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Research paper

HEI-OC1 cells as a model for investigating prestin function

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ABSTRACT

The House Ear Institute-Organ of Corti 1 (HEI-OC1) is a mouse auditory cell line that endogenously express, among other several markers of cochlear hair cells, the motor protein prestin (SLC26A5). Since its discovery fifteen years ago, and because of the difficulties associated with working with outer hair cells, prestin studies have been performed mostly by expressing it exogenously in non-specific systems such as HEK293 and TSA201, embryonic kidney cells from human origin, or Chinese Hamster Ovary (CHO) cells. Here, we report flow cytometry and confocal laser scanning microscopy studies on the pattern of prestin expression, as well as nonlinear capacitance (NLC) and whole cell-patch clamping studies on prestin motor function, in HEI-OC1 cells cultured at permissive and non-permissive conditions. Our results indicate that both total prestin expression and plasma membrane localization increase in a time-dependent manner when HEI-OC1 cells differentiate under non-permissive culture conditions. In addition, we demonstrate that HEI-OC1 cells have a robust NLC associated to prestin motor function, which decreases when the density of prestin molecules present at the plasma membrane increases. Altogether, our results show that the response of endogenously expressed prestin in HEI-OC1 cells is different from the response of prestin expressed exogenously in non-auditory cells, and suggest that the HEI-OC1 cell line may be an important additional tool for investigating prestin function.

1. Introduction

Prestin belongs to the mammalian SLC26 family of anion exchangers that mediate the translocation across the plasma membrane, among others, of chloride, bicarbonate, sulfate and oxalate (Alper and Sharma, 2013; Mount and Romero, 2004). In cochlear outer hair cells (OHCs) prestin acts as a motor protein, most likely by alternating between two structural conformations that occupy different cross-sectional areas within the membrane (Ashmore, 2008; Iwasa, 2001; Lim and Kalinec, 1998). Since its identification, prestin has been under intensive investigation because of its crucial role in the mechanism of cochlear amplification, the active mechanical process that increases hearing sensitivity by enhancing sound-induced vibration in the organ of Corti (Ashmore et al., 2010; Gorbunov et al., 2014; He et al., 2014).

Because of its location inside the temporal bone, the mammalian inner ear is not easily accessible. In addition, the organ of Corti of the most common laboratory animal, the mouse, contains only ~4000 OHCs. Moreover, they are structurally fragile, hard to isolate and transfect, and the few dozens that can be collected in good conditions from one animal survive only a few hours in vitro. Therefore, performing biochemical and molecular biology experiments directly in OHCs is not an easy task, and many prestin studies were based on the exogenous expression of this primarily auditory protein in non-specific systems such as HEK293 and TSA201, embryonic kidney cells from human origin, or Chinese Hamster Ovary (CHO) cells (see He et al., 2014 for a review). Among several specific OHCs' markers, House Ear Institute-Organ of Corti 1 (HEI-OC1) cells express prestin endogenously (Kalinec et al., 2003) but, to our knowledge, they have never been used to investigate prestin function. Moreover, some researchers mentioned, incorrectly, that HEI-OC1 cells do not express prestin (Ramamoorthy et al., 2013), probably indicating that the common use of this cell line as an in vitro system for ototoxicity studies may have concealed its potential value for other areas of hearing research.

Studies in many different cell systems have shown that integral proteins require a particular lipid environment adequate for its correct insertion and functioning (Ciesielska and Kwiatkowska,

Abbreviations: HEI-OC1, House Ear Institute-Organ of Corti 1; P-HEI-OC1, HEI-OC1 cells cultured at permissive conditions; NP-HEI-OC1, HEI-OC1 cells cultured at non-permissive conditions; NLC, Non-Linear Capacitance

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2015). A different lipid environment, with different lateral compressibility and fluid/gel ratio, could result in different aggregation patterns of prestin molecules as well as distinct motor responses to similar stimuli. Thus, simultaneous studies in cells expressing prestin exogenously, such as HEK293 cells, or endogenously, such as HEI-OC1 cells, could be important for establishing whether these potential differences indeed exist, and their eventual significance.

The purpose of this study was to investigate the suitability of HEI-OC1 cells for studying prestin function. With this purpose we used flow cytometry and confocal laser scanning microscopy techniques to describe the pattern of prestin expression in HEI-OC1 cells cultured at permissive (P-HEI-OC1) and non-permissive (NP-HEI-OC1) conditions. In addition, we measured non-linear capacitance (NLC), the electrical signature of prestin motor function (Ashmore, 1990; Santos-Sacchi, 1991), in P-HEI-OC1 and NP-HEI-OC1 as well as guinea pig OHCs, and compared the responses with those reported in the literature for mouse OHCs and transfected HEK293 cells. We show that HEI-OC1 cells have a robust NLC, that prestin translocate from the cytoplasm to the plasma membrane in a time-dependent manner when cells differentiate under non-permissive culture conditions, and that, in general, the response of endogenously expressed prestin in an auditory cell line is different from the response of prestin expressed exogenously in a non-auditory cell line. Thus, the comparative study of these distinct models could provide valuable information about structural requirements for prestin function.

2. Materials and methods

2.1. Cell culture

HEI-OC1 cells were cultured under permissive conditions (33 °C, 10% CO_2) in high-glucose Dulbecco's Eagle's medium (DMEM; Gibco BRL, Gaithersburg, Md., USA) containing 10% fetal bovine serum (FBS; Gibco BRL) without antibiotics. For prestin experiments, cells were moved to non-permissive conditions (39 °C in DMEM plus 10% FBS and 5% CO_2) at different periods. L-Thyroxine sodium salt pentahydrate (T4; Sigma) was added in different concentrations.

2.2. Flow cytometry

HEI-OC1 cells were grown at permissive conditions, then collected and counted using Cellometer Auto T4. To detect prestin expression, concentration of the cells was adjusted to 1.0×10^5 cells/well in a 6-well plate, and incubated at 33 °C overnight. After overnight incubation the cells were treated with DMEM media alone (control) or T4 for 72 h before flow cytometry. At the end of the treatment, cells were washed with PBS and detached with non-enzymatic cell dissociate solution (Sigma–Aldrich, 1×, Cat. #C5789). Cells were stained with anti-prestin (1:100) in PBS for 2 h, and washed 3 times with PBS. Cells were stained with Alexa 488 (1:500) in PBS for 30 min in the dark, and washed 3 times with PBS. Flow cytometry experiments were performed using a FACS Aria III® instrument (BD Biosciences, Erembodegem, Belgium) with 488 nm excitation (blue laser) and FL1: 530 ± 15 nm. In all cases, 10,000 cells were analyzed per experiment by using FACS Diva software (BD Biosciences, Erembodegem, Belgium).

2.3. Confocal microscopy

HEI-OC1 cells were incubated for variable periods in either DMEM media alone (control) or with thyroid hormone T4 (Sigma) on uncoated glass bottom dishes (MatTek, Ashland, MA). Cells were incubated at non-permissive conditions for different periods, then washed with PBS and fixed with 4% paraformaldehyde (EMS, Fort Washington, PA) in PBS for 2 h and processed for confocal microscopy. Anti-prestin (N-20) from Santa Cruz Biotechnologies (Santa Cruz, CA) and anti-Na^+K^+ATPase from Cell Signaling Technology (Beverly, MA) were used as primary antibodies at 1:100 and 1:200 dilutions, respectively. Alexa 488 or 549 (Molecular Probes-Invitrogen, Eugene, OR) were used as secondary antibodies at 1:500 and 1:1000 dilutions, respectively. Actin was stained with rhodamine phalloidin (Molecular Probes). Samples were observed with TSC-SP5 Broadband Spectra laser confocal microscope with an HCX-PL 63×/1.2 objective (Leica Microsystems, Deerfield, IL, USA).

2.4. Electrophysiological measurements

Patch electrodes were made from borosilicate capillary glass (G-1.5; Narishige, Tokyo, Japan) using a P-97 micropipette puller (Sutter Instruments, Novato, CA). Continuous perfusion of an external solution consisting of L-15 adjusted to 305–310 mOsm with distilled water was provided at a rate of 0.3 mL/min using a syringe pump. Internal (intrapipette) solution was composed of 150 mM KCl, 1 mM $MgCl_2$, 0.1 mM EGTA, 2 mM ATP-Mg, 0.1 mM GTP-Na, and 10 mM HEPES; pH was adjusted to 7.2 with Tris. Osmolarity of the intrapipette solution was adjusted to the same value of the control extracellular solution by adding glucose. The resistance between the patch electrode filled with this solution and the bath solution was 4–6 MΩ. Measurements of voltage-dependent nonlinear capacitance (NLC), the electrical signature of prestin (Santos-Sacchi, 1991), were performed using the software lock-in amplifier function included in the Pulse software (HEKA). Capacitance function was fitted to the first derivative of a two state Boltzmann function relating nonlinear charge to membrane voltage (Santos-Sacchi, 1991):

$$C_m = Q_{max}\frac{ze}{kT}\frac{b}{(1+b)^2} + C_{lin},$$

with

$$b = exp\left(\frac{-ze\left(V - V_{pkC_m}\right)}{kT}\right),$$

where $\boldsymbol{Q_{max}}$ is the maximum nonlinear charge moved, $\boldsymbol{V_{pkCm}}$ is voltage at peak capacitance, $\boldsymbol{V_m}$ is membrane potential, $\boldsymbol{z}$ is valence, $\boldsymbol{C_{lin}}$ is linear membrane capacitance, $\boldsymbol{e}$ is electron charge, $\boldsymbol{k}$ is Boltzmann's constant, and $\mathbf{T}$ is absolute temperature.

For capacitance measurements, cells were perfused with a blocking solution containing 100 mM NaCl, 20 mM CsCl, 20 mM tetraethylammonium-Cl, 2 mM $CoCl_2$, 1.52 mM $MgCl_2$, 5 mM glucose, and 10 mM HEPES, adjusted to pH 7.2 with Tris. The intrapipette solution consisted of 140 mM CsCl, 2 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.2 with Tris. Osmolarity of these solutions was adjusted to 300 mOsm with glucose or distilled water.

2.5. Statistics

Statistical analysis of the data, including One-way and Two-way ANOVA, was performed using JMP 9 software (SAS Institute, Cary, NC) and $P \leq 0.05$ as the criterion for statistical significance.