Original Article

Prestin forms tetramer with each subunit being mechanically independent

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Abstract Prestin is the motor protein of cochlear outer hair cells (OHCs). It is able to perform rapid and reciprocal electromechanical conversion that underlies OHC electromotility. Due to the inadequate size of a single prestin molecule to form the ~12 nm intramembraneous protein particles(IMPs) in the OHC lateral membrane(LM), the possibility of prestin oligomerization has been proposed. It has been suggested that prestin molecules form high-order oligomers, most likely as the tetramer, in heterologous systems. In OHCs, however, the oligomeric structure of prestin remains unclear. Here we calculated the prestin-related charge density in both gerbil and guinea pig OHCs through measuring their nonlinear capacitance (NLC) and LM surface area, showing that the average charge density(22,608 μm^{-2} in gerbils; 19,460 μm^{-2} in guinea pigs) is statistically 4 times the average density of IMPs (5,686 μm^{-2} in gerbils; 5, 000 μm^{-2} in guinea pigs). This suggests that each IMP contains four prestin molecules based upon the notion that each prestin transfers a single elementary charge, implying that prestin forms tetramers in OHCs. To determine whether the prestin tetramer functions as a mechanical unit, we subsequently compared the slope factors (α) of electromotility and NLC simultaneously measured from the same OHC, showing that the α values of the two are statistically the same. This suggests that each prestin molecule in the tetramer is mechanically independent and equally contributes to OHC electromotility.

Key words Prestin, oligomer, outer hair cells, electromotility, nonlinear capacitance, gerbil

Introduction

The outer hair cell(OHC) is a type of receptor cell in the organ of Corti that plays an important role in mammalian hearing. It is able to rapidly change its length [1, 2] and stiffness [3] when the transmembrane potential is altered. Such electrically evoked length change of OHCs was termed electromotility [4]. Associated with OHC electromotility is a gating current arising from the redistribution of charged voltage sensors across the cell plasma membrane[5], which confers the OHC a measurable voltage-dependent capacitance, called nonlinear capacitance (NLC) [6, 7]. The molecular substrate underlying the association between electromotility and NLC in OHCs is the motor protein prestin expressed in the cell lateral membrane (LM) [8]. It is generally believed that the membrane-bound prestin molecule acts as an electromechanical transducer by utilizing the intracellular anions (mostly Cl-ions) in a 1:1 ratio as the voltage sensor [9, 10]. Therefore, the total number of prestin molecules expressed in an OHC can be estimated from the value of its maximum charge movement (Qmax).

Freeze-fracture studies [11-13] showed that the OHC LM is highly packed with an array of large intramembraneous particles(IMP; ~12 nm in diameter), whose major component is believed to be prestin since the increase of particle density during the development [14] is in line with the onset and development of OHC electromotility [15]. Nevertheless, a single prestin molecule, consisting of 744 amino acids with the molecular mass of ~ 81.4

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kDa [8], is too small to account for the size of IMPs. Therefore, the possibility of prestin oligomerization is raised. Recent biochemical evidence suggests that prestin forms tetramers [16] or dimers [17] in the prestin-transfected mammalian cell lines. However, the oligomeric structure of prestin in the OHCs per se has not been explored. It is also unclear if prestin molecules in the putative oligomer kinetically interact with each other to form a mechanical unit.

Therefore, our aims in this study were to address these two questions, which would lead to increased understanding of the operational function of prestin in cochlear active mechanics. First, we calculated the prestin-related charge density (ρ_Q) in OHCs through measuring the NLC and the LM surface area, and compared the value of ρ_Q with the density of IMPs (ρ_{IMP}) reported in previous freeze-fracture studies. If ρ_Q was twice the value of ρIMP or greater, it would suggest that each IMP contains more than one prestin molecule based upon the notion that each prestin transfers one elementary charge [6, 7, 10]. Thus, the oligomeric structure of prestin could be speculated. On the other hand, if ρ_Q were less than double ρ_{IMP}, the result would not support prestin oligomerization. Second, we measured OHC electromotility and NLC simultaneously from the same OHC and compared their slope factors (α) according to the two-state Boltzmann function. The reason to do so is that the α value helps determine whether the two measurements have the same valences (z) ($\alpha = ze/kT$; see Eq. 3). If electromotility and NLC showed the same α values, it would suggest that the motility valence is close to 1 as is that of NLC [6, 7], i.e., each prestin molecule is mechanically independent. If, however, the α value of electromotility is twice of that of NLC or greater, it would suggest that the motility valence is ≧ 2, i.e., at least two prestin molecules function together to form a mechanical unit.

Materials and Methods

Preparation of isolated OHCs

Adult gerbils and guinea pigs (Charles River, MA, USA) of postnatal days 28 (P28) to 35 (P35) were used. The animals were decapitated following a lethal dose of sodium pentobarbital injection (150 mg / kg, i.p.). Both cochleae were removed and placed in fresh Leibovitz's L-15 medium (Gibco, OK, USA) at pH 7.6 and 300 ± 2 mOsm / kg. The entire basilar membrane on which the organ of Corti resides was pulled off from the modiolus and was incubated in the enzymatic digestion L-15 medium (collagenase IV, 1 mg / ml, Sigma, USA) at the room temperature (22 ± 2 ℃) for 10 min. Gentle trituration was applied on the tissue to obtain isolated OHCs. Only the OHCs showing a uniform width throughout its longitudinal axis and no signs of damages (e.g., swelling, blebbing, and / or dislocation of the nucleus) were selected for experimental measurements. Care and use of animals were approved by the NIDCD / NIH grants and the Institutional Animal Care and Use Committee of Creighton University.

Whole-Cell Voltage Clamp Recording

Isolated OHCs were placed in an experimental chamber containing fresh external solution on the stage of an inverted microscope (Olympus IX-71, PA, USA). The voltage protocols for measuring OHC electromotility and NLC were delivered by an Axopatch 200B amplifier (Axon Instruments, CA, USA). Patch electrodes were pulled from 1.5 mm borosilicate glass capillaries (A-M System Inc., USA) using a Flaming/Brown micropipette puller (Model P-97, Sutter Instruments, USA) with the initial resistance of 3-6 MΩ in the external solution. Seal resistance upon the establishment of whole-cell configuration exceeding 1 MΩ was accepted. The access resistance following the membrane rupture typically ranged between 6 and 12 MΩ. The uncompensated series resistance was corrected offline after data collection. Measurements were rejected if any visible cell damage occured during the recordings. The external solution contained (in mM): NaCl 99.2, TEA-Cl 20, CsCl 20, $CoCl_2$ 2.0, $MgCl_2$ 2.0, $CaCl_2$ 1.5, HEPES 10, and Glucose 5.0, whereas the internal (pipette) solution contained (in mM): CsCl 140, $MgCl_2$ 2.0, EGTA 10, HEPES 10. Both solutions were adjusted to a pH of 7.4 and 300 ± 2 mOsm / kg. The reason to use K^+ and Ca^{2+} channel blockers in both solutions was to eliminate contamination of the gating current intrinsic to the voltage-gated ion channels [6, 7, 18].

Charge movement and NLC measurements

The two-sine AC technique was used to evoke prestin-related gating currents and calculate the corresponding NLC in OHCs as previously described [19]. In brief, a continuous, high-resolution (2.56 ms sampling), two-sine voltage protocol lasting 500 ms (20 mV