Selective phototoxic destruction of quinacrine-loaded Merkel cells is neither selective nor complete

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Abstract Experiments were performed on slowly adapting type I mechanoreceptors in an isolated rat skin-nerve preparation (SA I receptors) and in an isolated rat sinus hair preparation (St I receptors). Merkel cells were stained in vitro with the fluorescent dye quinacrine and irradiated with ultraviolet (UV) light (2 mW for up to 1 h) while recording receptor responses to standard mechanical stimuli every 30 s. In addition, thresholds for electrically evoked action potentials were tested by applying electrical stimuli to the skin through the same stylus used for mechanical stimulation. UV irradiation resulted in abrupt failure to respond to mechanical stimuli in 73% of the SA I receptors examined (n=37) within less than 1 h. This confirms previous reports of phototoxic destruction of Merkel cells. However, several minutes after the receptors failed to respond to mechanical stimulation, thresholds for electrical stimuli applied to the receptive field increased sharply. About 40% of the St I receptors (n=13) irradiated with UV light following quinacrine staining stopped responding to bending of the hair within 1 h. In contrast, none of the seven St II receptors treated in the same way showed significant changes in the responses. Electron microscopic examination of sinus hairs after quinacrine staining alone showed slight changes in the appearance of Merkel cells, and in particular enlargement of the perinuclear space. These changes did not affect receptor responses. Electron microscopic studies of sinus hairs with receptors that had maintained normal responses to mechanical stimuli after quinacrine staining and 1 h of UV irradiation revealed that a substantial number of Merkel cells still had a normal ultrastructure while adjacent nerve terminals were severely swollen and partially compressing the Merkel cells. No changes were observed in lanceolate nerve terminals forming the morphological substrate of St II receptors. These results demonstrate that sensitivity to phototoxic destruction following quinacrine staining varies greatly among Merkel cells, with some maintaining normal function and ultrastructural appearance even after 1 h of UV irradiation. On the other hand there is clear evidence that the phototoxic damage affects the nerve terminals as well. Such experiments can therefore not provide conclusive proof about the role of Merkel cells in these mechanoreceptors.

Keywords Mechanoreceptors - Merkel cells - Vibrissae - UV light - Phototoxicity - Quinacrine

Introduction

Slowly adapting type I (SA I) mechanoreceptors in the skin and sinus hair type I (St I) receptors in vibrissae are the only types of mechanoreceptors consisting of specialized cells — Merkel cells — in close proximity to enlarged nerve terminals (Iggo and Muir 1969; Andres and von Düring 1973; Gottschaldt et al. 1973). The functional role of these Merkel cells is still controversial. Iggo and Findlater (1984) suggested that the mechano-electric transduction process occurred in the Merkel cells and involved chemical synaptic transmission to the nerve terminal. However, attempts to interfere with the putative synaptic transmission using specific antagonists have had no significant effect on receptor responses (Smith and Creech 1967; Gottschaldt and Vahle-Hinz 1982; Baumann and Chan 1993).

Quinacrine has been used to specifically stain Merkel cells in living tissues (Crowe and Whitear 1978). Such stained Merkel cells were shown to be particularly sensitive to the damaging effects of UV irradiation (Diamond et al. 1988; Ikeda et al. 1994; Mills and Diamond 1995). Therefore, following phototoxic destruction of these cells, receptors should no longer respond to mechanical stimulation if the assumption that Merkel cells play a vi-
tal role in the mechanoelectric transduction process is correct. Ikeda et al. (1994) reported that receptor failure consistently occurred within less than 30 min of UV irradiation in quinacrine-loaded SA I receptors. However, Diamond and coworkers observed mechanosensitivity of touch domes several days after photobleaching of quinacrine-stained Merkel cells (Diamond et al. 1988; Mills and Diamond 1995). The authors interpreted this finding as evidence against a mechanotransducer function of Merkel cells in these receptors.

The interpretation of these contradictory findings hinges strongly on the question of whether the photodestruction selectively affects Merkel cells only and destroys all of them but leaves the nerve terminals intact. UV irradiation of skin in vivo can induce inflammation resulting in hyperalgesia and changes in thresholds of cutaneous receptors (Eschenfelder et al. 1995). Therefore, it appeared appropriate to re-examine – in terms of both function and electron microscopic appearance – the effect of acute, low-intensity UV irradiation on quinacrine-loaded SA I and St I receptors using in vitro preparations. The results indicate that in contrast to responses of St II receptors, which remained unaffected by quinacrine loading and UV irradiation, toxic damage can cause failure of SA I and St I receptors to respond to mechanical stimuli. However, there is evidence that the phototoxicity was not limited to Merkel cells, but resulted in damage to nerve terminals also. Therefore, such experiments cannot provide conclusive information about the site of the mechanoelectric transduction process in type I receptors. Brief reports of these results have been published as abstracts (Baumann and Senok 1993; Baumann and Halata 1995).

Materials and methods

Isolated skin-nerve preparation

With the approval of the Animal Research Ethics Committee of the Chinese University of Hong Kong, Sprague-Dawley rats of about 400 g body weight were anaesthetised with urethane (20% w/v/s 6 ml/kg i.p.). The skin of one leg together with the saphenous nerve was carefully separated from the underlying muscles in the subcutaneous tissue layer and transferred to an isolated organ bath (Reeh 1986; Baumann and Tsu 1990). The skin was mounted with the corium side facing up, fixed with insect pins under slight tension to the Silgard bottom (Silgyl 184, Dow Corning, USA) of the bath and superfused with oxygenated synthetic interstitial fluid (SIF) at 28°C (Bretag 1969). The nerve was carefully guided into a paraffin-filled chamber with a dissection platform and the recording electrodes. Under a dissection microscope (Zeiss Stemi-SR), fine nerve strands were teased for single unit recordings from SA I receptors identified by their characteristic responses (Iigo and Muir 1969; Horch et al. 1974). Mechanical stimuli were applied every 30 s using a stylus with a spherical tip 1 mm in diameter attached to a force transducer (Statham UC2). Each stimulus (Fig. 1B, c) rose from a contact force of 0.5 mN within 200 ms to a plateau force of 15 mN which was kept constant for 2 s by feedback control (developed in conjunction with the Electronics Department of the Chinese University of Hong Kong). Displacements of the probe were measured simultaneously with a Sango D55 (Schlumberger) transducer. The stylus was made of silver with an insulated shaft enabling application of electrical stimuli (Digitimer DS2) to the receptor under investigation. The middle part of the Silgard bottom was made softer by including an air cushion to achieve displacements of about 0.5–1.0 mm comparable to those usually required in situ (Baumann et al. 1986).

Isolated sinus hair preparation

For the isolated sinus hair preparation rats were anaesthetised with urethane as described above and subsequently killed with an intracardiac injection of an overdose of the same drug. A whisker pad was quickly excised for dissection of sinus hairs including a short length of the deep vibrissal nerve. Single sinus hairs were fixed with insect pins on a Silgard platform and superfused with oxygenated SIF at 32°C (Bretag 1969). Under a dissection microscope (Zeiss SV-11), fine nerve strands were teased for single unit recordings from St I receptors identified by their slowly adapting responses and characteristic discharge pattern (Gottschaldt et al. 1973). A stainless steel hook was attached to the hair shaft about 10 mm from the root and standard mechanical stimuli were applied every 60 s by feedback-controlled bending of the hair in the most sensitive direction for 5 s. Each trapezoid stimulus had ramps of 500 ms and a plateau phase of 4 s during which the displacement of the hair was kept at 1 or 1.5 mm. The nerve strands were attached to the different recording electrode in the lower part of the chamber below the level of the Silgard platform which was filled with Fluorinert FC-40 (Sigma).

Recording of receptor responses

Nerve action potentials were recorded via Ag–AgCl electrodes, amplified (Neurolog NL4104), filtered (70–5000 Hz; Neurolog NL125) and processed by a spike discriminator (Digitimer DL130) before being displayed on an oscilloscope (Gould DSO1604 with integral plotter) to monitor the waveform of action potentials to ensure single unit recordings (Fig. 1A). TTL pulses representing action potentials together with the signals from force and displacement transducers were analysed and stored on hard disk using a 486-based computer in conjunction with a laboratory interface CED1401 (Cambridge Electronic Design) controlling all stimulation and response data using programs written in TurboPascal (Borland). Responses were measured as the total number of action potentials for each stimulus and also analysed separately for the dynamic phase (ramp) and static phase (plateau). Interstimulus interval (ISI) histograms were obtained for the adapted responses during the last second of the plateau phase using a bin width of 1 ms, and displayed on line on the computer screen together with the other stimulation and response parameters (Fig. 1B). Continuous monitoring of the typical irregular firing (Fig. 1B, a and b) and skewed ISI histograms (Fig. 1B, c) were used for controlling that we were actually recording from type I receptors. Both preparations were shown to have stable receptor responses to repetitive mechanical stimuli for at least 5 h (Baumann and Tsu 1990; Senok et al. 1995).

Quinacrine loading of Merkel cells and UV illumination

After steady receptor responses had been recorded for at least 30 min, quinacrine (for skin receptors, 10 μM; for sinus hair receptors, 20 μM) was added to the superfusing fluid for 30 min. Such concentrations were well above the minimum required for staining of Merkel cells in vitro (0.3 μM; Yamashita et al. 1992). Following a return to a normal superfusing solution the entire receptive field of the SA I receptor or hair bulb was irrigated for up to 1 h with UV light from a xenon lamp (XBO 150 W) using a light guide (LLG-2 from Hi-Tech, Salisbury, UK). UV intensities at the end of the light guide (2 mW) were measured with a UV radiometer (UVR-1 with sensor UVR-40, Topcon, Tokyo; most sensitive range 360–480 nm).
Fig. 1  A Sample oscillograms of 100 ms each from single unit recordings of action potentials from: a a type I receptor with typical irregular intervals between spikes and b a type II receptor firing much more regularly. B Computer screen picture for one stimulus applied to an SA I receptor in the isolated rat skin-nerve preparation. Trace a is the train of pulses representing spikes for the whole duration (2500 ms) of the stimulus, while b shows the spikes for the time 1000–2000 ms after the start of the stimulus with an expanded time scale. The time of the first spike at 24 ms in this example serves as a measure of the receptor threshold. Trace c shows displacement (in mm) and force (scale=10 in mN): the trapezoidal force profile is kept constant during the plateau phase at 15 mN by feedback control. At the bottom are three boxes for interspike interval (ISI) histograms during the interval between successive stimuli (d), the dynamic phase (e) and the last 1000 ms of the static phase (f), with mean interval, standard deviation (STD) and coefficient of variance (C-VAR) (typically well above 0.1 for type I receptors).

Electron microscopy

At the end of the electrophysiological experiments, selected sinus hairs (of control experiments, after exposure to quinacrine alone and sinus hairs that had maintained normal responsiveness throughout 1 h of UV irradiation following quinacrine staining) were removed from the organ bath and fixed in 6% glutaraldehyde in 0.1 M sodium phosphate buffer for 20 min at 4°C and post-fixed for 2 h in 1% OsO₄ in 0.1 M phosphate buffer with 1% sucrose. The specimens were stored in 0.1 M sodium phosphate buffer at 4°C for several weeks, cut in half longitudinally and dehydrated in alcohol. Embedding in Epon 812 was done according to the method described by Luft (1961).

Serial semithin sections (1 μm thick) were cut on an OmU4 ultramicrotome and stained with toluidine blue and pyronin red according to Laczkó and Levai (1975). Selected semithin sections were re-embedded for electron microscopy and thin sections stained with lead citrate according to Reynolds (1963). The electron microscope used was a Philips 300.

Results

Responses of cutaneous slowly adapting type I receptors

Quinacrine alone did not affect the responses of SA I receptors even if the concentration was increased to 100 μM. Low-intensity (2 mW) UV irradiation for 1 h without prior quinacrine loading also had no significant effect on receptor responses to standard mechanical stimuli applied every 30 s (Fig. 2A). In contrast, 29 of the 37 SA I receptors (73%) receiving UV irradiation following superfusion with quinacrine (10 μM for 30 min) developed abrupt receptor failure within less than 1 h after the start of exposure to UV light. Figure 2B shows examples of eight SA I receptors exposed to UV irradiation for an average of 27±4 min before ceasing to respond to mechanical stimuli. Static responses during the plateau phase of the mechanical stimuli decreased first, followed by a decline in responses during the dynamic phase as well. Eight of the 37 receptors examined showed no significant change in their responses to mechanical stimuli during 1 h of UV illumination following quinacrine staining.

Thresholds for electrically elicited action potentials were tested intermittently in 12 receptors while keeping
the stylus at a contact force of 0.5 mN. Great care was taken to ensure that the elicited action potentials had an identical shape to those in response to mechanical stimulation. No change in electrical threshold or latency between stimulus artefact and action potential was observed during UV illumination. However, after failing to respond to mechanical stimuli 75% of the receptors showed increases in electrical thresholds either immediately or within 10 min (Fig. 3B). The remaining 25% maintained normal electrical thresholds for at least another 30 min (Fig. 3A). There were no changes in latency in spite of increased thresholds as long as action potentials could be elicited by electrical stimuli.

Responses of sinus hair type I receptors

Similar to the observations on SA I receptors in the skin, neither quinacrine alone nor UV irradiation without prior quinacrine (20 µM) staining had significant effects on the responses of sinus hair type I receptors to standard mechanical stimuli. Also, not all receptors in the sinus

Fig. 2A, B Number of impulses from slowly adapting type I receptors of isolated rat skin in response to standard mechanical stimuli plotted against time. Responses to the last ten stimuli before the start of UV irradiation (time 0) were taken as reference (100%) and all responses are expressed as a percentage of this control value. A Mean±SEM of four receptors irradiated with UV light (2 mW) from minute 0 to 60 without prior staining with quinacrine. B Individual traces of eight experiments in which cells were pretreated with quinacrine (10 µM for 30 min) shortly before the start of UV irradiation. Receptor failure occurred on average after 27±4 (mean±SEM) min of UV irradiation

Fig. 3A, B Responses of slowly adapting type I receptors to standard mechanical stimuli (circles) and thresholds for electrically elicited action potentials (triangles) plotted against time. While some receptors maintained the same electrical threshold after failing to respond to mechanical stimuli (like the example in A), 9 of 12 receptors showed sudden increases in threshold within the following 10 min (like B)

hair stopped responding to mechanical stimulation during 1 h of UV irradiation following quinacrine staining (Fig. 4A, B). Five of 13 St I receptors irradiated after quinacrine loading developed receptor failure; the other eight maintained stable responses. Only sinus hairs in which receptors with unaffected responsiveness had been recorded from were chosen for electron microscopic examination of Merkel cells and nerve terminals.

For comparison, seven St II receptors were exposed to quinacrine and UV irradiation in the same way. None of these receptors showed a significant decrease in responsiveness.

Electron microscopy of UV-irradiated sinus hairs

Merkel nerve endings are found in the thickened middle portion of the hair follicle between the sebaceous gland and the ring bulge. They form a cuff under the glassy membrane separating them from the blood sinus (Andres and von Düring 1973). They are closely associated with nerve terminals from myelinated afferent axons (diameter 4–6 µm) entering from the connective tissue of the hair bulb. One axon innervates several Merkel cells, easily recognizable by their elongated shape and strongly
lobulated nucleus. Within the Merkel cell cytoplasm a large number of dense-cored granules with diameters of about 600 nm can be found polarized near the nerve terminal (Fig. 5A). Numerous finger-like protrusions or “spines” extend from the Merkel cell between the surrounding cells and penetrate the basal lamina, anchoring in the glassy membrane.

Electron microscopy of receptors maintaining normal responses to mechanical stimulation during exposure to quinacrine showed nerve terminals with a normal appearance. Merkel cells showed swelling of the perinuclear space, the cytoplasm appeared relatively pale and lacked free ribosomes (Fig. 5B).

As expected, damaged Merkel cells were found in sinus hairs fixed after 1 h of UV illumination following quinacrine staining, including those that maintained full responsiveness. However, Merkel cells with a normal appearance and intact granules in the cytoplasm were also commonly seen. Frequently nerve terminals with different degrees of swelling were observed. Some severely bloated nerve terminals were found adjacent to Merkel cells that appeared compressed but otherwise normal (Fig. 5C). The mitochondria in these damaged nerve terminals varied greatly in size, in contrast to the uniformity of the mitochondria in control nerve terminals (compare

Fig. 5A and C). Swollen nerve terminals were never seen in non-irradiated control receptors (n=10). In contrast, quinacrine exposure followed by 1 h of UV irradiation did not result in any ultrastructural changes in lanceolate terminals, which are the morphological substrate of St II receptors (Gottschaldt et al. 1973). Neither did we see any abnormalities in nerve fibres within the sinus hairs.

Discussion

The present experiments re-examined the effect of phototoxic destruction of Merkel cells on the responsiveness and ultrastructure of Merkel cell receptors in isolated rat skin-nerve and sinus hair preparations. Similar experiments had been carried out previously on rats in situ in an effort to shed light on the still controversial role of Merkel cells in these receptors, and resulted in contradictory interpretations (Diamond et al. 1988; Ikeda et al. 1994; Mills and Diamond 1995). Direct measurement of receptor potentials has not been possible due to the poor accessibility of Merkel cells and nerve terminals in these receptors. Various potential neurotransmitter substances have been found in Merkel cells (for review see English et al. 1992). However, attempts to interfere with the postulated synaptic transmission from Merkel cells to nerve terminals have not resulted in the functional identification of any neurotransmitter (Smith and Creech 1967; Gottschaldt and Vahl-Hinz 1982; Baumann and Chan 1993). Thus, in an effort to obtain information on their functional role in the mechanoelectric transduction process, experiments have concentrated on pharmacologically impairing Merkel cells (Pacitti and Findlater 1988; Baumann et al. 1990) or selectively destroying them (Diamond et al. 1988; Ikeda et al. 1994; Mills and Diamond 1995).

Electrophysiological identification of Merkel cell receptor responses

Different types of mechanoreceptors are found side by side in the skin (Jänic et al. 1968; Reeh 1986; Leem et al. 1993a) as well as around vibrissae (Andres and von Düring 1973; Halata and Munger 1980; Halata 1993). Impinging the skin with a fine probe of a mechanical stimulator will result in deformation of a skin area much larger than the tip of the probe, depending on the compliance of the skin and underlying tissue. Thus, a variety of receptors in the affected skin area may be activated if the stimulus strength exceeds their thresholds. The present study did not attempt to stimulate single Merkel cell–nerve terminal complexes by using ultra-fine stimulating probes. One afferent fibre innervates up to 90 Merkel cells in a touch dome about 250 μm in diameter (Yassargil et al. 1988). Deformation of the skin during mechanical stimulation of an intensity sufficient to reach the epidermo-dermal border where the Merkel cells are located will almost inevitably affect a signifi-
sponse to long-lasting mechanical stimuli decreased earlier than the dynamic responses. By using short mechanical stimuli such early signs of impaired receptor function may not be detected. In this study, each mechanical stimulus had a plateau phase of 2 s during which the force was maintained at 15 mN, enabling us to monitor the firing pattern of the receptors during adaptation directly and in the form of interspike interval histograms (Fig. 1B, b and f).

Thus, in addition to Diamond’s interpretation that the nerve terminals must be the mechano-electric transducers, we would find it difficult to exclude the possibility that the discrepancies between his findings, the present experiments and the findings of Ikeda et al. (1994) could be either the result of misinterpretation of responses from other types of receptors or due to incomplete elimination of all Merkel cells in the irradiated touch domes.

Selective staining and phototoxic destruction of Merkel cells

Antimalarial drugs such as quinacrine are selectively accumulated in some peptide-secreting cells and nerve terminals (Olson et al. 1976; Ekelund et al. 1980). In the skin, the fluorescent dye quinacrine was reported to be taken up only by Merkel cells and used as a vital staining technique for these cells (Crowe and Whitear 1978; Nurse et al. 1983). Electron microscopic examinations have clearly shown that irradiation of such quinacrine-loaded cells with UV light resulted in damaged Merkel cells (Diamond et al. 1988; Ikeda et al. 1994; Mills and Diamond 1995). Thus, the idea of testing mechanosensitivity of SA I receptors after selective photodestruction of Merkel cells appeared promising for determining the role of Merkel cells in the mecano-electric transduction process (Diamond et al. 1988). It is well known that UV irradiation alone can acutely impair cell function in living tissue, possibly through the absorption of the UV energy by chromophores resulting in the formation of various oxidants such as superoxides (for review see Tyrrell and Keyse 1990). Loading cells with fluorescent dyes dramatically increases their sensitivity to UV light and first signs of disturbed cellular functions have been reported after UV irradiation of 15 mJ/mm² (Grapengieser 1993). One hour of irradiation in our setup delivered about 150 times that amount of energy to the tissue. Thus, it was surprising that some receptors were still able to function normally after such levels of irradiation. On the other hand, lanceolate nerve terminals do not accumulate quinacrine. Thus, it was expected that type II receptors would remain both functionally and ultrastructurally unaffected in the same way as those St I receptors that were not stained with quinacrine prior to UV irradiation.

Different approaches have been used in previous studies: While Diamond’s group (Diamond et al. 1988; Mills and Diamond 1995) examined the receptors several days after UV irradiation, Ikeda et al. (1994) irradiated rat skin in situ while recording SA I responses. The present

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**Fig. 5** A Control. Longitudinal section of a Merkel nerve ending from an untreated sinus hair. The Merkel cell (m) has elongated shape and lobulated nucleus. The cytoplasm contains rough endoplasmic reticulum, mitochondria and osmophilic granules which are mainly concentrated in the part of the cell facing the nerve terminal. Cytosplasmic processes of up to 15 μm from the Merkel cell extend into the connective tissue of the hair follicle (asterisk) and can be seen penetrating the basal lamina (arrow). The disc-shaped nerve terminal (t) is rich in mitochondria, neurotubules and neurofilaments. Magnification x10 000. B Cross-section of a Merkel nerve ending from a sinus hair exposed to quinacrine for 30 min. Hemidesmosomes (arrows) can be seen between Merkel cell (m) and basal lamina and the contact area appears larger than in untreated samples, not interrupted by cell processes from the basal epithelial layer as in A. Filaments are found in the cytoplasm of the Merkel cell, which is less rich in contrast and the perinuclear space is enlarged (asterisk). The density of the osmophilic granules and their orientation towards the nerve terminal are unchanged. No alterations can be seen in the nerve terminal (t). Magnification x8000. C Cross-section of a Merkel nerve ending from a sinus hair after exposure to UV irradiation following quinacrine staining. The nerve terminal (t) is swollen and contains mitochondria, neurotubules and empty vesicles. The Merkel cell (m) is flattened (possibly as a result of the swelling of the nerve terminal) but appears otherwise normal. Magnification x8000.
study monitored responses from SA I and St I receptors during UV irradiation in vitro, thereby reducing the possibility of interference from reactions to tissue damage which may cause oedema and changes in the thresholds of “silent” receptors as mentioned above (Baumann et al. 1991; Eschenfelder et al. 1995). Such tissue reactions may have been responsible for the transient increase in receptor responses observed during the early stages of UV irradiation by Ikeda et al. (1994). It had been well established that UV irradiation can cause the death of quinacrine-stained Merkel cells.

However, evidence for normal functioning of the nerve terminals associated with Merkel cells at the time when receptors stopped responding to mechanical stimuli is essential in order to prove that the mecano-electric transduction process occurs in the Merkel cells. On the other hand, maintained responsiveness of SA I receptors after phototoxic destruction of Merkel cells can only serve as proof that the transduction process occurs in the nerve terminals if it can be unequivocally shown that all Merkel cells have been effectively destroyed and that receptor responses are identical to those of SA I receptors under control conditions. This would require electron microscopic examination of at least 250 serial sections per touch dome. The present study did not attempt to quantify the damage to Merkel cells or to relate receptor responses to single Merkel cell–nerve terminal complexes. Electron microscopic examinations were carried out on sinus hairs, where large numbers of Merkel cells are found in close proximity in the middle portion of the hair follicle. Thus, a clear idea of the general condition of Merkel cells can be reached after examining a much smaller number of sections than would be necessary for touch domes.

The UV intensity (2 mW) used in the present experiments was similar to that used by Diamond’s group, but much lower than the 250 mW used by Ikeda et al. (1994). In the latter study, the quinacrine-pretreated SA I receptors usually stopped responding after less than 20 min of UV irradiation. Our data show development of sudden receptor failure in 73% of the skin receptors after an average irradiation time of about 30 min. Only 2 of 29 SA I receptors in the isolated skin-nerve preparation (Fig. 2B) stopped responding within 10 min of exposure to UV light. Thus, 5–10 min of UV illumination as used by Diamond et al. (1988) and Mills and Diamond (1995) is not sufficient for acute phototoxic destruction of all Merkel cells in the irradiated touch domes. In our in vitro preparations, the extent of damage to Merkel cells developing several days after irradiation could not be examined. The present results would suggest that irradiation of more than 10 min is necessary to ensure complete destruction of the up to 90 Merkel cells in a dome (Yassargil et al. 1988). Receptor responses in sinus hairs were found to be more resistant than those in the skin, with about 60% of the St I receptors compared with 27% of SA I receptors still responding normally after 1 h of irradiation. This may be due to the much larger number of Merkel cells or to a different degree of branching of the afferent nerve fibres. In monkeys, each sinus hair has up to 2000 Merkel cells supplied by about 50 afferent nerve fibres (Halata and Munger 1980; Halata 1993); quantitative data from rats are not available. Thus, the chances of a few Merkel cell–nerve terminal complexes “surviving” the irradiation and responding normally are likely to be higher in sinus hairs than in the skin. This may also be the cause for the more gradual decline of responses in St I receptors in contrast to the rather abrupt development of receptor failure found in SA I receptors (compare Figs. 2B and 4B).

In most SA I receptors, the thresholds for electrically elicited action potentials remained initially unchanged following failure to respond to mechanical stimuli but increased rapidly within the next 10 min (Fig. 3B). This suggests that when the mecano-electric transduction process had failed, the nerve terminals or at least the peripheral parts of the afferent nerve were still able to conduct action potentials. On the other hand, the fact that 75% of receptors eventually also stopped responding to electrical stimuli suggested that the phototoxic destruction did also cause damage to the nerve terminals and was possibly spreading along the terminal branches of the afferent nerve, rather than destroying only the Merkel cells. This could be due to loss of trophic influences from Merkel cells which were suggested to sustain type I nerves (English et al. 1984) or caused by substances released from damaged Merkel cells. Therefore, special attention was paid to examining the electron micrographs to detect possible ultrastructural signs of damage to nerve terminals associated with the Merkel cells as substrate of the functional impairments.

Electron microscopic findings of damaged nerve terminals

Previous studies by Ikeda et al. (1994) and Mills and Diamond (1995) have shown electron microscopic pictures clearly demonstrating the damaging effects of UV irradiation on quinacrine-stained Merkel cells. This could be confirmed in the present study. In addition, our results indicate that staining with quinacrine alone (without UV irradiation) can affect the ultrastructure of Merkel cells and cause mild swelling of the peri-nuclear space (Fig. 5B). However, these slight morphological abnormalities did not result in measurable changes in receptor function. Photosensitization with quinacrine was found to be essential for both the functional impairment and ultrastructural signs of cell damage. One hour of UV irradiation without prior quinacrine exposure did not result in significant damage to Merkel cells. Equally, no morphological changes were found in lanceolate terminals and nerve fibres in sinus hairs irradiated after quinacrine, as these structures do not take up the fluorescent dye.

It had been argued that the nerve terminals were unaffected by the UV irradiation of quinacrine-stained receptors (Diamond et al. 1988; Ikeda et al. 1994), although the study by Mills and Diamond (1995) conceded that
damaged nerve terminals were seen after “over-irradiation”, resulting in total abolition of receptor responses.

The present electron microscopic findings are the first to demonstrate that nerve terminals can develop severe morphological changes after UV irradiation of quinacrine-stained St I receptors while adjacent Merkel cells were mainly unaffected (Fig. 5C). Such changes were not seen in any of the ten control receptors examined. These changes are consistent with the finding that in most experiments, electrical stimuli failed to trigger action potentials in the afferent nerve several minutes after the receptors stopped responding to mechanical stimuli. Swollen nerve terminals were seen next to flattened Merkel cells which appeared otherwise normal. These tissue samples were taken from sinus hairs with St I receptors still responding normally in spite of 1 h of UV irradiation. Thus, not all Merkel cells are equally sensitive to phototoxic destruction. On the other hand, our electrophysiological results and these electron micrographs clearly show that the assumption of selective phototoxic destruction cannot be confirmed. As shown in Fig. 5C, some nerve terminals are more sensitive than the associated Merkel cells and may in turn compress Merkel cells as they swell.

In conclusion, the present results confirm previous findings by Ikeda et al. (1994) that phototoxic destruction of Merkel cells can cause failure of SA I and St I receptors to respond to mechanical stimuli. Following failure to respond to mechanical stimulation, electrical stimuli could still trigger action potentials for several minutes before the threshold increased rapidly. This suggests that the phototoxic damage also affected the nerve terminals or peripheral parts of the afferent nerve fibres. Electron microscopy revealed that a significant proportion of Merkel cells maintained normal ultrastructure after 1 h of UV irradiation following quinacrine staining while adjacent nerve terminals showed signs of severe damage. Therefore, phototoxic destruction of Merkel cells is evidently neither selective nor complete and such experiments cannot distinguish whether the mechano-electric transduction process occurs in the Merkel cells or in the nerve terminals.

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References


Jänig W, Schmidt RF, Zimmermann M (1968) Single unit responses and the total afferent outflow from the cat's foot upon mechanical stimulation. Exp Brain Res 6:100–115


