Merkel cells are not the mechanosensory transducers in the touch dome of the rat

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Summary

The identity of the mechanosensory transducing elements in the vertebrate touch receptors that contain Merkel cell-neurite complexes is unknown. The Merkel cells, however, have long been the favoured candidates. We have now selectively eliminated the Merkel cells from rat touch domes by first loading them with quinacrine, and then irradiating the domes with near-UV light. Mechanical stimulation of these domes revealed a range of mechanosensory function, evaluated qualitatively, that varied from non-responsive to normal. Since irradiation eliminated the quinacrine fluorescence, the status of the Merkel cells was evaluated by EM. In both responsive and unresponsive domes fixed for EM immediately following irradiation, the Merkel cells and associated nerve endings appeared to be normal. After 2 or more days, even in domes that continued to be normally responsive, there was a striking reduction in the normal complement of about 90 Merkel cells, and most of the remaining Merkel cells appeared to be degenerating. However, numerous 'isolated' (Merkel cell-free) nerve endings remained in the basal epidermis. A few of these nerve endings showed signs of damage, but in the non-responsive domes abnormal nerve endings were routinely observed. The EM studies did not exclude the possibility that a few surviving innervated Merkel cells, or even one such, had escaped detection and were responsible for a persisting mechanosensitivity. To resolve this issue a mechanical stimulating technique with a spatial resolution of 55 btm was used to map the mechanosensory profile of a single responsive dome irradiated 2.75 days earlier. This dome was then serially sectioned for EM study. Only seven Merkel cells had survived which appeared to be both viable and innervated, but almost all of the tested sites were normally responsive. When the correlation was made, seven of these sites were located 55-100 btm away from the nearest surviving Merkel cell, four were 110-165 btm away, and three were more than 165 btm away. Even when allowance is made for errors in the positioning of the stimulus, the responses at the last seven sites cannot be attributed to the presence of underlying Merkel cells. We conclude that mechanosensory transduction within touch domes is not a function of the Merkel cells, but must reside in the associated nerve endings.

Introduction

The Merkel cell was discovered as a consequence of a deliberate search for specialized end-organs in skin that would subserve 'touch' (Merkel, 1875, 1880). Merkel cell-neurite complexes, each comprised of a single Merkel cell and its abutting sensory nerve ending(s), have now been described in the skin of humans and all other vertebrate species studied (e.g., Merkel, 1875, 1880; Breathnach, 1971, 1977; Munger, 1977; Fox & Whetar, 1978; English et al., 1980; Saxod, 1980; Tachibana & Nawa, 1980; Nurse et al., 1983). Whether individually dispersed across the skin as in the salamander (Parducz et al., 1977), organized into identifiable arrays as in the rodent footpad (Mills et al., 1989), or grouped within sensory end-organs as in the touch dome (Iggo & Muir, 1969), the Merkel cell-neurite complexes are generally assumed to contribute to the 'light touch' modality (Johansson & Valbo, 1979, 1983; Iggo & Andres, 1982). Although Merkel himself never explicitly made the attribution, it has long been accepted that the process of mechanosensory transduction occurs in the Merkel cell itself (e.g. Iggo & Muir, 1969; Kurosumi et al., 1969; Mihara et al., 1973; Horch et al., 1974; Winkelmann, 1977; Hartschuh & Weihe, 1979; Hartschuh et al., 1985; Baumann et al., 1990; Tsu & Baumann, 1991). The role of the Merkel cell would then be to convert the primary mechanical stimulus into a form that would lead to the initiation of impulses in the associated nerve ending. Consistent with this view of Merkel cell function are EM observations suggestive of a chemical synaptic transmission step from Merkel cell to nerve ending (Munger, 1965, 1977; Iggo & Muir, 1969;
Parducz et al., 1977; Hartschuh & Weihe, 1979; Yasargil et al., 1988).

These observations notwithstanding, the concepts both of a transducer role for Merkel cells, and of chemically-mediated synaptic links with their associated nerve endings, have not gone unchallenged (Gottschaldt & Vahle-Hinz, 1981; Diamond et al., 1986; Meanow & Diamond, 1988); in the last of these studies Merkel cells were eliminated from the skin of Xenopus by irradiation after quinacrine-loading of the Merkel cells. The present study has also used the quinacrine-loading approach to address the question of Merkel cell involvement, but with a more rigorous protocol anticipated to eliminate possible ambiguities in the earlier experiments. The preparation used was the touch dome of the rat back, which contains 90 or so Merkel cells (Nurse et al., 1983, 1984a,b). Critical to the proposed investigation were (i) a high-resolution mapping technique which provides the mechano-sensory profile across a single touch dome (Yasargil et al., 1988), and (ii) the serial sectioning of an irradiated and mapped dome to identify by EM any Merkel cells that might have survived the treatment. The results clearly point to the nerve ending, rather than the Merkel cell, as the site at which mechano-sensory transduction must occur in this sensory end-organ. A brief report of some of these findings has been published (Diamond et al., 1988).

**Materials and methods**

**IRRADIATION PROTOCOL**

Female Wistar albino rats (175-225 g) were injected intraperitoneally (i.p.) with 15 mg ml⁻¹ of quinacrine dihydrochloride dissolved in distilled water, a procedure which effectively labels the Merkel cells within the touch domes of the hairy skin of the rat (Nurse et al., 1984a). One day later they were anaesthetized with 45 mg kg⁻¹ of sodium pentobarbital (somnotol) i.p., the fur of the back was shaved and a depilatory, Neet, was briefly applied to the skin. The light source used to irradiate the touch domes was a 50 W mercury HBO fitted with a heat filter and a BG 12 Exciter filter which allows the passage of 330-550 nm light. Since the excitation peaks for quinacrine are 285 and 420 nm (Nurse et al., 1985), the second quinacrine excitation peak at 420 nm passed unattenuated. The intensity of this ‘near-UV’ radiation was measured at the skin surface with a radiometer as 1.875 Mw, and when focused the light provided a spot size of 3 mm diameter. The animals were anaesthetized throughout the irradiation procedure.

Domes within the mechanosensory fields of either the left, or right, medial branches of the dorsal cutaneous nerves (mDCNs) T13-L3 were identified under a low power dissecting microscope (Yasargil et al., 1988), and 10-30 of them were ringed with permanent blue ink using a fine-tipped felt marker. Half of these were used as experimental domes while the other half served as controls; generally domes were matched so that, for each dome irradiated, at least one of its closest neighbour domes was a non-irradiated control. A permanent map showing the distribution of the domes was made on a clear acetate sheet moulded over the back of the animal.

Just prior to irradiation a 1 cm diameter flexible plastic donut-shaped shield was fitted around the dome; the ‘hole’ of the donut included the dome together with a small annulus of surrounding skin. To facilitate the fit of this shield, petroleum jelly was smeared on its under surface. The animal was then placed on a platform, and the 50 W HBO light source was focused on the selected dome. The minimum time required to bleach all quinacrine fluorescent cells (QFC) within a dome ranged from 2.5 to 5.5 min; this ‘minimum bleaching time’, which was fairly constant for all the domes of a particular animal, was determined by irradiating a series of test domes in that animal for progressively longer time periods (starting at 1 min), and examining them for QFC (see below). When QFC were observed, the exposure time was increased on the next dome by 30 s. This procedure was repeated until the minimum bleaching time was established. Since quinacrine is normally cleared from Merkel cells in 24-48 h the fate of the Merkel cell population was followed over longer periods of time by giving another dose of quinacrine 24 h before examining the domes.

**IDENTIFICATION OF TOUCH DOMES INNERVATED BY A SINGLE AXON**

The DCNs of segments T13-L3 were dissected free over a 1-2 cm length, ligated close to the body wall, and then cut above the ligature. For extracellular recording of afferent impulses the nerves were draped over platinum hook electrodes and coated with petroleum jelly. Signals were amplified, displayed on a storage oscilloscope, and fed into an audio monitor. The mechanosensory fields of each mDCN were mapped (Jackson & Diamond, 1984) to establish the innervation source for all of the domes selected for irradiation. The characteristic slowly adapting (irregular) discharge of a touch dome was conveniently evoked by the application of a stiff bristle. Domes that were innervated by more than one axon (deriving either from a single DCN or from adjacent DCNs), were identified as previously described (Yasargil et al., 1988), and were excluded from the present study.

**EVALUATION OF TOUCH DOME MECHANO-SENSITIVITY**

**Mechanical stimulation**

The skin region containing the domes of interest was stabilized by gently stretching it over a horizontal plexiglass platform. The mechanical stimulus was provided by a vertical ‘prodder’, a tungsten wire etched to a tip diameter of either 16 or 120 μm. The prodder was mounted on a piezoelectric crystal clamped to a micromanipulator. To stimulate a dome that prodder was first lowered until the tip was just touching the dome’s surface; thereafter vertical displacements of the prodder tip were achieved either by voltage pulses applied to the crystal, or by transient manual adjustments of the micromanipulator. All displacements...