRP-IR Merkel cells can be seen in the bottom of a palatal epithelial rete peg surrounded by a peripapillary plexus of CGRP-IR nerve fibres. **C, D** CGRP-IR Merkel cells located in touch domes. In **C**, the touch dome is near a hair exit (*h*, hair shaft). CGRP-IR nerve fibres are present in the dermis and some of them penetrate the epithelium ending freely in it (*arrow*). Most of the Merkel cells (*M*) show the greatest immunoreactivity in their basal cytoplasm (*arrowheads*). In **D**, several CGRP-IR nerve fibres are related to dermal core capillaries (*arrow*). Scale bars: A 100 µm; B 25 µm; C–D 50 µm

fibres lie in focal planes of the 60 µm sections outside the skin epithelia. Photomontage reconstructions were needed in order to appreciate the full extent of the CGRP-immunoreactive peripapillar fibre network (Fig. 1 B).

In touch domes of hairy skin, CGRP-IR Merkel cell clusters were located in the basal layer of the epithelium (Fig. 1 C, D). Usually they were associated with the exits of some hair shafts (Fig. 1 C). Some CGRP-IR nerve fibres penetrate the epithelium near CGRP-IR Merkel cell clusters and end freely within it (Fig. 1 C). Other fibres were present surrounding the capillaries of the touch dome dermal cores (Fig. 1 D). Similar intraepithelial and perivascular CGRP-

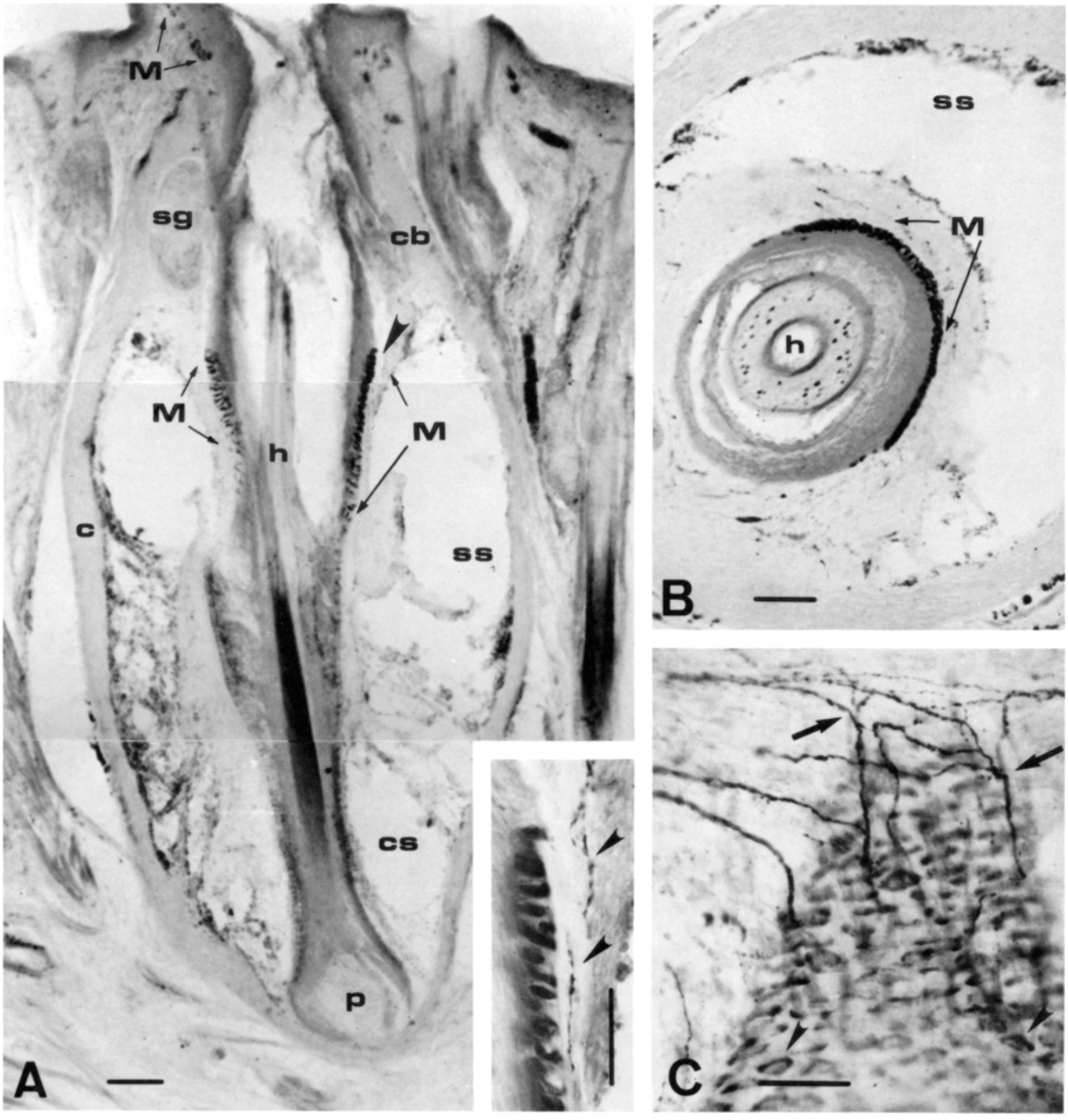


Fig. 2. CGRP-IR Merkel cells (*M*) and nerve fibres in vibrissae follicles. **A** Longitudinal section of a vibrissae follicle. CGRP-IR Merkel cells occur in the superior enlargement adjoining the superior sinus (*ss*) and in the exit of the hair shaft (h); *cb* indicates the conical body; *sg* sebaceous gland; *p* the dermal papillae; *cs* the cavernous sinus and *c* the conjuntive capsule. The *inset* shows the area labelled with an *arrowhead*. Some CGRP-IR nerve fibres (*arrowheads*) are present in the conjuntive tissue of the superior enlargement below CGRP-IR Merkel cells located in the basal layer of the external root sheath. **B** Transverse section of a vibrissae follicle with CGRP-IR Merkel cells (*M*) surrounding the hair shaft (*h*). **C** CGRP-IR nerve fibres in a tangential section through the boundary between the superior enlargement (lower portion of the micrograph) and the conical body (upper portion). Merkel cells show the greatest CGRP-immunoreactivity in the cytoplasm side facing the hair exit (*arrowheads*). Numerous CGRP-immunoreactive nerve fibres run in the conical body and enter the superior enlargement after changing their course 90° (*arrows*). Scale bars: A 100 μm, *inset*, 20 μm; B 100 μm; C 50 μm

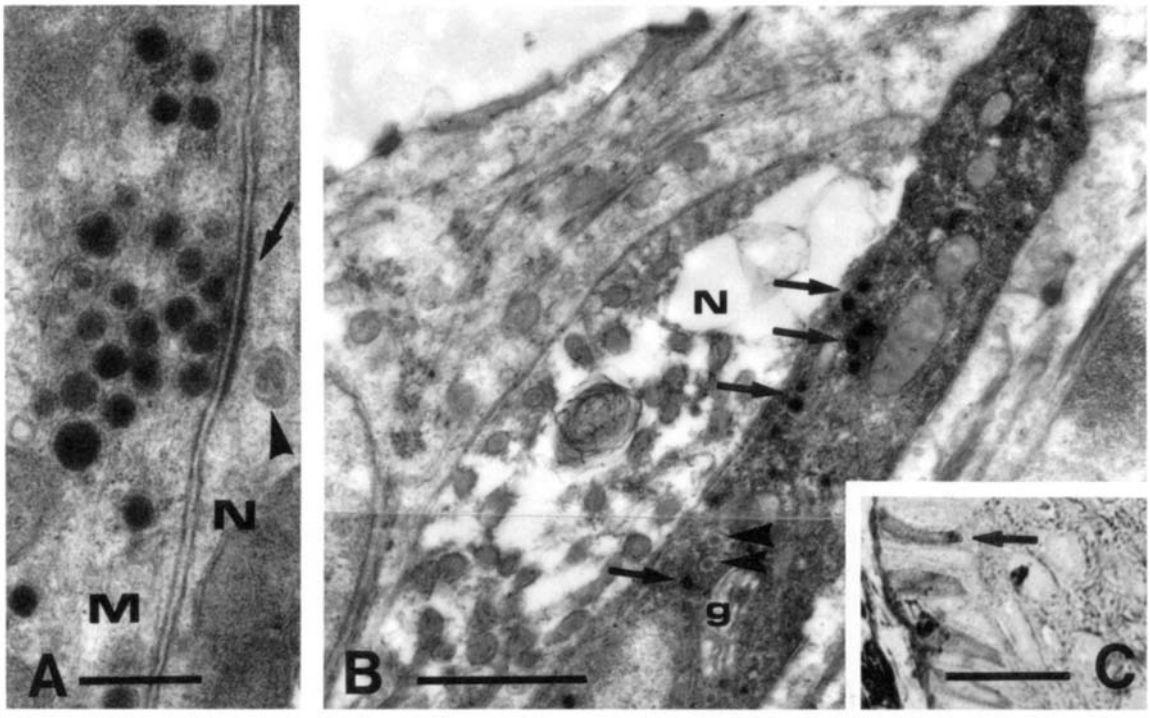


Fig. 3. Merkel cell ultrastructure in vibrissae follicles. **A** A synaptic-like structure (*arrow*) between a Merkel cell and a neurite. An accumulation of dense-cored vesicles can be seen on the Merkel cell side (*M*) while less electron-dense vesicles (*arrowhead*) are present in the neurite side (*N*). **B** CGRP-IR Merkel cell and its associated neurite (*N*). The Merkel cell cytoplasm appears dark due to the DAB precipitate. It contains different types of electron-dense vesicles (*arrows* and *arrowheads*) and a non-immunostained Golgi complex (*g*). **C** Semithin section of the vibrissae follicle showing the position (*arrow*) of the CGRP-IR Merkel cell shown in B. Scale bars: A 0.25 μm; B 1 μm; C 50 μm

IR nerve fibres were found randomly in areas where no Merkel cells were observed.

A large number of CGRP-IR Merkel cells were present piled up in the basal layer of the external root sheath of vibrissae follicles (Fig. 2A, B). They were located in the superior enlargement of the follicle throughout all the extension of the superior sinus and disappeared at the level of the cavernous inferior sinus. Some CGRP-IR Merkel cells were also observed in the epithelium surrounding the whisker shaft exit (Fig. 2A).

Although intensely immunoreactive Merkel cells appeared uniformly stained, different zones of immunostaining were observed inside many lightly immunoreactive Merkel cells. Epidermal Merkel cells showed the strongest immunoreactivity preferently in the cytoplasm nearest to the basal membrane of the epithelia (Fig. 1C). Vibrissae follicle Merkel cells showed the greatest immunoreactivity usually on the cytoplasm side facing the exit of the vibrissae hair shaft (Fig. 2C).

All Merkel cells found in semithin sections cut from the first microns of 40 μm immunostained sections were immunostained in all three zones studied (hard palate epithelial rete pegs, upper lip hairy skin touch domes and vibrissae follicles).

Merkel cell neurites always appeared unstained in material processed for electron microscopic immunocytochemistry (Fig. 3B). Occasionally some synaptic-like structures between Merkel cells and neurites were observed in ultrathin sections obtained from deeper levels of the 40 μm cryostat sections. These levels had the best ultrastructural preservation but showed almost no immunostaining (Fig. 3A).

Numerous CGRP-IR nerve fibres running longitudinally in the superior enlargement were observed with light microscopy (Fig. 2A, inset). These nerve fibres enter the superior enlargement from the conical body where they follow a circular course (Fig. 2C). Additional CGRP-IR nerve fibres were observed in relation to the sebaceous gland and the cavernous inferior sinus of the vibrissae follicle. The latter were seen entering the follicle inside thick nerve bundles that traverse the capsule near the dermal papilla of the follicle, while the former enter the vibrissae follicle through the conical body. Sparse CGRP-IR nerve fibres were seen in the dermal papillae of the vibrissae follicles.

In semithin sections, isolated CGRP-IR fibres were seen ending in the glassy membrane but never traversing it (Fig. 4). In addition, when examined ultrastructurally, the thick CGRP-IR nerve fibres observed with light microscopy appear as bundles of unmyelinated fibres with a high proportion of immunoreactive fibres (Fig. 5). These bundles were devoid of any endoneural or perineural envelope and were surrounded by a basal membrane in direct contact with the collagen fibrils of the connective tissue. In some places the Schwann cell investment was incomplete leaving the axolemma of some nerve fibres abutting against the basal membrane.

VIP-immunoreactivity

VIP-IR Merkel cells were found in the same areas and share the same morphological characteristics as those described for CGRP (Fig. 6A–D).

VIP-IR nerve fibres were present near immunoreactive Merkel cells, over superficial skin capillaries, in touch dome

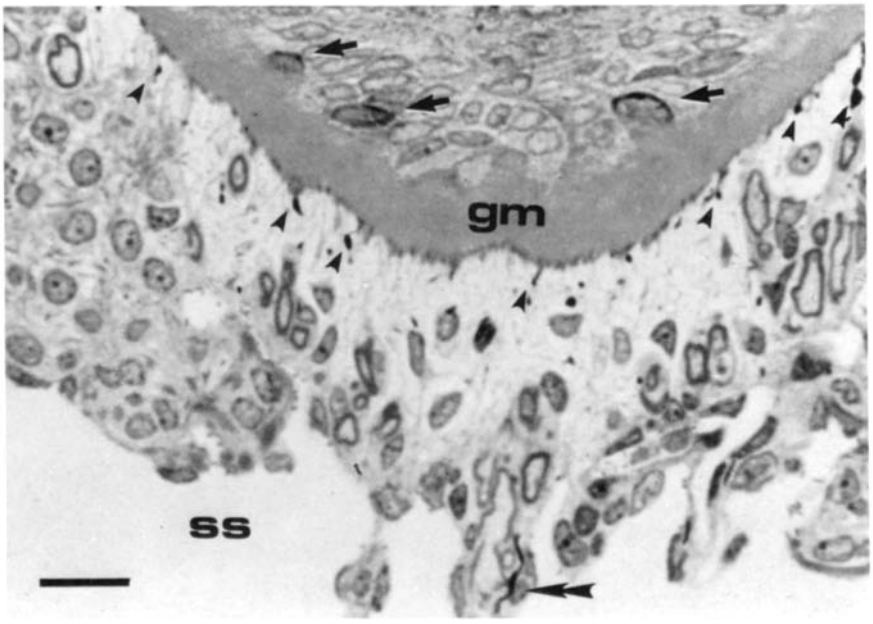


Fig. 4. Semithin section of a vibrissae follicle immunostained with CGRP antisera and counterstained with toluidine blue. CGRP-IR Merkel cells (*arrows*) located in the external root sheath appear darker (brown in the original section due to the DAB precipitate) than surrounding keratynocytes (blue in the original section). In the conjunctive tissue of the superior enlargement (*ss*, superior sinus) some CGRP-IR nerve fibres (*double arrow*) run near unstained thick myelinated fibres. CGRP-IR nerve fibres (*small arrowheads*) end attached to the glassy membrane (*gm*), but they never traverse it. Scale bar: 50 μm

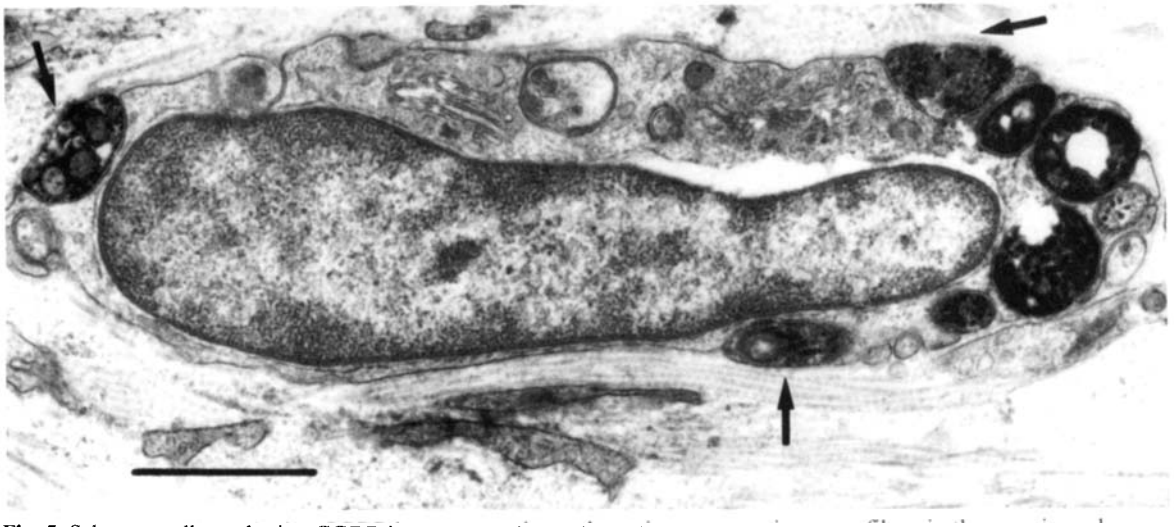


Fig. 5. Schwann cell enveloping CGRP-immunoreactive and non-immunoreactive nerve fibres in the superior enlargement of the vibrissae follicle. Some CGRP-immunoreactive nerve fibres make direct contact with the basal membrane of the bundle (*arrows*). Scale bar: 1 μm

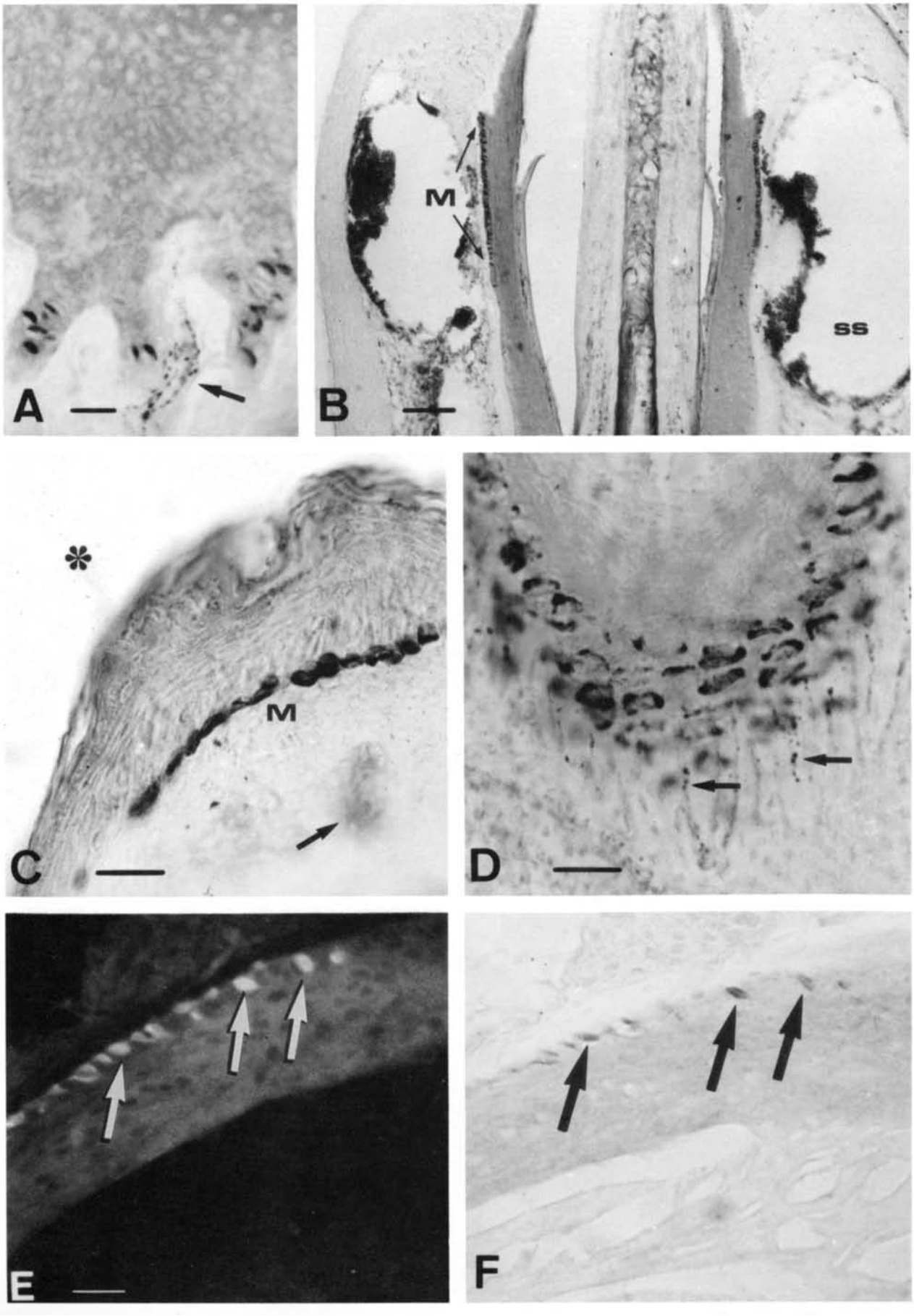
dermal cores and around epithelial rete pegs but were scarce in number compared with CGRP-IR fibres (compare Fig. 6A with Fig. 1B and Fig. 6C with Fig. 1C, D). However a dense network of VIP-IR perivascular fibres was located over the blood vessels of the deep vascular plexus.

VIP-IR nerve fibres were found in the superior enlargement of the vibrissae follicle but were less abundant and thinner than CGRP-IR fibres (compare Fig. 6D with Fig. 2C). Electron microscopy revealed that they are localized in bundles of unmyelinated fibres which are similar to the CGRP-IR nerve fibre bundles except that, within each bundle, only a few fibres are immunostained. VIP-IR nerve fibres were also present in the adjoining sebaceous gland and the trabeculeae of the inferior sinus.

Coexistence of VIP and CGRP in Merkel cells

Double immunostaining was carried out on sections of vibrissae follicles where Merkel cells are numerous and easy to find. All CGRP-IR Merkel cells (Fig. 6E) were also posi-

Fig. 6. VIP-IR Merkel cells (*M*) and neighbouring VIP-IR nerve fibres in various cutaneous areas. **A** VIP-IR Merkel cells in the bottom of palatal epithelial rete pegs. Some VIP-IR nerve fibres enter a dermal papillae (*arrow*). **B** VIP-IR Merkel cells in the vibrissae follicle superior enlargement (*ss*, superior sinus). **C** VIP-IR Merkel cells in relation to a hair shaft exit (*asterisk*). The subepidermal blood vessels (*arrow*) are devoid of VIP-IR perivascular nerve fibres. **D** High magnification micrograph of Merkel cells and nerve fibres (*arrows*) immunoreactive to VIP in the superior enlargement. **E, F** Double immunostained section with immunofluorescence against CGRP (**E**) and PAP against VIP (**F**). Merkel cells stained for both CGRP and VIP are indicated by *arrows*. Scale bars: B 100 μm; A, C, D 50 μm; E, F 20 μm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tive for VIP (Fig. 6F), indicating that VIP and CGRP are co-localized in the same Merkel cells.

Discussion

Merkel cells

We have shown that cat Merkel cells contain CGRP in addition to VIP and that both substances are co-localized in the same cells. The VIP results are in agreement with previous reports describing VIP-immunoreactivity in cat Merkel cells (Hartschuh et al. 1983, 1984). CGRP has not previously been described in Merkel cells.

CGRP- and VIP-immunoreactivity was observed in Merkel cells found in hard palate epithelial rete pegs, upper lip hairy skin touch domes and vibrissae follicles. Thus in cat, VIP and CGRP appear to be found in all Merkel cells. However in rat Hartschuh and colleagues (1979) have shown that Merkel cells are not VIP-immunoreactive. Our unpublished data confirms this observation and shows also that rat Merkel cells are not immunoreactive to CGRP. This species difference is intriguing since no morphological or physiological differences have been reported between cat and rat Merkel cells.

At light-microscopic level CGRP- and VIP-immunoreactivity appeared concentrated to one side of the Merkel cells and a similar observation has been reported previously for VIP-immunoreactivity (Hartschuh et al. 1984). The highly immunoreactive sites correspond to areas of Merkel cell cytoplasm which are associated with adjoining neurites. For example, Merkel cells showing the highest immunoreactivity in their basal cytoplasm were located in skin epithelia, where neurites lie under the Merkel cell cytoplasm near to the basal membrane of the epithelium. In contrast Merkel cells in vibrissae follicles usually showed high immunoreactivity in the cytoplasm facing the whisker exit and their neurites correspondly lie over dorsal aspects of the cells. These results suggest that, if CGRP and VIP are released from Merkel cells, they may exert actions over the associated neurites. In our material, staining was not concentrated over particular vesicles, but occurred diffusely in the cytoplasm. This is a common result with pre-embedding staining (Priestley and Cuello 1983), but unfortunately means it is not possible to determine which type of vesicle contains VIP or CGRP.

The presence of two coexisting neuropeptides inside Merkel cells accords with the fact that a variety of functions have been suggested for putative Merkel cell neurochemicals. Some authors have proposed that Merkel cells act as transducers of mechanical stimuli and that they initiate nerve potentials in their associated neurites via a chemical synapse (Horch et al. 1974). In fact, Merkel cell-neurite synaptic structures have been reported by Hartschuh and Weihe (1980). We also found synaptic-like structures in some Merkel cell-neurite complexes but they were very scarce. Gottschaldt and Vahle-Hinz (1981, 1982) deny the chemosynaptic hypothesis and propose a trophic action for the neuropeptides found in Merkel cells. Our morphological data only address the question of which neurochemicals Merkel cells may use and physiological work is needed in order to establish their roles.

Other authors relate Merkel cells to the APUD system and propose endocrine or paracrine functions for them (Fujita 1977; Hartschuh and Grube 1979; Hartschuh et al. 1984; Gu et al. 1981). Epithelial cells displaying CGRP-immunoreactivity have also been found in respiratory airways (Rodrigo et al. 1985; Cadieux et al. 1986), but their functions are unknown. CGRP has potent vasodilator effects (Brain et al. 1985) and this suggests that subepithelial vasculature may be a possible target for CGRP released from Merkel cells. Such an arrangement has been proposed previously for VIP (Hartschuh et al. 1984).

CGRP- and VIP-immunoreactive nerve fibres and terminals

The skin of the cat was found to be extensively innervated by VIP- and CGRP-immunoreactive nerve fibres and this is in agreement with previous studies (Hartschuh et al. 1984; Rodrigo et al. 1985; Gibbins et al. 1985; Landis and Fredieu 1986; Alvarez et al. 1988). The focus of this study was the immunoreactive fibres present in the subepithelial plexus in the vicinity of Merkel cells. In this location CGRP-immunoreactive fibres were more abundant than those immunoreactive to VIP antiserum.

Our results suggest that these nerve fibres do not actually contact Merkel cells. Thus, most of the CGRP- and VIP-immunoreactive fibres seen with light microscopy over Merkel cell clusters lie in focal planes outside the epithelium. In addition, the myelinated fibres which give rise to Merkel cell neurites (Iggo and Muir 1969), always appeared unstained in ultrathin and semithin sections of vibrissae follicles. Finally, Merkel cell neurites did not appear immunostained with CGRP and VIP antisera at the ultrastructural level.

Some unmyelinated CGRP- and VIP-immunoreactive fibres end freely in vibrissae follicles. Their ultrastructural morphology resembles the fibre terminals shown by Munger and Halata (1983) in the monkey vibrissae follicle (compare our Fig. 4D with their Figs. 5B, C and D) and fulfill all the criteria proposed by these authors for free nerve endings. The fibres do not enter the epithelial layers of the external root sheath but end abutting the glassy membrane in a pattern similar to the penicillate endings described by Cauna (1973) in the basal membrane of human hairy skin epithelium.

The morphology and distribution of CGRP- and VIP-immunoreactive nerve fibres which end freely in the skin suggests that they are sensory cutaneous afferents. This is consistent with the fact that CGRP and VIP-IR cell bodies have been shown in sensory ganglia (Ju et al. 1987). No sensory function has been described for the free nerve endings located in vibrissae follicles. Outside the vibrissae follicles many free nerve endings in cat skin are thought to be thermoreceptive or nociceptive (Hensel et al. 1974; Kruger et al. 1981). However, many low threshold mechanoreceptive afferents with unmyelinated axons occur in hairy skin of the cat (Iggo 1960). Thus although the CGRP and VIP fibres may belong to nociceptive or thermoreceptive afferents the possibility that they are mechanoreceptive should not be excluded.

In summary, we have shown that CGRP and VIP coexists in Merkel cells. Immunoreactivity is observed also in neighbouring nerve fibres which do not contact the Merkel cells but which end as various types of free nerve endings.

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