Extraction of Prestin-Dependent and Prestin-Independent Components from Complex Motile Responses in Guinea Pig Outer Hair Cells

Nozomu Matsumoto and Federico Kalinec
Section on Cell Structure and Function, Gonda Department of Cell and Molecular Biology, House Ear Institute, Los Angeles, California 90057

ABSTRACT Electromotility of cochlear outer hair cells (OHC) is associated with conformational changes in the integral membrane protein prestin. We have recently reported that electrical stimulation evokes significant prestin-dependent changes in the length, width, and area of the longitudinal section of OHCs, but not in their volume. In contrast, prestin-independent responses elicited at constant membrane potential are associated with changes in cell length, width, and volume without significant changes in their longitudinal section area. In this report we describe a novel analytical technique, based on a simple theoretical model and continuous measurement of changes in cell length and longitudinal section area, to evaluate the contribution of each one of these mechanisms to the motile response of OHCs. We demonstrate that if the relative change in OHC length (L) during the motile response is expressed as $L = A^2 \times V^{-1}$ (with A and V being the relative changes in longitudinal section area and volume, respectively), A^2 will describe the contribution of the prestin-dependent mechanism whereas V^{-1} will describe the contribution of the prestin-independent mechanism. Thus, relative changes in any two of these cellular morphological parameters (L, A, or V) would be necessary and sufficient for characterizing any OHC motile response. This simple approach provides access to information previously unavailable, and may become a novel and important tool for increasing our understanding of the cellular and molecular mechanisms of OHC motility.

INTRODUCTION

Cochlear outer hair cells (OHC) are cylindrical, with a constant diameter of ~9 μm and a length ranging from ~10 μm in the basal, high-frequency turn of the cochlea to ~100 μm in the apical, low-frequency region (1). Three rows of colonnaded OHCs support the organ of Corti's reticular lamina and regulate its distance to the basilar membrane. Whereas passive mechanical properties of OHCs are crucial for the structural integrity of the cochlea, their ability to reversibly change their shape in response to external stimuli is responsible for the exquisite sensitivity of mammalian auditory organ (2–5). This reversible change in shape is known as ''OHC motility'' (6).

OHC motility has been classically divided into fast and slow, corresponding to time courses in the order of microseconds and seconds, respectively (5). Slow motile responses can be induced by a variety of mechanical and chemical stimuli, and they are usually both ATP and Ca^{2+} dependent (5,7). Fast motility, on the other hand, is frequently identified as ''electromotility'' and associated with voltage-dependent conformational changes in the integral membrane protein ''prestin'' (1–3,8–11). Portraying electromotility only as a fast response, however, may be misleading. For instance, slow changes in the ionic environment of the OHC's plasma membrane may induce slow electromotile responses due to fluctuations in membrane potential (12,13). Recently, we suggested that confusion could be avoided by categorizing OHC motility as either prestin-dependent or prestin-independent (12). Moreover, prestin-dependent changes in OHC length were always associated with changes in cell width and longitudinal section area but without detectable variations in cell volume. In contrast, prestin-independent changes in OHC length were associated with changes in cell width and volume without detectable variations in the area of the longitudinal section. These differences not only support the proposed classification, but also confirm previous assumptions about OHC motility and suggest that an experimental approach could be developed for investigating the simultaneous occurrence of both types of OHC motility and for evaluating the individual contribution of each mechanism to the total cell movement (12).

In this study, we extend and complete our previous work on characterizing prestin-dependent and prestin-independent responses, and describe a novel approach for evaluating their contribution to OHC motility either when present alone, or as a combination. With the single assumption that OHCs' shape can be approached by a cylinder, we show theoretically and experimentally that changes in the relative values of OHC volume (V) and longitudinal section area (A) can be estimated from measurements of relative values of cell length (L) in ''pure'' prestin-dependent or prestin-independent responses. Moreover, relative changes in any two of these cellular morphological parameters (L, A, or V) would be necessary and sufficient for characterizing any OHC motile response. These results expand our understanding of OHC motility and provide a new conceptual and experimental

Submitted April 12, 2005, and accepted for publication September 20, 2005.

Address reprint requests to Federico Kalinec, PhD, Section on Cell Structure and Function, Gonda Dept. of Cell and Molecular Biology, House Ear Institute, 2100 West Third St., Los Angeles, CA 90057. Tel.: 213-353-7030; Fax: 213-273-8088; E-mail: fkalinec@hei.org.

0006-3495/05/12/4343/09 $2.00

doi: 10.1529/biophysj.105.064626

framework for analyzing cell mechanical responses combining both motile processes.

MATERIALS AND METHODS

Isolation of guinea pig outer hair cells

Guinea pigs (200–300 g) were euthanized with CO_2 following procedures approved by the Institutional Animal Care and Use Committee. The cochlear spiral was removed from otic bullae and placed in Leibowitz L-15 (Gibco, Gaithersburg, MD) containing 1 mg/ml collagenase (type IV, Sigma, St. Louis, MO), incubated at 31°C for 3 min, and transferred to a recording chamber (PCCS1, Bioscience Tools, San Diego, CA) on an inverted microscope (Axiovert 135TV, Zeiss, Thornwood, NY). OHCs were mechanically dissociated as previously described (14) in the recording chamber filled with L-15, and observed with Nomarski differential interference contrast optics and a 63×/1.2 C-Apochromat objective (Zeiss). Only cells that met the established criteria for healthy OHCs (15) and were not attached to the bottom of the chamber were used in this study.

Special solutions were used instead of L-15 in some experiments, as indicated in the text. The standard external solution contained (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES. The high K^+ external solution contained (in mM): 5 NaCl, 150 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES. These solutions were in turn subdivided into other two by adjusting their osmotic pressures to either standard (324 mOsm with ~10 mM glucose) or hypotonic respect to the standard (~310 mOsm with ~0.5 mM glucose) values. The osmolarity of every solution was measured with a μOsmette 5004 freezing-point osmometer (Precision Systems, Natick, MA), and their pH adjusted to 7.4 with Tris(hydroxymethyl) aminomethane (Tris).

Electrophysiology

Whole-cell voltage clamp was achieved under conventional whole-cell patch techniques at room temperature using an EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany). Patch electrodes were made from borosilicate capillary glass (G-1.5, Narishige, Tokyo, Japan) using a P-97 micropipette puller (Sutter Instruments, Novato, CA). Intrapipette solution was composed of (in mM): 150 KCl, 1 $MgCl_2$, 0.1 EGTA, 2 ATP-Mg, 0.1 GTP-Na, and 10 HEPES, pH of which adjusted to 7.2 with Tris. Osmolarity of the intrapipette solution was adjusted with glucose to a value slightly higher than that of the control extracellular solution (typically 314 mOsm), increasing the turgor of the cells being recorded and contributing to prevent OHC's attachment to the substrate. The resistance between the patch electrode filled with this solution and the bath solution was 4–6 MΩ.

Capacitance measurement

Measurements of voltage-dependent nonlinear capacitance (NLC) were performed using the ''software lock-in amplifier'' function included in the Pulse software (HEKA, Lambrecht, Germany). OHCs for capacitance measurements were perfused with a blocking solution containing (in mM): 100 NaCl, 5 KCl, 20 CsCl, 20 tetraethylammonium-Cl, 2 $CoCl_2$, 2 $MgCl_2$, 10 glucose, and 10 HEPES, pH of which adjusted to 7.4 with Tris. The intrapipette solution consisted of (in mM): 140 CsCl, 2 $MgCl_2$, 10 EGTA, and 10 HEPES, pH of which adjusted to 7.2 with Tris. The osmolarity of these solutions was adjusted to 310 mOsm with glucose.

Video analysis and data handling

Images of isolated OHCs were captured in QuickTime video format at standard video resolution (720 × 480 pixels) and frequency (30 frames/s) as previously described (12). The obtained video images were then analyzed offline using Dynamic Image Analysis System (DIAS) software (Soll Technologies, Iowa City, IA) running on a Macintosh G4 computer. First, the cell image was rotated as needed, to place it horizontally on the screen. Then, the DIAS software automatically detected the cell edge, found the cell centroid, and measured its geometrical parameters (Fig. 1). Cell length was defined as the maximum horizontal length (the command ''x bounded width'' in DIAS software) of the detected image. Cell width was measured at the cell centroid (command ''central width''). The area of the optical section was measured as the internal area of the detected cell image (command ''area''). The volume of the cell in each analyzed frame was estimated using the model described in Results (Eq. 4; see also Matsumoto and Kalinec (12)). Data was analyzed using Excel (Microsoft, Redmond, WA) and IGOR Pro (Wavemetrics, Lake Oswego, OR) software.

RESULTS

The model

We assume that OHCs are cylinders (Fig. 1). Microscopic images represent optical sections of the cylinders (OHCs), where their lengths, widths, and section areas can be measured directly. The longitudinal section area (a) at the axis of the cylinder (Fig. 1 *A*, *shaded region*), can be calculated from the values of OHC length (l) and width (w) by

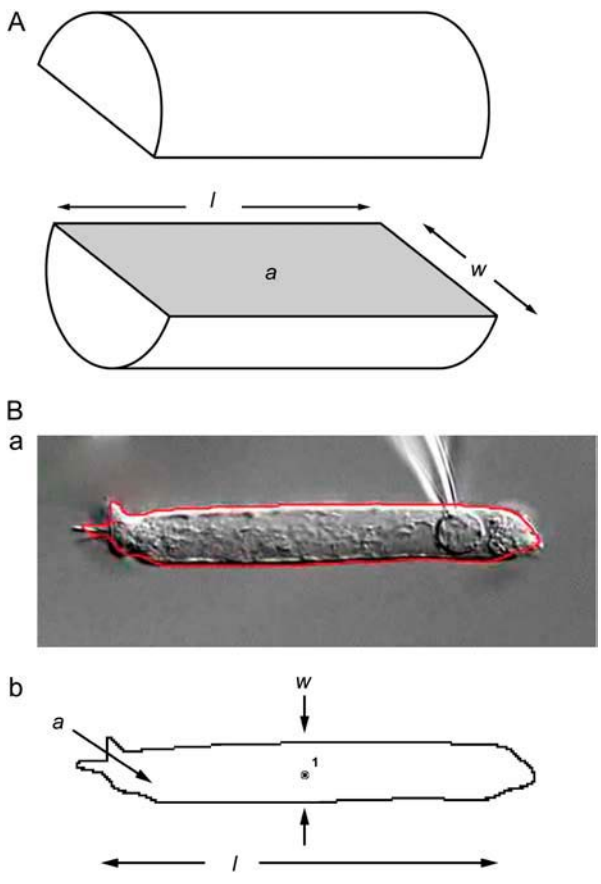

FIGURE 1 Definition of parameters. (*A*) OHCs are modeled as cylinders, and the parameters length (l), width (w), and longitudinal section area (a) are defined as shown in the diagram. (*B*, *a*) Actual picture of an isolated OHC with its automatically defined boundary delineated in red; (*B*, *b*) the automatically defined boundary and the centroid of the cell as measured by DIAS software. The cell width (w) is measured at the cell centroid.