Merkel Cells, Merkel Cell Carcinoma and Neurobiology of the Skin.
H. Suzuki and T. Ono, editors.

Calcium influx and calcium-induced calcium release in mechanically stimulated Merkel cells of rat sinus hair type I mechanoreceptors

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Abstract. The present experiments measured free calcium concentrations in Fluo-3 loaded Merkel cells of rat sinus hairs using confocal microfluorimetry. During mechanical probing, increases in cytosolic calcium concentrations to about 125% of the control level were observed. Similar increases were also seen while exposing the sinus hairs to hyposmotic solutions. Reducing the osmolarity to about 95% of normal by omitting gluconate and sucrose in the bathing solution, results in reproducible increases of cytosolic calcium. Such increases cannot be observed without calcium in the bathing solution or after addition of 1mM amiloride. In contrast, addition of 10 mM caffeine stongly enhances the hyposmotically induced calcium increases reaching peak values in the range of 300% of the control level. In another series of experiments, 10 mM caffeine increases responses of sinus hair type I receptors to about 175% of control responses.

The present results confirm previous findings of mechanically induced increases of cytosolic calcium in Merkel cells. These can be enhanced through CICR resulting in increased receptor responses. Thus, our results suggest that the Merkel cell plays an important role in the mechanoelectric transduction process in slowly adapting type I mechanoreceptors, especially during the adapted phase of responses to mechanical stimuli.

Introduction

Merkel cell receptors are the only type of mechanoreceptor in the skin, in which the terminal of the afferent nerve fibre gets into close contact with specialised cells, the Merkel cells. These receptors — also referred to as slowly adapting type I (SA I) mechanoreceptors [1] — are located at the epidermal-dermal border, making them inaccessible for microelectrode recordings without prior destruction of their normal anatomical assembly. The lack of direct evidence about the role of Merkel cells in intact SA I receptors has so far resulted in controversial views about the site of the mechanoelectric transduction process in these receptors [2–5]. A similar arrangement of Merkel cells and nerve terminals is also found in sinus hairs (whiskers) of most mammals and called sinus hair type I (St I) receptor [6]. Here, only a 1 μm thin glassy membrane separates the Merkel cells from the blood sinus [7]. Careful dissection of rat sinus hairs allows the isolation of these receptors in a functionally intact preparation. The free intracellular calcium concentration of Merkel cells can be monitored by micro-

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fluorimetry through the glassy membrane while simultaneously recording receptor responses from the afferent nerve [8]. The present study shows mechanically induced increases of cytosolic calcium in Merkel cells in this isolated sinus hair preparation through the influx of calcium from the extracellular fluid and release from intracellular stores. Thus, the results support the hypothesis, that the Merkel cell plays a vital part in the mechanoelectric transduction process.

Methods

With prior approval from the Animal Research Ethics Committee of the Chinese University of Hong Kong, whisker pads were removed from Sprague-Dawley rats (250 to 300 g) for isolation of single sinus hairs. The technique for isolating single vibrissae and recording from type I and type II slowly adapting mechanoreceptors has been described in detail by Baumann et al. [8]. A whisker pad was rapidly excised and placed in a pool of (SIF) Synthetic Interstitial Fluid [9], containing (in mM) Na^+ 145, K^+ 3.5, Ca^{2+} 1.5, Mg^{2+} 0.69, Cl^- 114, HCO_3^- 26.2, PO_4^{2-} 1.7, SO_4^{2-} 0.69, gluconate 9.6, glucose 5.55 and sucrose 7.6. The solution was continuously bubbled with a gas mixture of 95% O_2/5% CO_2 to achieve a pH of 7.4. Single vibrissae were dissected from the whisker pad with a short length of the deep vibrissal nerve (DVN) still attached. The thick capsule enclosing the blood sinus was slit longitudinally and the blood washed off.

For electrophysiological recordings, a hair follicle was mounted on a Sylgard platform using fine insect pins and continuously superfused with SIF at 33–35°C using a peristaltic pump. Fine strands were teased from the nerve and attached to a pair of silver wire electrodes in Flourinert and tested for mechanosensory activity by mechanically displacing the hair shaft while monitoring spike activity on an oscilloscope and through loudspeaker. When suitable single unit responses were obtained, the hair shaft was firmly attached to the probe of a mechanical stimulator about 0.5 cm from the hair bulb. Standard feedback controlled displacements were applied to the hair shaft for 5 s (with 500 ms ramps and 4000 ms plateau) every 30 s. St I (Merkel cell) and St II receptors were functionally distinguished on the basis of their response characteristics [5,6].

For microfluorimetric measurements of free intracellular calcium in Merkel cells, the sinus hair follicles were incubated in fluo-3/AM (2 μm in SIF) supplemented with Pluronic F-127 for 45 min at room temperature followed by wash-off of the incompletely de-esterified dye in normal SIF. Hair follicles were mounted on a coverslip-bottomed bath and continuously superfused with SIF. Using the BioRad MRC 1000 confocal system attached to the inverted microscope (Nikon Diaphot TMD-EF) with a high NA (1.3) 60× oil immersion objective, Fluo-3 labelled Merkel cells were visualised in the hair follicles by optical sectioning to the deeper layers of the hair follicle beyond the mesenchymal tissue and the glassy membrane. Merkel cells were distinguished from surrounding keratinocytes (which took up little Fluo 3 under the loading conditions used) by their characteristic shape and arrangement in the hair follicle as well as their

ability to selectively take up the fluorescent dye quinacrine). The 488-nm (visible) laser line was used for excitation while the resultant single wavelength emission was collected.

Mechanical stimuli could be applied either through fine glass probes (see [10]) or by exposing the preparation to slightly hypotonic (95% of normal) solution prepared by omitting gluconate and sucrose (equivalent to 17.2 mosm reduction in osmolarity) from the normal SIF. We checked electrophysiologically that this small change in osmolarity was not deleterious to continued function of the receptors.

Results

Direct mechanical stimulation of the area of sinus hairs containing Merkel cells with a fine micromanipulator driven glass rod results in transient increases of the cytosolic calcium concentration to about 125% of the control level (Fig. 1). Similar increases are also observed when the isolated sinus hair preparation is exposed to slightly hyposmotic solution (Fig. 2). However, such increases cannot be seen if the hyposmotic solution is made calcium free (Fig. 2). Also the presence of amiloride (1 mM) abolishes such increases in cytosolic calcium (Fig. 3). In contrast, exposure to hyposmotic solution in the presence of caffeine (10 mM) strongly enhances the increase in cytosolic calcium to about 300% of the control level (Fig. 4).

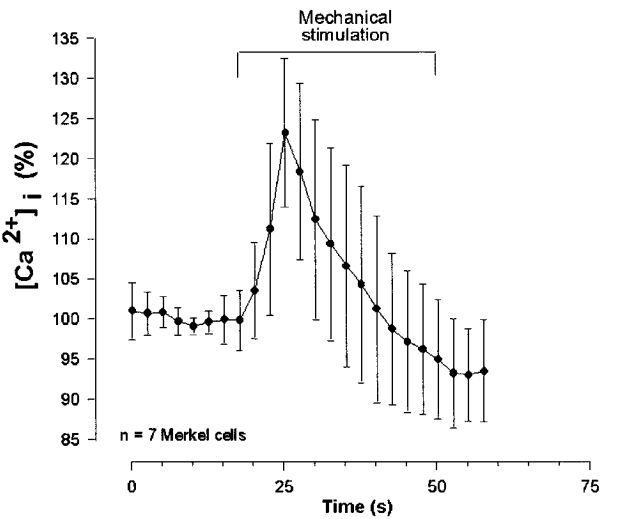

Fig. 1. Effect of mechanical stimulation on cytosolic calcium in Fluo-3 loaded Merkel cells. A micromanipulator driven glasrod was carefully pushed against Merkel cells and kept in position during the time indicated by the bar at the top. (Mean ± SEM of seven Merkel cells.)

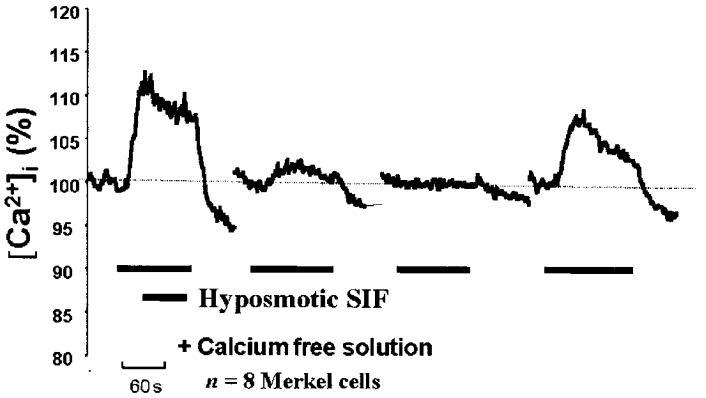

Fig. 2. Effect of hyposomotic swelling on cytosolic calcium in Merkel cells using confocal microfluorimetry. Absence of calcium in the extracellular fluid abolishes the intracellular calcium increase suggesting an influx of calcium through the membrane. (Mean ± SEM of eight Merkel cells.)

Monitoring receptor responses to standard mechanical stimulation shows a dose dependent effect of caffeine on St I responses. The number of spikes recorded from the afferent nerve significantly increases during periods of exposure to caffeine. This effect is much more pronounced for the static discharge during the adapted phase of responses than for the dynamic phase (Fig. 5). The increase in receptor responses during caffeine exposure is specific to St I receptors. In contrast, responses of slowly adapting type II (St II) receptors decrease under the influence of caffeine (Fig. 6).

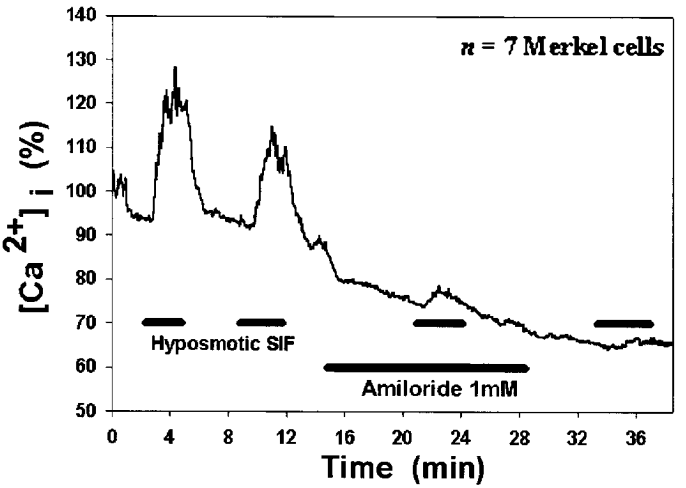

Fig. 3. Effect of hyposmotic swelling on cytosolic calcium in Merkel cells using confocal microfluorimetry. Addition of 1 mM amiloride in the bathing solution almost abolishes the intracellular calcium increase. (Mean ± SEM of seven Merkel cells.)

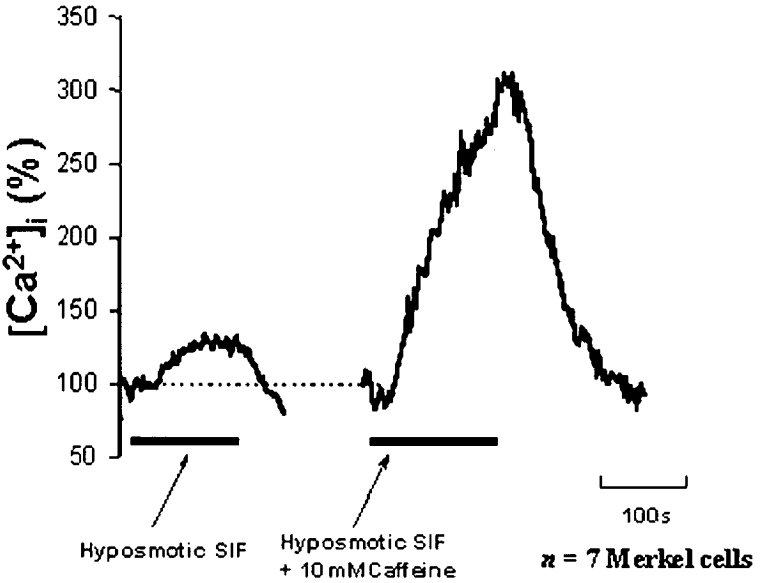

Fig. 4. Effect of hyposmotic swelling on cytosolic calcium in Merkel cells using confocal microfluorimetry. Addition of 10 mM caffeine in the bathing solution strongly enhances the intracellular calcium increase. (Mean ± SEM of seven Merkel cells.)

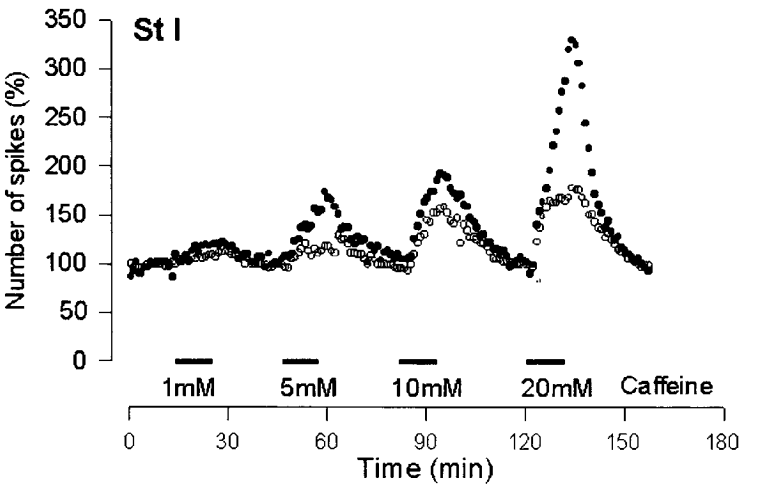

Fig. 5. Effect of different concentrations of caffeine in the bathing solution on the responses of a sinus hair type I receptor to repeated standard mechanical stimuli. The number of spikes in normal solution during the dynamic phase (○, first 500 ms of individual stimuli) and static phase (●, last 1,000 ms of the plateau phase) are plotted as percentage of their respective control values (responses to 10 stimuli immediately preceding the first caffeine dose). Caffeine increases receptor responses in a dose-dependent manner. This effect is more pronounced during the static than during the dynamic phase of stimuli.

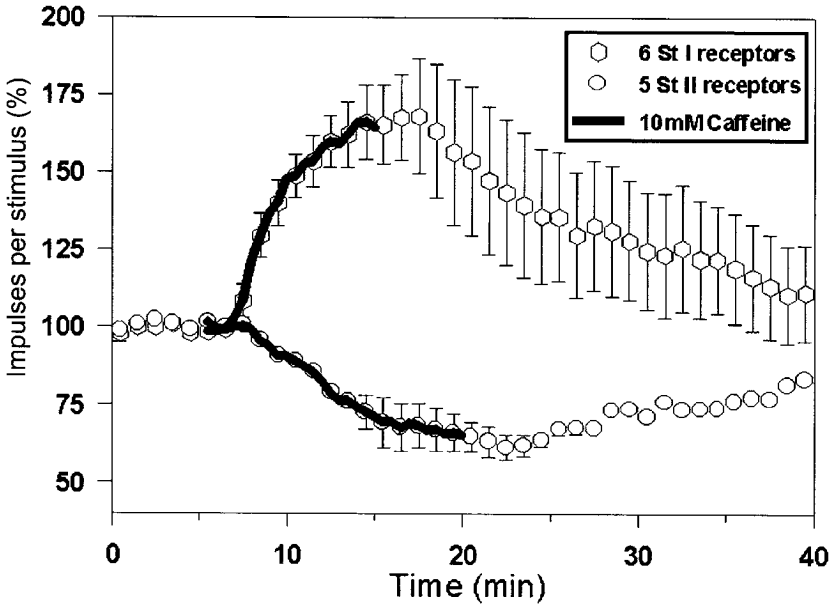

Fig. 6. Comparison of the caffeine effect on responses to standard mechanical stimuli of six St I and five St II receptors (mean ± SEM) plotted as percentage of individual control responses prior to caffeine exposure. The bold lines indicate the periods of application of 10 mM caffeine. Increases in receptor responses can only be seen in type I but not in type II receptors.

Discussion

The present report demonstrates that mechanical stimulation causes an increase in free cytosolic calcium of functioning Merkel cells in an isolated rat vibrissal preparation. The increase is mostly in the order of 20—40% above the control level without mechanical stimulation (usually around 100 nM). There is no obvious difference in the level of increase, whether these stimuli are applied by pushing the cells with fine microprobes or through increased tension over the whole membrane of the Merkel cells in the course of swelling in hyposmotic solution. Removal of extracellular calcium in the bathing solution abolishes these increases. This suggests, that mechanically gated channels cause an influx of calcium through the membrane. Similar results were obtained on single Merkel cells isolated from the hamster cheek pouch [11]. Also in this preparation the intracellular calcium increase depended on the presence of extracellular calcium and blocking of these channels with Gd^{3+} strongly inhibited the increase.

In contrast, previous patch clamp studies by Nurse and Cooper [12] found only voltage gated, nonselective cation channels in the membrane of Merkel cells, which did not respond to mechanical stimuli. Therefore, the authors argued, that Merkel cells could not have a role in the mechanoelectric transduction process. However, Ogawa's group [13] showed evidence for voltage gated calcium

channels in the Merkel cell membrane, but could not demonstrate calcium currents in response to mechanical stimuli. This may have been the result of changes in the shape of Merkel cells following their isolation.

Iggo and Findlater [2] speculated, that the characteristic finger-like cytoplasmic processes of intact Merkel cells may be associated with mechanically gated ion channels. Such processes can be found regularly in Merkel cells irrespective of their location (see, e.g., [14]). Isolated Merkel cells have lost their longitudinal shape and become round. Thus, these processes can no longer be observed and mechanical stimulation of such cells could easily miss the sites of mechanosensitivity. In contrast, slight swelling of Merkel cells during exposure to hyposmotic solutions is likely to stretch the entire cell membrane. In addition, highly sensitive calcium indicator dyes are now available for the measurement of changes in free intracellular calcium concentrations in living cells. Thus, different laboratories have now succeeded with these methods to show mechanically induced calcium influx into Merkel cells [10,11], contradicting the above mentioned previous reports [12,13].

Amiloride has been used to block mechanotransduction channels in inner ear hair cells [15,16]. However, this is the first report showing that the mechanically induced increase of intracellular calcium in Merkel cells can be blocked by amiloride. These experiments cannot differentiate whether amiloride blocks channels allowing the influx of calcium directly (see [15]) or whether the calcium influx is secondary. The situation may be similar as in hair cells where the influx of ions through mechanically gated channels causes depolarisation and opening of voltage gated calcium channels [16]. Further studies will be needed to identify the nature of the channels in Merkel cells.

However, the data further support the previous finding from our laboratory of a calcium-induced calcium release in Merkel cells [17]. Merkel cells show a much greater increase in cytosolic calcium during exposure to hyposmotic solution with caffeine than without (Fig. 4). Also, receptor responses are significantly increased in the presence of caffeine (Fig. 5) especially during the adapted phase, while dynamic responses are less affected. In addition, ryanodine has been shown to increase receptor responses, whereas blocking of the calcium influx abolishes the increased responses during caffeine exposure, strongly suggesting a calcium-induced calcium release [22]. In contrast, the other type of slowly adapting mechanoreceptor found in sinus hairs (St II) does not increase responses to standard mechanical stimuli during caffeine exposure (Fig. 6).

Thus, the present data provide evidence for increases in free intracellular calcium concentration of Merkel cells during mechanical stimulation, which requires the influx of calcium through the Merkel cell membrane and can cause a further increase in calcium through a calcium-induced calcium release. This would provide a mechanism for a longer lasting increase in cytosolic calcium, than could be expected from the influx through membrane channels alone, likely to inactivate rapidly [18]. Thus, the increase in cytosolic calcium appears to be essential for the characteristic slowly adapting response of Merkel cell receptors.

The fact, that most mechanosensitive channels inactivate rapidly, may well be the reason behind the failure to measure mechanically induced ion currents in Merkel cells using patch-clamp recordings [19]. Microfluorimetry does not cause strain to the cell membranes prior to mechanical stimulation and is obviously not affected by those technical limitations.

Conclusion

Our data are in line with the hypothesis that the Merkel cell is involved in the mechanoelectric transduction process in SA I and St I receptors. As suggested by Ogawa [20], Merkel cells may be particularly important for the slowly adapting response to maintained mechanical stimuli. The calcium-induced calcium release within Merkel cells in response to a mechanically induced calcium influx may be the requirement for the maintained release of neurotransmitter from the Merkel cell to the associated nerve terminal [17]. In spite of intensive investigation to identify the neurotransmitter substance, which may be released at this putative synapse, convincing results are still lacking. Various candidates had been found immunohistochemically in the dense core granules of Merkel cells [21,22] without clear evidence for a neurotransmitter function of any of these substances [23–25]. Most recent results from Cahusac's lab show, that blocking glutamate receptors with kynurenic acid, reduces responses of St I receptors especially during the adapted state [26].

Acknowledgements

This work was supported by the Research Grants Council of Hong Kong.

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