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Chloroquine specifically impairs Merkel cell mechanoreceptor function in isolated rat sinus hairs

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Abstract

The function of Merkel cells in mechanotransduction has remained controversial. Single unit recordings were made from Merkel cell receptors (sinus hair type I, St I) and another slowly adapting mechanoreceptor (sinus hair type II, St II) in isolated rat sinus hairs by applying controlled mechanical displacements to the hair shaft. Chloroquine (50–300 μM) caused a concentration dependent inhibition of Merkel cell receptor responses to mechanical stimulation. In contrast, both stimulated and spontaneous spike activity of St II receptors was increased by the same concentrations of chloroquine. Ultrastructural examination of chloroquine treated sinus hairs revealed swollen Merkel cells with multiple vacuoles and randomly distributed granules while other neural and surrounding structures showed no striking morphological changes. These results suggest that the Merkel cell plays a mechanotransducer role in Merkel cell receptors.

Keywords: Vibrissae; Merkel cells; Slowly-adapting mechanoreceptors; Mechanotransduction

The Merkel cell nerve ending is unique among mechanoreceptors in the skin and its appendages in that it has a specialized cell with which the expanded nerve terminal makes synaptiform contact. In sinus hairs Merkel cell nerve endings are found in the external root sheath of the hair follicle at the level of the ring sinus, lying next to the glassy membrane. They are innervated by myelinated fibres of the deep vibrissal nerve (DVN), with one fibre supplying anything from 8 to 50 Merkel cells (for review see [8]). A single Merkel cell receptor unit (sinus hair type I, St I) consists of a cluster of Merkel cells innervated by terminal endings arising from one myelinated afferent axon. A second slowly adapting receptor called sinus hair type II (St II, [7]) is thought to be the lanceolate terminals which form a palisade around the hair follicle in the mesenchymal sheath at the level of the ring sinus, outside the glassy membrane. St I and St II receptors can be functionally distinguished in the isolated rat sinus hair preparation on the basis of their response characteristics on mechanical stimulation [12,13].

The function of Merkel cells in the St I receptor unit remains unresolved. It is yet to be firmly established whether the Merkel cell or the expanded nerve terminal is the site of mechanoelectric transduction (e.g. [9,10,13]). The present report documents a specific effect of the 4-aminoquinoline antimalarial drug chloroquine (CQ) on the function of St I receptors and the morphology of Merkel cells in isolated rat sinus hairs. The specific action suggests a mechanotransducer function of Merkel cells in the St I.

The method of isolating and recording mechanoreceptor activity from single sinus hairs has been described in greater detail elsewhere [2,13]. Briefly, sinus hairs were removed from the whisker pads of anaesthetized (urethane; 20% w/v 6 ml kg^{-1} i.p.) adult male Sprague–Dawley rats weighing about 300 g, complete with a short length of the deep vibrissal nerve attached. The thick capsule enclosing the hair follicle was slit to release the blood from the sinus. The hair was then mounted on a silgard platform using fine insect pins. Continuous superfusion (1.25 ml/min) of synthetic interstitial fluid (SIF; [3]) at

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33–35°C was maintained throughout. Electrophysiological recordings were made from fine strands of nerve teased from the DVN using silver wire electrodes. Action potentials in response to bending of the hair shaft were monitored on a digital storage oscilloscope and through a loudspeaker. When a single unit response was obtained, the hair shaft was firmly attached to the probe of a feedback controlled mechanical stimulator which was then used to apply controlled displacement in the most sensitive direction. Stimuli were applied for 5 s (500 ms ramps and 4000 ms plateau) every 30 s. Responses were determined as the number of action potentials during the 5 s stimulus duration. Spontaneous receptor activity between stimuli and interspike interval data, were also monitored online [13]. The St I and St II receptors were distinguished one from the other by their characteristic discharge patterns [7,12,13]. The St I receptor displayed an irregular discharge pattern with broad interspike interval histograms while the St II receptor had a regular discharge pattern with tall, narrow interspike interval histograms. All data were digitized using a CED 1401 + (Cambridge Electronic Design, UK) laboratory interface with a 80486-based PC and stored on hard disk for later off-line analysis.

After steady responses were obtained for at least 20 min, CQ (Sigma Chemical Co.) was prepared in SIF at the appropriate concentrations and superfused by switching the normal SIF for CQ-containing solution for 15–20 min. Some CQ treated hairs were fixed for electron microscopy at different intervals after commencement of drug superfusion: when receptor responses started going down, during maximal inhibition, and after recovery of responses. Hairs dissected and subjected to the same recording conditions, but not treated with CQ were also fixed as controls. Processing for electron microscopy was carried out as described in [13].

On application of CQ-containing SIF, St I response to mechanical stimulation was reversibly and dose dependently inhibited (Fig. 1a).CQ (10 μM) had virtually no effect while 300 μM often resulted in complete inhibition of responses. Spontaneous firing (where present) was also inhibited. Fig. 1c shows the effect of 100 μM CQ on seven St I receptors. Responses usually took 30–45 min to recover. In contrast to the effect on St I receptors, superfusion of CQ on St II receptors resulted in a small but significant increase in stimulated responses (Fig. 1b,d). The effect of CQ on the spontaneous activity of St II receptors was more dramatic (Fig. 1b). Receptors that were 'silent' before CQ application developed spontaneous firing, often as high as 40 spikes/s, while any pre-existing spontaneous activity was further increased. CQ-induced spontaneous firing was unrelenting and could persist for hours after discontinuing CQ superfusion. The effect of CQ on the St II was also dose dependent (Fig. 1b). Concentrations of CQ $\geq$ 300 μM could block both types of receptors. St I receptors completely inhibited by this high concentration of CQ (as shown in Fig. 1a), had a

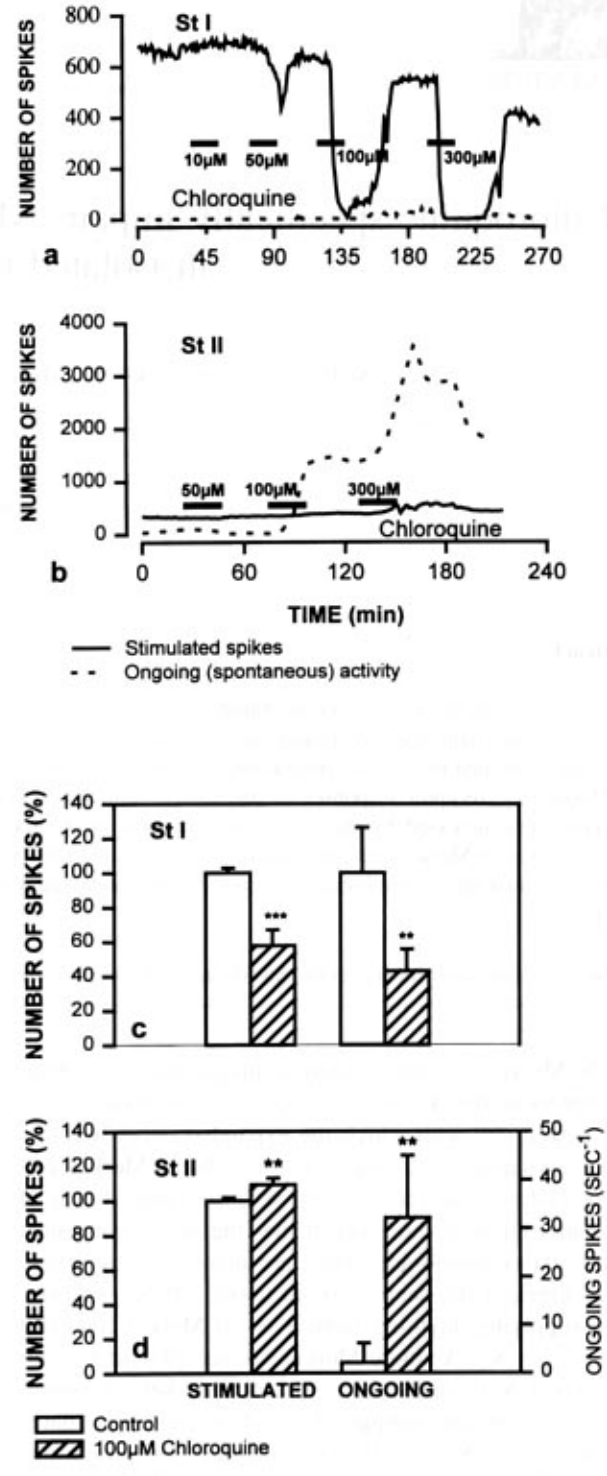

Fig. 1. Effect of CQ on St I and St II function. (a) CQ dose dependently inhibits St I responses to mechanical stimulation. Note that the low level spontaneous activity later in the experiment was also inhibited by the next (300 μM) application of CQ. Effect of CQ on St II is also concentration dependent. CQ-induced spontaneous firing was persistent. Summary of seven experiments showing inhibition of both stimulated and ongoing (spontaneous) activity of St I receptors by 100 μM CQ. St II responses to mechanical stimulation are increased slightly but consistently by 100 μM CQ. The change in ongoing activity is greater and more variable ($n = 7$). The control period is taken as the 10 stimuli preceding drug application. ($^{**}P < 0.001$, $^{***}P < 0.0001$).

recovery time of 45–60 min while the St II responses (if suppressed) recovered within 10–15 min (data not shown).

Electron microscopy showed that hairs fixed when responses started declining had pale Merkel cells with haloes round the dense cored granules (Fig. 2b). Hairs fixed when responses were severely depressed had swollen Merkel cells with vacuoles, disruptions of the cytoplasmic membrane and randomly distributed granules (Fig. 2c). Minimal changes were observed in nerve terminals associated with Merkel cells or in lanceolate nerve terminals nearby (Fig. 2b–d). Although St I responses recovered, there was incomplete reversal of morphological changes within the 45–60 min recovery time used. Merkel cells still had vacuoles and apparently fewer dense cored granules (Fig. 2d).

Thus CQ specifically impaired the function of St I receptors while exerting the opposite effect on St II receptors. Morphologically, CQ apparently spared the neural structures and surrounding keratinocytes while causing extensive vacuolation and intracellular disorganization in the Merkel cells. The exact mechanism by which CQ exerts its inhibitory action on St I mechanoreceptor function is unclear. As a lysosomotropic agent [5,6], it may inhibit the processing and/or release of transmitter from the Merkel cells in a manner similar to its known inhibition of hormonal secretion from the pituitary [11]. CQ is also known to interfere with calcium dependent processes [1]. At higher concentrations CQ also acts as a local anesthetic [4,14], inhibiting the generation and propagation of action potentials in excitable cells. It is unlikely that this was the mechanism behind the inhibition of St I receptor function. St II receptors should have been blocked at the same con-

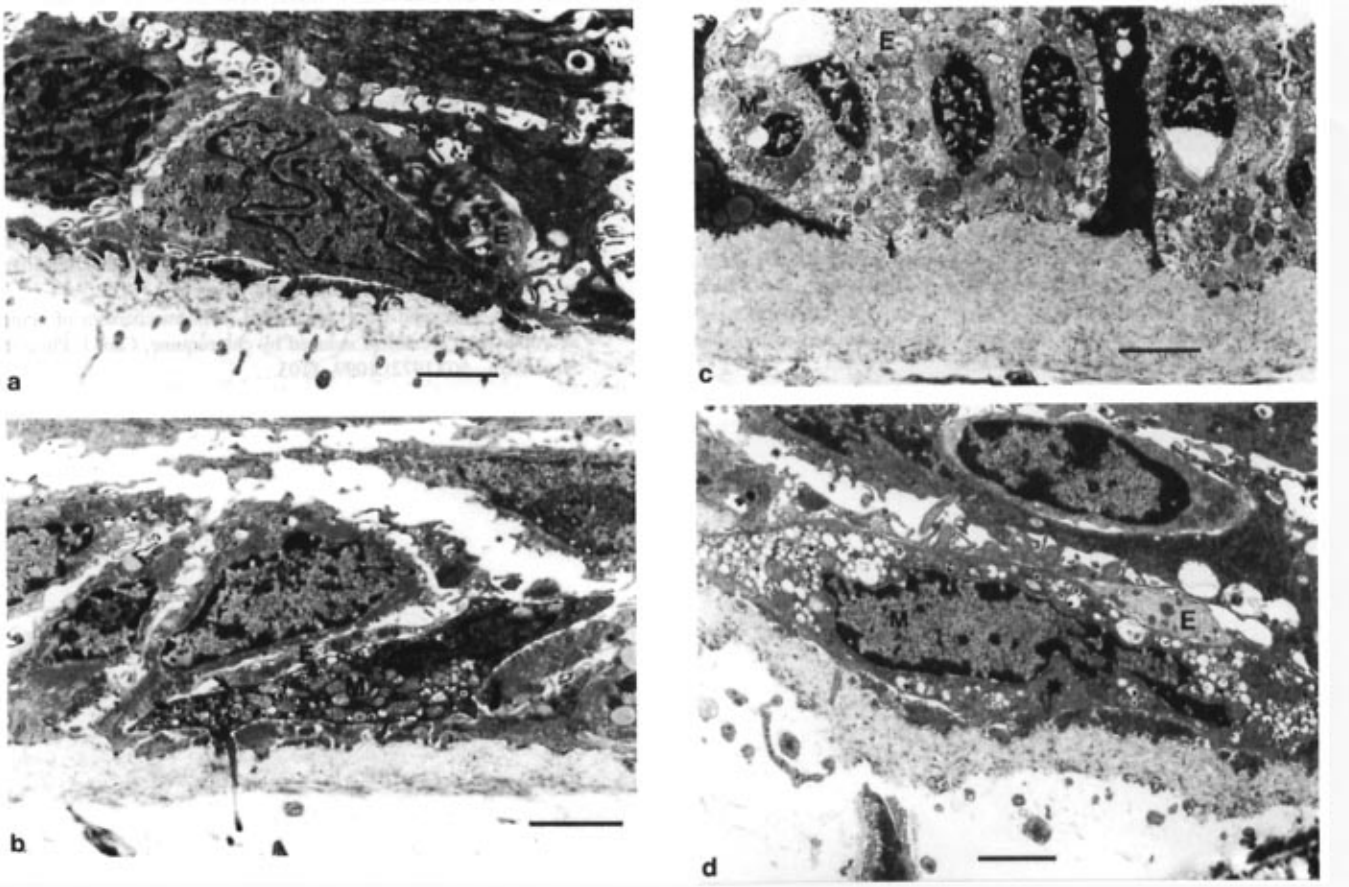

Fig. 2. (a) Control, longitudinal section of a Merkel nerve ending from an untreated sinus hair. The Merkel cell (M) is typically elongated with a lobulated nucleus. The cytoplasm contains osmiophilic granules which are mainly concentrated in the part of the Merkel cells facing the nerve terminal. Cytoplasmic processes from the Merkel cell extend into the connective tissue of the hair follicle and can be seen penetrating the basal lamina (arrow). The disc-shaped nerve terminal (E) is rich in mitochondria, neurotubules and neurofilaments. (b) Longitudinal section of a Merkel nerve ending taken from a sinus hair exposed to chloroquine (100 μM) and fixed shortly after receptor responses started to decline. Size and form of the Merkel cell are unchanged. The granules are surrounded by light haloes (arrows). Some empty vesicles can be found. In the nerve terminal abnormal mitochondria can be found next to normal ones. (c) Cross section of a Merkel nerve ending taken from a sinus hair exposed to chloroquine (100 μM) and fixed when receptor responses had decreased to less than 30% of the control value. The cytoplasma of the Merkel cells contains more filaments, lysosomes and empty vesicles while the number of osmiophilic granules is reduced. The perinuclear space is enlarged. "Spiral bodies" (arrow) and electron empty vesicles can be found in the nerve terminal between normal mitochondria. (d) Longitudinal section of a Merkel nerve ending taken from a sinus hair exposed to chloroquine (100 μM) and fixed at the time when receptor responses had recovered to 80% of the control level. Many vesicles appear devoid of electron dense material. The small nerve terminal contains mitochondria, sparse empty vesicles and neurofilaments.(*a kinocilium penetrating the basal lamina). Scale bar, 2 μm.

centrations since the two receptor type are supplied by myelinated axons of the same calibre. It would however explain the inhibition of St II receptor function at higher concentrations. Moreover, the much faster recovery of St II responses following CQ (300 μM) treatment suggests that the action of CQ on nerve conduction is relatively short-lived as compared to the action on the receptors (both St I and St II).

In conclusion these findings demonstrate that the transduction mechanisms in St I and St II mechanoreceptors are basically different and lends further support to the notion that Merkel cells are the mechanotransducers in St I receptors. In addition, CQ provides the first pharmacological tool to exert qualitatively different effects on St I and St II mechanoreceptors. The underlying mechanism for the observed CQ action on both receptors warrants further investigation.

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