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Plenary Article

Piezo2 channel conductance and localization domains in Merkel cells of rat whisker hair follicles

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ABSTRACT

We have recently shown that Merkel cells transduce tactile stimuli via Piezo2 channels to initiate the sense of touch. Here we performed patch-clamp recordings to assess single channel activity on the membranes of Merkel cells in whisker hair follicles. Under the cell-attached configuration, most Merkel cell membrane patches showed large outward unitary currents with single channel conductance being ~200 pS. The outward unitary currents were not affected by negative pressures up to 150 mmHg when applied to the membrane patches. The application of negative pressures up to 190 mmHg also could not directly elicit any inward unitary current in the membrane patches. However, after establishing the whole-cell configuration, mechanically activated currents (MA) that resembled Piezo2 currents could be elicited by membrane displacements in every Merkel cell tested. While the MA current decayed rapidly, a small steady-state current component with significant channel noise could be observed. Applications of stationary and non-stationary fluctuation analyses to the MA currents yielded single channel conductance of 32.5 ± 3.8 and 54.0 ± 5.3 pS, respectively. The lack of mechanical responses under the cell-attached configuration and the existence of Piezo2 MA currents under the whole-cell configuration raised a possibility that Piezo2 channels are preferentially located on Merkel cell processes, the membrane domains inaccessible by recording electrodes.

1. Introduction

Mammals rely on tactile end-organs such as Merkel discs, Pacinian corpuscles, Meissner's corpuscles, and Ruffini endings for environmental exploration, social interaction, tactile discrimination and other tasks in life [11]. Merkel discs, also known as Merkel cell–neurite complexes, are located in high abundance in fingertips of humans, whisker hair follicles of non-human mammals [7,14], and other touch-sensitive spots throughout mammalian body [8,15]. Although discovered 139 years ago [14], only recently have the mechanisms underlying tactile transduction in Merkel discs been uncovered [9,13,19]. Merkel cells have now been established as primary sites of tactile transduction and Piezo2 channels are mechanotransducers in Merkel cells [9,13,19]. Furthermore, it has also been found that Piezo2 channels transduce tactile stimuli into Ca^{2+}-action potentials in Merkel cells, which drives Aβ-afferent nerve endings to fire slowly adapting impulses and initiate the sense of touch [9]. However, details about Piezo2-mediated tactile transduction in Merkel cells remain to be explored.

In heterologous expression system that expressed Piezo1 or Piezo2 channels, membrane displacements evoked whole-cell MA currents (mechanically activated currents) [3]. Piezo1 single channel activity could be elicited by stretching membrane patches using negative pressures when recordings were performed under the cell-attached configuration; the single channel conductance of Piezo1 was measured to be ~23 and ~30 pS [3,4]. While whole-cell Piezo2 currents have been observed in heterologous expression system, sensory neurons [3], and Merkel cells [9,13,19], single channel properties of Piezo2 channels remain unknown.

Single channel properties of ion channels can be directly studied by single channel recording techniques when the channels are accessible by patch-clamp electrodes. When ion channels are not accessible by patch-clamp electrodes, e.g. the channels that are localized at distal domains such as synaptic sites, single channel properties can only be assessed by channel noise fluctuation analyses of whole-cell (macroscopic) currents [10,17]. Structurally, each Merkel cell has a number of finger-like processes that protrude into adjacent epidermal cells [8,14]. It has been thought that these processes might be primary sites of mechanotransduction.

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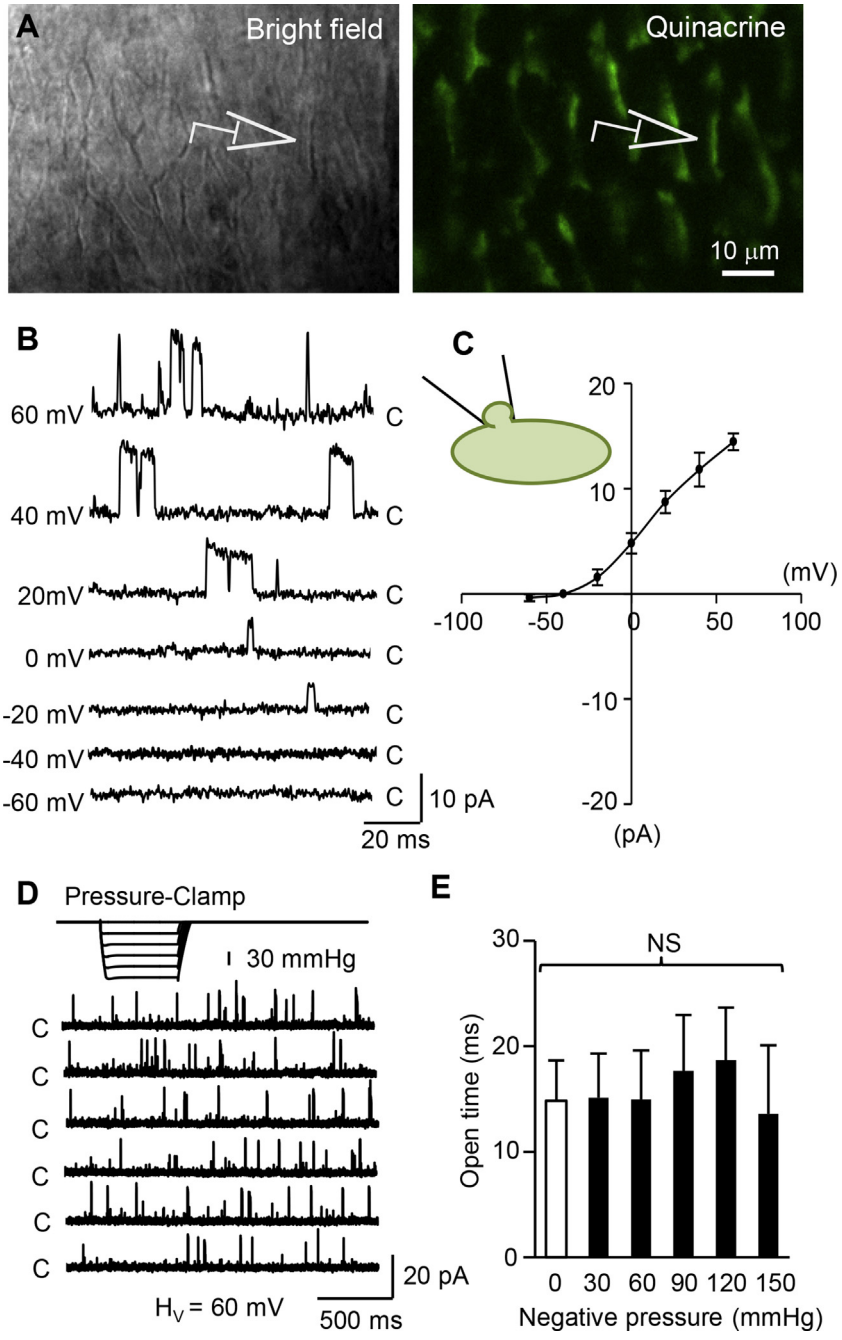

Fig. 1. Merkel cell membrane patches under the cell-attached configuration display outward unitary currents with large single channel conductance. (A) Images show Merkel cells in a rat whisker hair follicle. Merkel cells were vital-stained by the fluorescent dye quinacrine and observed under bright field (left panel) and fluorescent field (right). Merkel cells are in green color in the right panel. (B) Sample traces show outward unitary currents recorded from a Merkel cell membrane patch under the cell-attached configuration. The membrane patch was held at trans-membrane potentials from 60 to −60 mV (indicated on the left side of each trace). Channel closing state is indicated by the letter c on the right of each trace. (C) Summary data of *I–V* relationship of the outward unitary currents ($n = 31$). (D) Sample traces show outward unitary currents recorded under different negative pressures. The negative pressures were applied using a high-speed pressure-clamp (HSPC) device, and the pressure steps were indicated on the top of recording traces. The Merkel cell was held at 60 mV. (E) Summary data ($n = 20$) show the channel open time for the outward unitary currents at different negative pressures. Data represent mean ± SEM, NS, not significantly different from the controls recorded without negative pressures (0 mmHg).

The processes usually became lost in acutely dissociated Merkel cells, which might account for the lack of mechanical sensitivity in the acutely dissociated Merkel cells [18,20]. Merkel cell processes are too fine to be directly studied for their mechanical sensitivity by electrophysiological methods. Piezo2-mediated mechanical responses in Merkel cells were detected based on the macroscopic MA currents recorded from the cell bodies of Merkel cells. These macroscopic currents may represent the activity of Piezo2 channels that are located on the cell bodies and/or the processes of Merkel cells.

2. Materials and methods

Animal care and use conformed to NIH guidelines for care and use of experimental animals. Experimental protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Merkel cells in situ preparations were made as described in our previous study [9]. In brief, Sprague Dawley rats aged 10–18 days were used. Whisker hair follicles were dissected out from whisker pads and the capsule of each follicle was removed. The follicles were then fixed in a recording chamber with