Selective phototoxic destruction of rat Merkel cells abolishes responses of slowly adapting type I mechanoreceptor units

Isamu Ikeda*†, Yoshiro Yamashita*, Tomomichi Ono† and Hisashi Ogawa*

*Department of Physiology and †Department of Dermatology, Kumamoto University School of Medicine, Honjo 2-2-1, Kumamoto 860, Japan

1. The fluorescent dye quinacrine which accumulates in Merkel cells in touch domes was administered to rats and the effects of excitation light irradiation on the mechanical responses of slowly adapting (SA) type I units innervating the touch domes were investigated.

2. Histological examination showed that after 10 min of irradiation degeneration was specifically localized to Merkel cells loaded with quinacrine. Nerve terminals associated with Merkel cells remained intact, even after treatment.

3. In SA type I units, responses to standard stimulation (a 100 ms ramp followed by a 2·9 s plateau of 400 μm constant displacement) decreased significantly after irradiation of the domes with quinacrine-excitation light through a 'B' filter ('B' light). With 5 min irradiation, the response decreased to $52 \pm 7\%$ ($n = 10$, mean ± S.E.M.) of the pretreated value, to $17 \pm 4\%$ with a 10 min treatment and practically disappeared within 20 min.

4. In SA type I units with non-loaded Merkel cells, the response increased to $119 \pm 8\%$ ($n = 13$) with 5 min irradiation and was $99 \pm 9\%$ with the 10 min treatment. At around 15 min after the onset of irradiation there was a gradual decrease and within 60 min the response disappeared.

5. When responses were divided into phasic (0–120 ms after the onset of stimulation) and tonic (120–3000 ms) components, 'tonic' responses were more affected than 'phasic' ones in quinacrine-loaded SA type I units.

6. Stimulus–response curves shifted to the right and downwards in SA type I units with quinacrine-loaded Merkel cells after irradiation, but no significant change was seen in SA type I units without quinacrine.

7. Our observations are consistent with the hypothesis that Merkel cells are responsible for mechanoelectric transduction in SA type I units.

Merkel cells are present at the basal layer of the epidermis in vertebrates from fish to mammals and are characterized by an interdigitated nucleus and large dense-cored granules (Cauna, 1962; Iggo & Muir, 1969; Fox & Whitear, 1978). Clusters of Merkel cells are associated with mechanosensitive spots and are prominent at touch domes in the hairy skin of mammals (Haarscheiben) (Iggo & Muir, 1969; Munger, Pubols & Pubols, 1971; Smith, 1977). Merkel cells make synaptic contacts with cutaneous afferent fibres to form Merkel cell–neurite complexes which are physiologically identified as slowly adapting type 1 (SA type 1) units (Iggo & Muir, 1969).

Based on these findings it has been suggested that Merkel cells are mechanoelectric transducers, though no direct evidence has supported this thesis. Merkel cells are assumed to be targets of regenerating nerve endings in the denervated skin of the salamander (Scott, Cooper & Diamond, 1981) or paracrine regulators of the surrounding epidermal and adnexal structures (Weber, Hartschuh, Feurle & Weihe, 1980). In the latter, the nerve terminals making contact with Merkel cells are considered to be true transducers (Diamond, Mills & Mearow, 1988).

A relatively new approach is being used to kill carcinoma cells; i.e., irradiation by excitation light of carcinoma cells loaded with fluorescent dyes (Dougherty, Kaufman, Goldfarb, Weishaupt, Boyle & Mittelman, 1978; Manyak, Russo, Smith & Glatstein, 1988; Gomer, Rucker, Ferrario & Wong, 1989). Merkel cells can be loaded with the fluorescent dye quinacrine (Crowe & Whitear, 1978) *in vivo* and hence selective destruction of these cells is feasible.

The mechanical responses of SA type I units before and after selective destruction of Merkel cells loaded with quinacrine were examined to observe whether Merkel cells have a mechanoreceptive function. We also histologically examined the Merkel cells in the touch dome to search for possible degeneration.

METHODS

Animals and surgery

Albino Sprague–Dawley rats of either sex, weighing 180–420 g, were used. The animals were anaesthetized with urethane (1 g kg^{-1}, I.P.) and placed prone on a plate equipped with a micromanipulator for recording electrodes. Hair on the dorsum was clipped off, and a parasagittal incision 4–5 cm long was made at the left side of vertebrae. A small strand of the dorsal cutaneous nerves from L1 to L6 segments was then isolated from the surrounding connective tissue and placed on a pair of Ag–AgCl electrodes in a paraffin pool. Impulse discharges of single slowly adapting (SA) type I units in response to mechanical stimulation of touch domes were recorded using conventional physiological equipment consisting of a preamplifier and a cathode-ray oscilloscope.

Recording

Impulse discharges were fed into a microcomputer (NEC PC-8001 MKII) through a window discriminator. The responses were also recorded on FM instrumentation tape, together with trigger pulses for subsequent off-line data analysis. Identification of SA type I units was made when the units had receptive fields localized on touch domes, and showed irregular discharges in the tonic component of responses to maintained displacement at the touch domes (Iggo & Muir, 1969). Touch domes innervated by the unit under study were located with calibrated von Frey hairs.

Mechanical stimulation

We used a glass rod with a tip diameter of 500 μm, which is large enough to cover one whole touch dome and to activate SA type I units. The rod was attached to a moving coil transducer (Ling MSE 101 vibrator) that provided controlled mechanical stimulation (Iggo & Muir, 1969; Ogawa, Morimoto & Yamashita, 1981).

The standard mechanical stimulus used was a 100 ms ramp followed by a 2·9 s plateau of 400 μm constant displacement. Mechanical thresholds of SA type I units were examined with calibrated von Frey hairs.

Photoexcitation procedure

In rats administered quinacrine dihydrochloride (MW 472·9; Sigma, USA), we gave the drug dissolved in physiological saline intraperitoneally at a dose of 10–15 mg (kg body weight)$^{-1}$, 1 or 2 days before the start of experiments.

Excitation of fluorescent dye which had accumulated at Merkel cells in the touch domes was achieved by illuminating the touch domes of the rat skin with excitation light from the surface side. We obtained excitation light emitted from a fluorescent microscope with a 200 W ultra-high pressure mercury lamp (VFD-R, Nikon). This microscope gave a light spot of 2 mm diameter on the rat skin and the beam intensity was 0·25 J s^{-1} (measured with a UV radiometer (UVR-1; (Topcon, Tokyo) equipped with a sensor having a sensitive range of 360–480 nm (UVR-40; Topcon)). The light was filtered through a 'B' filter (passing wavelengths ranging from 420 to 490 nm) to obtain excitation light ('B' light), which includes one of the excitation wavelength maxima for quinacrine (excitation wavelength maxima for quinacrine are 285 and 420 nm; Udenfriend, Duggan, Vasta & Brodie, 1957). As controls, 'UV' or 'G' excitation light was also obtained by passing the light through a 'UV' filter to give wavelengths of 315–400 nm or a 'G' filter to give wavelengths of 500–550 nm.

Protocol of experiment

The standard protocol used was as follows. First, we recorded responses (at least 3 times) to the standard mechanical indentations applied to one of the touch domes innervated by an SA unit under study, prior to irradiation. The intertrial interval was 1 min or longer. After irradiation of the dome with an excitation light for 5 or 10 min, we again recorded responses to the standard displacements. We repeated this protocol until the spike disappeared, or until the total irradiation time had reached 60 min.

Data analysis

The numbers of spikes and interspike intervals were analysed using a microcomputer. All results were individually normalized to the control responses obtained before irradiation, to eliminate inherent variations between individual mechanoreceptors. Student's t test was used for statistical analysis.

Histology

The touch domes were marked with pairs of tungsten wires (100 μm in diameter) and resected, the tissues were transferred to a fixative (4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer), then to acetate-buffered 2% osmium tetroxide for post-fixation and embedded in Epok 812 (Oken Shoji, Japan) after removing the wires. Semithin sections (1 μm) were obtained using a Porter-Blum MT2-B ultramicrotome and stained with Toluidine Blue. Ultrathin sections mounted on grids were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (HU-12A, Hitachi).

In some experiments, intact touch domes were marked with pairs of tungsten wires, resected, and fixed immediately after several minutes of irradiation.

RESULTS

Histological changes

Light microscopy (semithin section)

In both control and treated specimens, Merkel cells were identified on the basis of the following criteria: cells were elliptical with lobulated nuclei, lightly stained with Toluidine Blue and located just at the basal cell layer with their main axis usually parallel to that of the epidermis (Iggo & Muir, 1969).

In comparison to findings in non-irradiated touch domes (shown by arrowheads, Fig. 1*A*), all the Merkel cells from touch domes irradiated for 10 min had an expanded cytoplasm and changed from the elliptical to the round form, thereby indicating cellular swelling (shown by arrowheads, Fig. 1*B*).

Electron microscopy

An electron microscopic picture of a normal touch dome is presented in Fig. 2*A*. A typical Merkel cell with a lobulated nucleus, cytoplasmic processes between keratinocytes and numerous dense-cored granules in the cytoplasm is seen near the basement membrane. Between the Merkel cell and the basement membrane, a nerve terminal rich with mitochondria is identified.

After irradiation with excitation light, quinacrine-loaded Merkel cells exhibited drastic changes, though they were still identifiable with typical granules. Such Merkel cells are presented in Fig. 2*B* (after 5 min irradiation) and *C* (after 10 min irradiation). The Merkel cell (Me) in Fig. 2*B* was heavily swollen with reticular cytoplasm, and the cell size was increased and invaginated by an associated nerve terminal (Ne), whereas another Merkel cell in Fig. 2*C* had small vacuoles in the periphery of the cytoplasm and a pyknotic nucleus, signs of irreversible degeneration. However, in both *B* and *C* no obvious sign of degeneration was noted in the associated nerve terminal, nor in the surrounding keratinocytes.

Physiological changes

Effects of irradiation on quinacrine-loaded and non-loaded Merkel cells

Ten slowly adapting (SA) type I units with touch domes with quinacrine-loaded Merkel cells (Q(+)) and thirteen SA type I units with non-treated Merkel cells (Q(−)) were examined for the effects of 'B' light irradiation on neural responses to mechanical stimulation. Before irradiation, all these units produced a burst of discharges followed by maintained irregular discharges in response to mechanical stimulation. There was no significant difference in the number of discharges between Q(+) and Q(−) units.

Typical neural responses of SA type I units with Q(+) or Q(−) Merkel cells are shown in Fig. 3. In Q(+) units, the

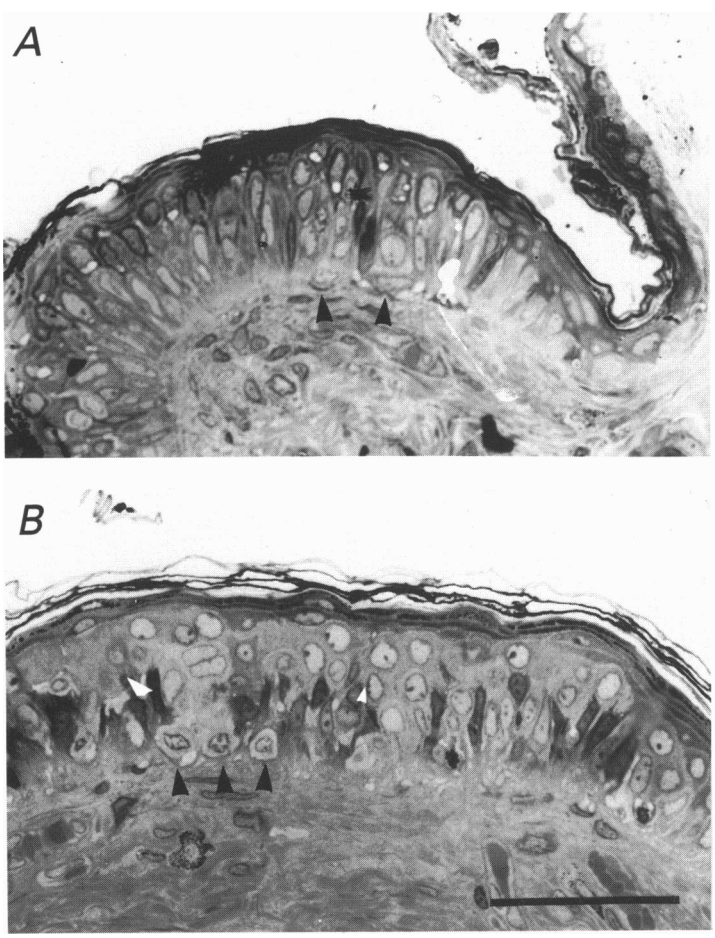

Figure 1. Light microscopic photographs of Merkel cells in the touch dome before and after treatment (semithin section; Toluidine Blue staining)
Scale bar represents 50 mm. *A*, photo from intact specimen. Arrowheads indicate Merkel cells. *B*, a specimen from quinacrine-loaded rat after irradiation with 'B' excitation light. Arrowheads indicate Merkel cells with enlargement of the cytoplasmic volume.

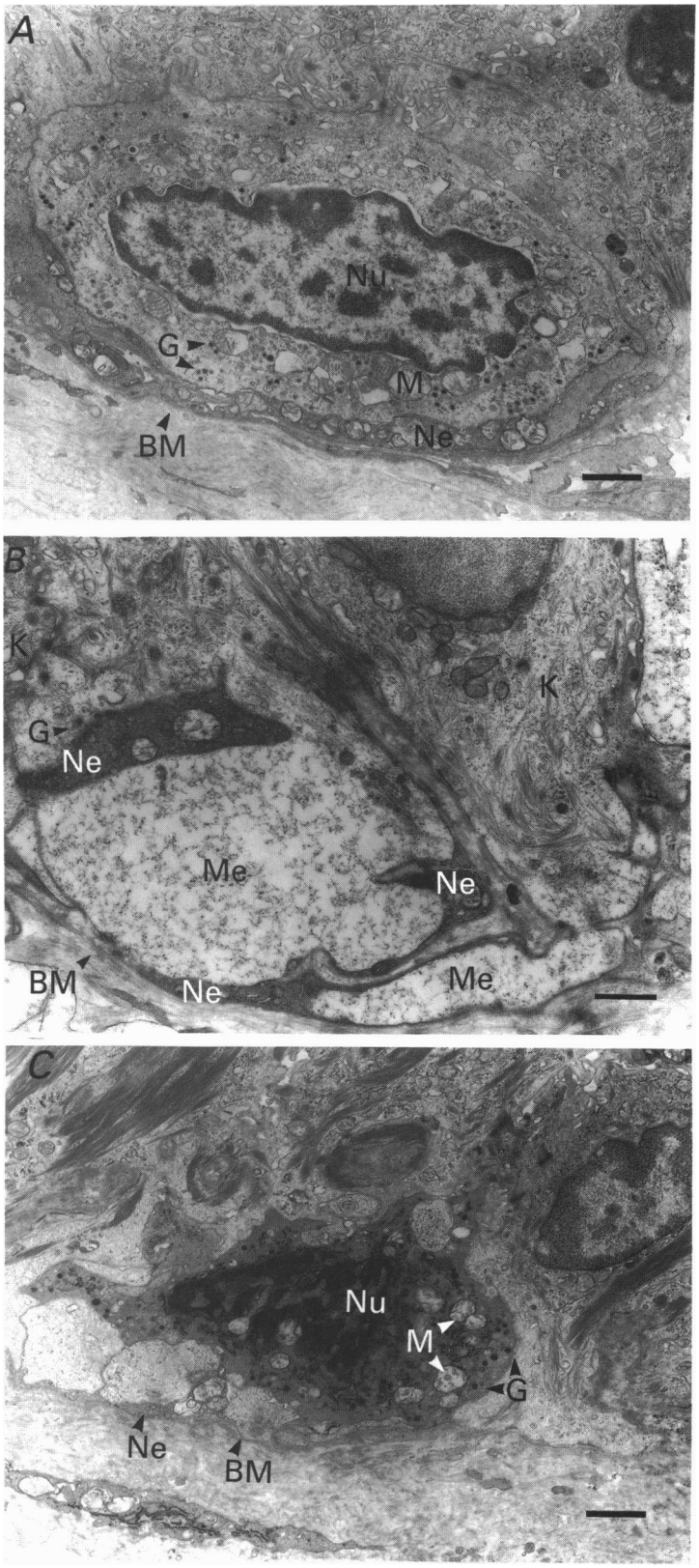

Figure 2. Electron microscopic (EM) pictures of quinacrine-loaded Merkel cells in the touch dome before and after irradiation with 'B' excitation light

Scale bars represent 1 mm. *A*, EM picture of an intact Merkel cell. *B*, EM picture of a degenerated Merkel cell after 5 min irradiation. An intact nerve ending appeared at three points, invaginating the swollen Merkel cell. *C*, EM picture of a degenerated Merkel cell after 10 min irradiation. Taken from the same specimen as in Fig. 1*B*. BM, basement membrane; K, keratinocyte; M, mitochondria; Me, Merkel cell; Nu, nucleus; G, dense-cored granules; Ne, nerve ending.

number of discharges decreased, especially in the tonic component after irradiation. The average response in the 3 s of stimulation was reduced to 52 ± 7% (mean ± S.E.M.) of the control value with a 5 min irradiation and to 17 ± 4% with a 10 min treatment (Fig. 4). The prolonged irradiation made SA type I units behave like rapidly adapting units, by totally eliminating the tonic responses. With 50 min of irradiation, SA type I units showed no neural responses. In contrast, the responses of Q(−) units were not affected by irradiation for up to 10 min and even increased at 5 min (119 ± 8% of the control with 5 min irradiation and 99 ± 9% with 10 min), though the increase was not statistically significant ($P > 0.05$). Prolonged irradiation (longer than 15 min) affected the responses: eleven of thirteen units. All units failed to respond after 60 min of irradiation. There were statistically significant differences in response between the two groups of units with Q(+) or Q(−) Merkel cells at 5, 10 and 15 min ($P < 0.001$ at 5 and 10 min, $P = 0.001$ at 15 min, and $0.01 < P < 0.05$ at 20 min).

Mechanical thresholds did not change in all the thirteen Q(−) units until total loss of responses had occurred. However, among the nine Q(+) units studied, increases in threshold by more than 1 mg, when measured by von Frey hairs, were seen in four units at 5 min and seven units at 10 min. The difference of frequency in threshold increase between Q(−) and Q(+) units was statistically significant ($0.025 < P < 0.05$ at 5 min and $P < 0.001$ at 10 min, χ^2 test).

Phasic and tonic responses

Close examination of Fig. 3 shows that the reduction of responses in SA type I units with Q(+) Merkel cells mainly occurred >120 ms after the onset of stimulation. After dividing a train of impulses into two parts, phasic (0–120 ms after the onset of stimulation) and tonic (120–3000 ms), we examined the effects of excitation light on the two components of responses in Q(+) or Q(−) Merkel cells (Fig. 5).

In Q(+) units, phasic responses were reduced, depending on the cumulative amount of irradiation, but survived

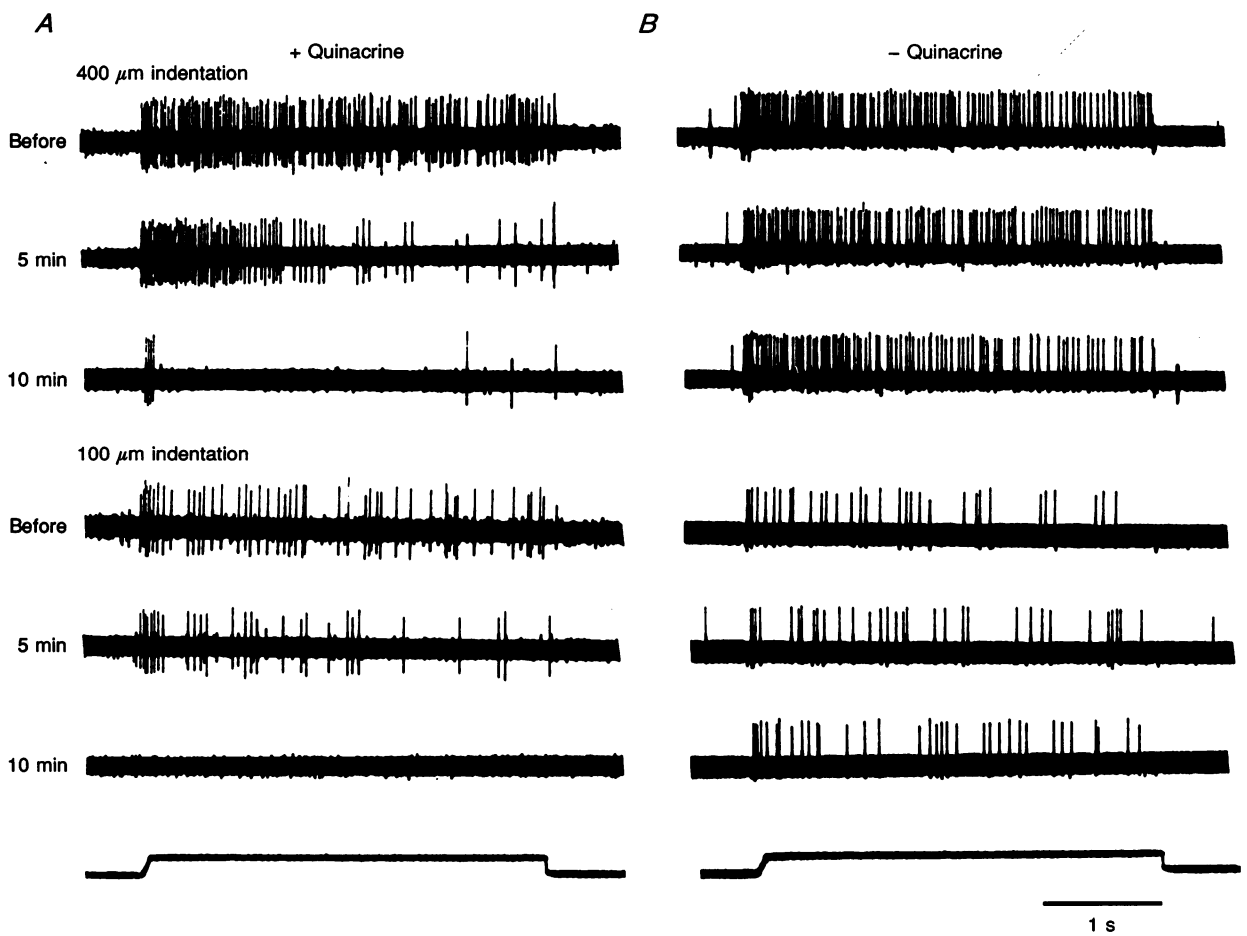

Figure 3. Effects of 'B' excitation light irradiation on afferent discharges in response to mechanical indentation (400 μm and 100 μm) of cutaneous slowly adapting type I units
Responses from units innervating quinacrine-loaded Merkel cells (*A*) and non-loaded control cells (*B*). Bottom traces show the time course of standard stimuli (a 100 ms ramp followed by a 2·9 s plateau of 400 μm constant displacement). Duration of irradiation is shown on the left of each trace.

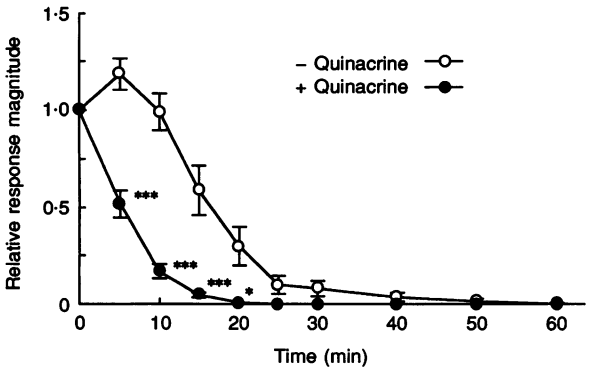

Figure 4. Changes in responses to standard mechanical stimulation (400 μm) during irradiation with 'B' excitation light

○, mean responses of 13 SA type I units without quinacrine; ●, mean responses of 10 SA type I units innervating Merkel cells loaded with quinacrine. Vertical bars represent S.E.M. The number of asterisks indicates degrees of significant mean difference between control and treated groups: * significant at the level of 5% (Student's *t* test); *** significant at the level of 0·1% (Student's *t* test).

25 min of irradiation. The tonic responses were abolished within 15 min.

The difference between SA type I units with Q(+) and Q(−) cells was statistically significant during both phasic and tonic components (Fig. 5*A* and *B*; $0{\cdot}01 < P < 0{\cdot}05$ at 5 min and $0{\cdot}001 < P < 0{\cdot}01$ at 10, 15 and 20 min for phasic responses; $P < 0{\cdot}001$ at 5–15 min and $0{\cdot}01 < P < 0{\cdot}05$ at 20 min for tonic responses). As in Q(+) units, however, the percentage decrease in the responses of Q(−) units was larger at the tonic than at the phasic component; for example, 55 *vs.* 13% at 5 min, 87 *vs.* 51% at 10 min.

Stimulus–response curve

Responses to a smaller indentation (e.g. 100 μm) were more easily affected than those to 400 μm indentation, as shown in Fig. 3. We studied the relationship between indentation magnitudes and responses in six Q(+) and five Q(−) units before and after irradiation with 'B' excitation light (Fig. 6).

Reduced responses were seen at all indentation magnitudes in units with Q(+) Merkel cells. With increased periods of irradiation, the indentation magnitude–response curve shifted to the right and downward. In units with Q(−) Merkel cells, the relationship was not affected by 10 min or less of irradiation.

Wavelength of excitation light

To assess the action point of excitation light in the Merkel cell–neurite complex, we examined the effects of excitation light with different spectra of wavelength: excitation light with longer ('G' light) or shorter wavelengths ('UV' light).

In the SA type I units with Q(+) Merkel cells (Fig. 7*A*), irradiation with 'G' light ($n = 5$) did not affect the responses even after 60 min of radiation. The difference

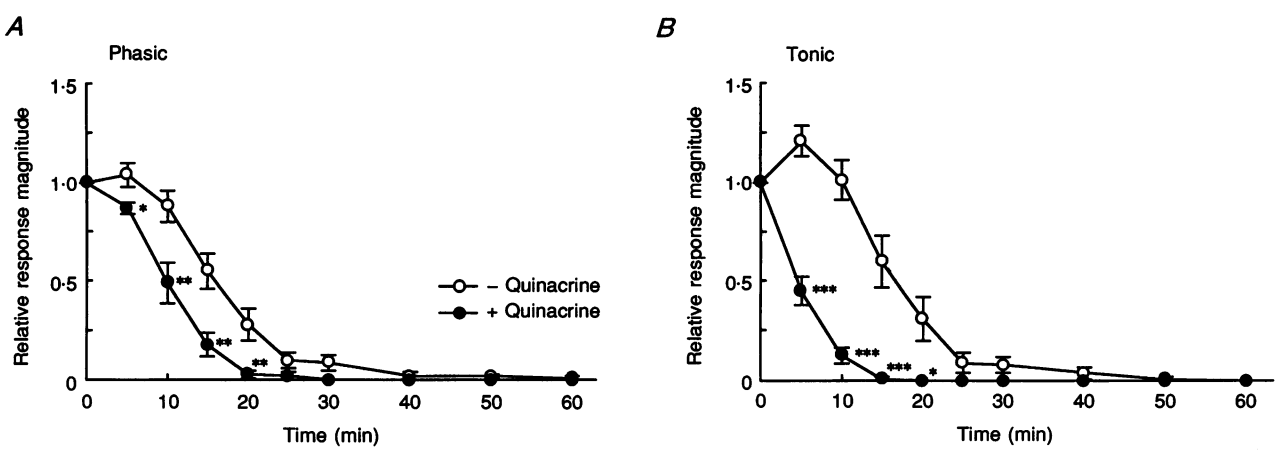

Figure 5. Changes in phasic (*A*) and tonic (*B*) responses of SA type I units after irradiation

Discharges in 0–120 ms and 120–3000 ms after the onset of mechanical stimulation were referred to as phasic and tonic responses. Data shown in Fig. 4 are re-plotted. Symbols are the same as those in Fig. 4. * significant at the level of 5% (Student's *t* test); ** significant at the level of 1% (Student's *t* test); *** significant at the level of 0·1% (Student's *t* test). Vertical bars represent S.E.M.

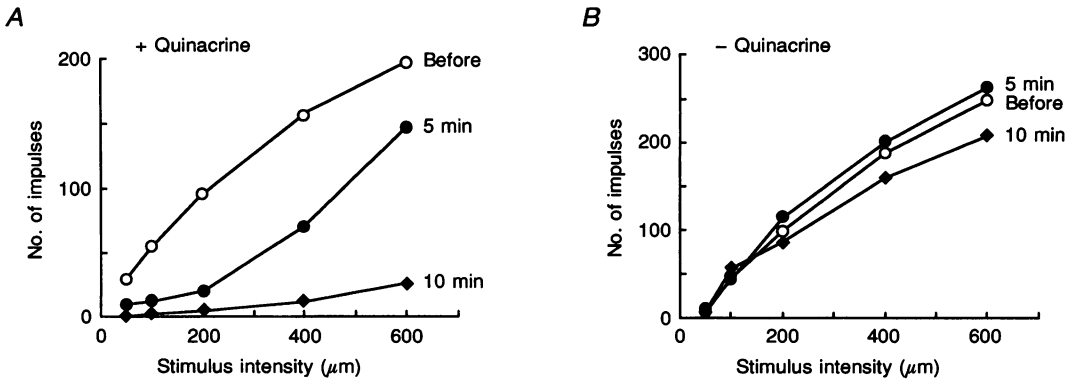

Figure 6. Effects of irradiation on stimulus–response curves in SA type I units with quinacrine-loaded Merkel cells (*A*) and with non-loaded cells (*B*)
The total number of impulses discharged during stimulation is plotted against stimulus intensity. Different symbols indicate different durations of irradiation.

between 'B' and 'G' light-irradiated units was statistically significant at all observed points ($P < 0{\cdot}001$ at 5 min and other points, Student's t test). Responses from units irradiated with 'UV' light ($n = 4$) began to decrease at 10 min and were abolished within 40 min. In comparison with 'G' light irradiation, effects of 'UV' light were significant at 15–60 min ($P < 0{\cdot}001$). However, the effect of 'UV' light was significantly smaller than that of 'B' light over 5–25 min ($0{\cdot}001 < P < 0{\cdot}01$ at 5 min, $P < 0{\cdot}001$ at 10–20 min, $0{\cdot}001 < P < 0{\cdot}01$ at 25 min).

In the Q(–) group (Fig. 7*B*), 'G' irradiaton for 60 min produced no reduction in responses in any unit studied ($n = 4$). The difference in response between units irradiated with 'B' and 'G' excitation light was significant at 20 min and later periods ($0{\cdot}001 < P < 0{\cdot}01$ at 20 min and $P < 0{\cdot}001$ at 25–60 min). The response of all the five 'UV'-irradiated units was reduced at 20 min and became progressively smaller with increased periods of irradiation. The response disappeared at 60 min in two of the five units. The difference in responses between 'UV' and 'G' irradiated groups was significant at 20 min and later periods ($P < 0{\cdot}05$ at 20 min, $P < 0{\cdot}01$ at 25 and 30 min, $P < 0{\cdot}001$ at 40–60 min). There was a significant difference in responses between the 'B' and 'UV' light-irradiated units at 25, 30 and 40 min ($P < 0{\cdot}001$ at 25 min, $0{\cdot}001 < P < 0{\cdot}01$ at 30 and 40 min).

Spontaneous discharges during irradiation

We recorded spontaneous discharges during irradiation with 'B' excitation light in three Q(+) units to examine

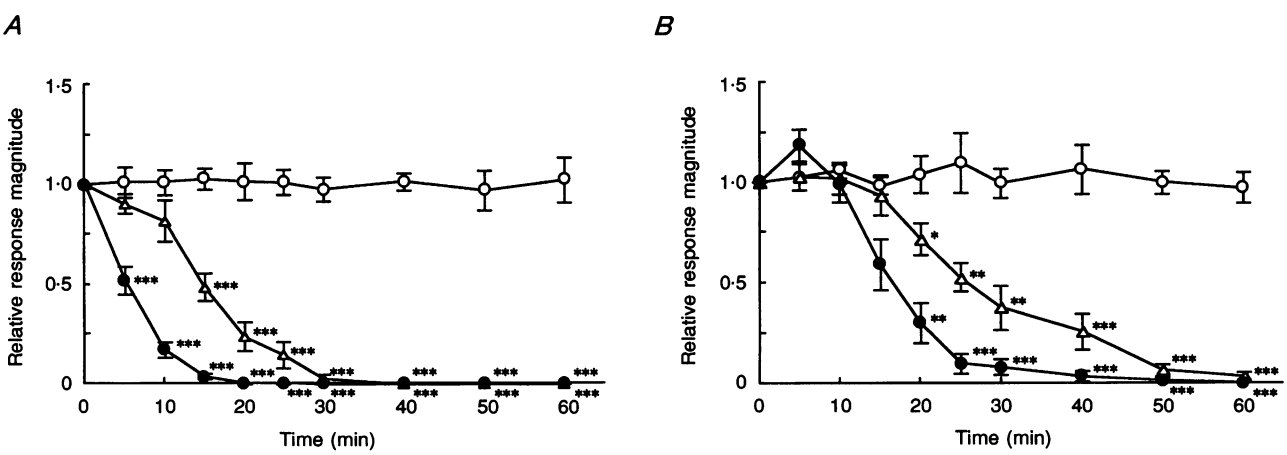

Figure 7. Effects of excitation light with various wavelength spectra on mechanical responses of SA type I units with quinacrine-loaded (*A*) and unloaded (*B*) Merkel cells
○, 'G' filtered light (500–550 nm); △, 'UV' filtered light (315–400 nm); ●, 'B' filtered light (420–490 nm). Different numbers of asterisks indicate different degrees of mean difference in mean responses between a pair of groups irradiated by 'G' and 'UV' light or a pair of groups irradiated by 'G' and 'B' light: * significant at the level of 5% (Student's t test); ** significant at the level of 1% (Student's t test); *** significant at the level of 0·1% (Student's t test). Vertical bars represent S.E.M.

whether changes in discharges would occur during irradiation. No evident change was detected in the rate of spontaneous discharges during irradiation.

DISCUSSION

Discrepancy between present and previous studies

In the present study, the responses of slowly adapting (SA) type I units to mechanical stimulation of touch domes in rats were abolished by excitation of quinacrine previously loaded into Merkel cells. Our results are not compatible with the conclusions of Diamond and colleagues in studies on *Xenopus* (Mills, Mearow, Visheau & Diamond, 1985; Mearow & Diamond, 1988) and rats (Mills *et al.* 1985; Diamond *et al.* 1988). They stated that the response threshold remained at the 'near-normal' level after phototoxic lesioning of Merkel cells and concluded that Merkel cells were not involved in mechanoreception.

The discrepancy between our results and theirs is partly due to differences in experimental procedure. Firstly, they used a small dose of excitation light (intensity of 1·875 mW for 5–10 min for *Xenopus*; Mearow & Diamond, 1988) to injure dye-loaded cells immediately, a dose which was at least 67 times weaker than ours (75 J cm^{-2} of 'B' light irradiation for 5 min). A few seconds' irradiation with our present dose was sufficient to bleach the fluorescent dye phosphine in isolated rat alveolar type II cells (DeCoursey, Jacobs & Silver, 1988). Data on excitation energy are not available for their experiments using rats. Secondly, they examined mechanical thresholds by recording a single spike discharged in response to a small mechanical pulse of a short duration (2 ms), whereas we measured the total number of impulses evoked in response to a 3 s constant displacement.

With a small dose of irradiation, Diamond and others observed no immediate effect on mechanical thresholds of Merkel cell-associated units or on the morphology of Merkel cells. In the present study, however, the immediate effect of irradiation with the large dose of excitation light on the SA units of rats was evident . The mechanical thresholds of the units were also changed in our experiment. Mechanical thresholds after 5 min irradiation did not change noticeably, despite a 50% reduction in total responses to a 3 s displacement, but 10 min irradiation raised the mechanical threshold and 20 min irradiation totally abolished the responses, including the phasic components.

In the experiments of Diamond and others, several days after irradiation, most Merkel cells had degenerated but mechanical thresholds were almost normal (Mearow & Diamond, 1988; DeCoursey *et al.* 1988). Despite their extensive survey of Merkel cells using electron microscopy, we think that some of the Merkel cells may have physiologically survived this small dose of irradiation.

Mechanism of degeneration of quinacrine-loaded Merkel cells by excitation light

Quinacrine-loaded Merkel cells were selectively destroyed by irradiation of touch domes, using light wavelengths which maximally excite quinacrine ('B' light). Ultrastructural examination demonstrated that these Merkel cells had degenerated cytoplasm and pyknotic nuclei, a sign of extensive damage to organelles and nuclear structures. There was no obvious damage in the nerve terminals or adjacent keratinocytes.

Such histological findings have been observed in mice tumour tissues loaded with a photosensitizer which easily permeates the cell membrane and becomes exposed to excitation light (Peng, Moan, Nesland & Rimington, 1990). Several types of organelles and nuclear structures were damaged by direct phototoxic action within 1 h of the treatment. The photosensitizers generate singlet oxygen on irradiation, and singlet oxygen damages cell structures, e.g. cell membranes and DNA *in vivo* (Ito, 1978). In contrast, another type of photosensitizer which does not easily permeate the cell membrane damaged the vascular structure around the tissue (Peng *et al.* 1990).

Quinacrine is a 9-alkylaminoacridine and a photosensitizer (Oster, Bellin, Kimball & Schrader, 1959), as are other members of the acridine group, e.g. acridine orange or proflavine (Ito, 1978). Quinacrine accumulates in Merkel cells in the epidermis (Nurse, Mearow, Holmes, Visheau & Diamond, 1983). The selective degeneration of Merkel cells in our experiments means that the intracellularly accumulated quinacrine became phototoxic on irradiation with excitation light, as is the case with other photosensitizers.

The brightest fluorescence of quinacrine is known to be localized around the periphery of the cytoplasm, an area where the Merkel cell granules accumulate (Nurse *et al.* 1983). No degeneration was found in the cytoplasm rich with cored vesicles. Many cored vesicles were clearly evident after photoexcitation.

As nerve endings probably share ultrastructural features with Merkel cells, it may be that the nerve endings take up quinacrine and undergo cellular destruction following irradiation with excitation light. However, we found no damaged nerve terminals in our ultrastructural examinations.

Causes of decreased responses of SA type I units in rats without quinacrine after irradiation with excitation light

Irradiation with 'B' light lasting up to 15 min did not affect responses of units not treated with quinacrine, but when the irradiation period exceeded 15 min the responses began to decrease, as with Q(+) units. These observations suggest the existence of photosensitive molecular mechanisms within the cells. Taking account of the wavelength spectra of excitation light, it is suggested that

several photosensitive agents with excitation maxima around the 'UV' or 'B' range are playing an important role in the metabolism of Merkel cells.

Some components of living cells are known to act as photosensitizers *in vivo*. 'Endogenous' photosensitizers with emission maxima around 290–400 nm include riboflavin, NADH, NAD^+ and others (Peak, Peak & MacCoss, 1984). In experiments on human keratinocytes in culture, irradiation with 15 J cm^{-2} (total dose) of ultraviolet light with spectra similar to our 'UV' light (315–400 nm) caused significant changes in the activities of antioxidant enzymes (Punnonen, Jansén, Puntala & Ahotupa, 1991). The amount of 'UV' light we used was calculated to be 6·5 J min^{-1} cm^{-2}. Thus, irradiation of light near the UV range at a high intensity for a long period ($\geqslant$10 min) may excite endogenous photosensitizers and damage cells, even those not loaded with exogenous photosensitizers.

Correlation between damaged Merkel cells and altered responses of SA type I units: functions of Merkel cells

Closely correlated with selective destruction of quinacrine-loaded Merkel cells in touch domes, irradiation of the touch domes with excitation light abolished the mechanical responses of SA type I units. It would thus appear that Merkel cells are essentially involved in mechanoelectric transduction.

Reduction of the responses was more severe during the tonic than during the phasic component in units with Q(+) Merkel cells, a finding compatible with observations in frogs (Yamashita, Ogawa & Taniguchi, 1986), in which Ca^{2+} blockers affected the tonic responses of mechanoreceptor units comparable to mammalian SA type I units. The present results are compatible with the two transduction site hypothesis, i.e. one at the Merkel cells and one at the nerve terminal (Yamashita & Ogawa, 1991). Our EM findings showed that the nerve terminals did survive the treatment of 5 and 10 min irradiation when mechanical responses were significally decreased to *ca.* 50 or 20% of the control value. The abolition of mechanical responses of SA type I units with 20 min or more of irradiation suggests the final involvement of the nerve terminals, in addition to Merkel cells.

The reduction in tonic responses may be partly due to depletion of transmitters by irradiation of excitation light as in the case of depletion of substance P with capsaicin (Theriault, Otsuka & Jessell, 1979; Gamse, Molnar & Lembech, 1979). When we examined changes in spontaneous discharge rates in three SA type I units during irradiation, we found no change in the rates. Histologically, abundant synaptic vesicles were observed in the damaged Merkel cells, in accordance with physiological findings.

The present findings are consistent with the hypothesis that Merkel cells are mechanoreceptor cells. This notion is compatible with a vast amount of previous circumstantial evidence, e.g. irregular discharge patterns of cat SA type I units suggesting release of transmitters from Merkel cells (Horch, Whitehorn & Burgess, 1974); selective decreases of mechanical responses of SA type I units by perfusion of Ca^{2+} channel blockers (Yamashita *et al.* 1986); correlation of decreased responses of SA type I units with reduction in Merkel cell granules in a hypoxic environment (Findlater, Cooksey, Anand, Paintal & Iggo, 1987); susceptibility of SA type I units to neomycin suggesting the presence of functional synapses from Merkel cells to nerve terminals (Baumann, Hamann & Leung, 1990). The possibility has been raised that the nerve terminal is mechanoreceptive under certain conditions, such as mechanical stimulation with high frequency components (Gottschaldt & Vahle-Hinz, 1981). Whether or not the nerve terminals themselves are involved in mechanoreception remains to be determined.

REFERENCES

Baumann, K. I., Hamann, W. & Leung, M. S. (1990). Acute effects of neomycin on slowly adapting type I and type II cutaneous mechanoreceptors in the anaesthetized cat and rat. *Journal of Physiology* **425**, 527–544.

Cauna, N. (1962). Functional significance of the submicroscopical, histochemical and microscopical organization of the cutaneous receptor organs. *Anatomische Anzeiger* **111**, suppl. 2, 181–187.

Crowe, R. & Whitear, M. (1978). Quinacrine fluorescence of Merkel cells in *Xenopus laevis*. *Cell and Tissue Research* **190**, 273–283.

DeCoursey, T. E., Jacobs, E. R. & Silver, M. R. (1988). Potassium currents in rat type II alveolar epithelial cells. *Journal of Physiology* **395**, 487–505.

Diamond, J., Mills, L. R. & Mearow, K. M. (1988). Evidence that Merkel cell is not the transducer in the mechanosensory Merkel cell–neurite complex. *Progress in Brain Research* **74**, 51–56.

Dougherty, T. J., Kaufman, J. E., Goldfarb, A., Weishaupt, K. R., Boyle, D. & Mittelman, A. (1978). Photoradiation therapy for the treatment of malignant tumors. *Cancer Research* **38**, 2628–2635.

Findlater, G. S., Cooksey, E. J., Anand, A., Paintal, A. S. & Iggo, A. (1987). The effects of hypoxia on slowly adapting type I (SAI) cutaneous mechanoreceptors in the cat and rat. *Somatosensory Research* **5**, 1–17.

Fox, H. & Whitear, M. (1978). Observation of Merkel cells in amphibians. *Biologie Cellulaire* **32**, 223–232.

Gamse, R., Molnar, A. & Lembech, F. (1979). Substance P release from spinal cord slices by capsaicin. *Life Sciences* **25**, 629–636.

Gomer, C. J., Rucker, N., Ferrario, A. & Wong, S. (1989). Review: properties and applications of photodynamic therapy. *Radiation Research* **120**, 1–18.

Gottschaldt, K. M. & Vahle-Hinz, C. (1981). Merkel cell receptors: structure and transducer function. *Science* **214**, 183–186.

Horch, K. W., Whitehorn, D. & Burgess, P. R. (1974). Impulse generation in type I cutaneous mechanoreceptors. *Journal of Neurophysiology* **37**, 267–281.

Iggo, A. & Muir, A. R. (1969). The structure and function of a slowly adapting touch corpuscle in hairy skin. *Journal of Physiology* **200**, 763–796.

Ito, T. (1978). Cellular and subcellular mechanisms of photodynamic action: the $^1O_2$ hypothesis as a driving force in recent research. *Photochemistry and Photobiology* **28**, 493–508.

MANYAK, M. J., RUSSO, A., SMITH, P. D. & GLATSTEIN, E. (1988). Photodynamic therapy. *Journal of Clinical Oncology* **6**, 380–391.

MEAROW, K. M. & DIAMOND, J. (1988). Merkel cells and the mechanosensitivity of normal and regenerating nerves in *Xenopus* skin. *Neuroscience* **26**, 695–708.

MILLS, L., MEAROW, K. M., VISHEAU, B. & DIAMOND, J. (1985). Are Merkel cells the mechanosensory transducers in rat touch domes and *Xenopus* touch spots? *Society for Neuroscience Abstracts* **15**, 36.3.

MUNGER, B. L., PUBOLS, L. M. & PUBOLS, B. H. (1971). The Merkel rete papilla, a slowly adapting sensory receptor in mammalian glabrous skin. *Brain Research* **29**, 47–61.

NURSE, C. A., MEAROW K. M., HOLMES M., VISHEAU B. & DIAMOND, J. (1983). Merkel cell distribution in the epidermis as determined by quinacrine fluorescence. *Cell and Tissue Research* **228**, 511–524.

OGAWA, H., MORIMOTO, K. & YAMASHITA, Y. (1981). Physiological characteristics of low threshold mechanoreceptor afferent units innervating frog skin. *Quarterly Journal of Experimental Physiology* **66**, 105–116.

OSTER, G., BELLIN, J. S., KIMBALL, R. W. & SCHRADER, M. E. (1959). Dye sensitized photoöxidation. *Journal of the American Chemical Society* **81**, 5095–5099.

PEAK, J. G., PEAK, M. J., MACCOSS, M. (1984). DNA breakage caused by 334-nm ultraviolet light is enhanced by naturally occurring nucleic acid components and nucleotide coenzymes. *Photochemistry and Photobiology* **39**, 713–716.

PENG, Q., MOAN, J., NESLAND, J. M. & RIMINGTON, C. (1990). Aluminum phthalocyanines with asymmetrical lower sulfonation and with symmetrical higher sulfonation: a comparison of localizing and photosensitizing mechanism in human tumor LOX xenografts. *International Journal of Cancer* **46**, 719–726

PUNNONEN, K., JANSÉN, C. T., PUNTALA, A. & AHOTUPA, M. (1991). Effects of *in vitro* UVA irradiation and PUVA treatment on membrane fatty acids and activities of anti-oxidant enzymes in human keratinocytes. *Journal of Investigative Dermatology* **96**, 255–259.

SCOTT, S. A., COOPER, E. & DIAMOND, J. (1981). Merkel cells as target of the mechanosensory nerves in salamander skin. *Proceedings of the Royal Society* **211**, 455–470.

SMITH, K. R. JR (1977). The Haarscheibe. *Journal of Investigative Dermatology* **69**, 68–74.

THERIAULT, E., OTSUKA, M. & JESSELL, T. (1979). Capsaicin-evoked release of substance P from primary sensory neurons. *Brain Research* **170**, 209–213.

UDENFRIEND, S., DUGGAN, D. E.,VASTA, B. M. & BRODIE, B. B. (1957). A spectrophotofluorometric study of organic compounds of pharmacological interest. *Journal of Pharmacology and Experimental Therapeutics* **120**, 26.

WEBER, U., HARTSCHUH, W., FEURLE, G. E. & WEIHE, E. (1980). Met-enkephalin-like immuno- and bioreactivity in extracts from skin containing Merkel cells. *Neuroscience Letters* **20**, 201.

YAMASHITA, Y., OGAWA, H. & TANIGUCHI, K. (1986). Differential effects of manganese and magnesium on two types of slowly adapting cutaneous mechanoreceptor afferent units in frogs. *Pflügers Archiv* **406**, 218–224.

YAMASHITA, Y. & OGAWA, H. (1991). Slowly adapting cutaneous mechanoreceptor afferent units associated with Merkel cells in frogs and effects of direct currents. *Somatosensory and Motor Research* **8**, 87–95.

Acknowledgements

We thank M. Ohara for reading drafts of the manuscript.

Received 21 January 1993; accepted 28 January 1994