Original Article

Measurement of Ca^{2+} Flow in Cochlear Cells Using Non–Invasive Micro–Test Technique

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Abstract Objective To test Calcium ion (Ca^{2+}) flow at the head and end of outer hair cells (OHCs) in resting state and in response to Nimodipine treatment. Methods Non–invasive micro–test techniques were used to study Ca^{2+} in isolated OHCs in adult guinea pigs. Results Four types of Ca^{2+} transport were identified in OHCs on basilar membrane tissue fragments: influx at the head of with efflux at the bottom(type 1): efflux at the head of OHCs with influx at the bottom (type 2); influx at the both head and bottom (type 3); and efflux at the both head and bottom (type 4). However, only type 1 and type 3 of Ca^{2+} ion transport were detected in the cochlea. We propose that Ca^{2+} ion transport exists in adult guinea pig cochlear OHCs in resting state and is variable. Ca^{2+} flow in OHC can be inhibited by Nimodipine in resting state.

Keywords Guinea pig, outer hair cells, Ca^{2+} ion, non–invasive micro–test technique, nimodipine.

Introduction

Normal mammalian hearing relies upon active cochlear function. Outer hair cells(OHCs) play a critical role in the sensitivity of mammalian hearing because they generate mechanical forces for the amplification of acoustic stimulation[1]. Their ability to contract or elongate following changes of the intracellular potential is called electr–omotility [2]. There is a close relationship between auditory mechanisms and ion exchange in outer hair cells. One important ion is Ca^{2+} [3]. Previous research has focused on the ion and ion channels involved in mechano–electrical transducer mechanisms by patch–clamp techniques, but the mechanism of ion transport and their overall levels in resting OHCs are not yet known. In this study, we studied Ca^{2+} ion flow and the overall level of ions transport in OHCs using a non–invasive micro–test technique (NMT).

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Materials and Methods

Animal Materials

Healthy adult guinea pigs(250–300g) were purchased from Beijing Keyu Animal Breeding Center. HEK293T cells were purchased from Biohermes Biomedical Science & Technology Corp., Shanghai.

Cochlea and OHCs Isolation

The animals were decapitated after intraperitoneal injection of a lethal dose of chloral hydrate (500 mg/Kg). The temporal bones were removed and the cochlea was dissected in an artificial extracellular solution(142 mM NaCl, 5.37 mM KCl, 1.47 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES,

300 mOsm and pH 7.2) using a standard technique[4–5]. The cochlea was opened and the basilar membrance was isolated using a sharpened needle and then incubated with collagenase Ⅳ in the standard extracellular solution for 5–7 min to dissociate OHCs. The dissociated OHCs were transferred to the 35 mm glass bottom dishes for recording. All steps were performed at room temperature (20–23℃).

Measurements of Net Ca^{2+} Fluxes with NMT

Net fluxes of Ca^{2+} were measured noninvasively using NMT (BIO–IM, YOUNGERUSA, LLC, Amherst, MA 01002, USA) as described by Newman[6]. Briefly, when Ca^{2+} is absorbed by cells, Ca^{2+} concentration increases gradually from near the cell membrane to the periphery. In contrast, when Ca^{2+} is released from cells, Ca^{2+} ion concentration reduces gradually from near the cell membrane to the periphery, resulting in an electrochemical gradient. The gradient direction depends on the direction of Ca^{2+} move– ment and its amplitude depends on the net flow per unit time. In this study, the direction of Ca^{2+} efflux was designated as positive and the direction of Ca^{2+} inflow as negative. Prepulled and silanized glass micropipettes(tip diameter 2– 4 microns, XYPG120–2; Xuyue Science and Technology Co., Ltd.) were first back filled with 100mM/L $Cacl_2$ to a length of approximately 1 cm, then front filled with approximately 15–mm columns of selective liquid ion–exchange cocktails(LIX 21048; Ca^{2+} ionophore I – cocktail A; Sigma–Aldrich, Louis, MO 63103, USA). An Ag / AgCl electrode wire(XYEH01–1; Xuyue Science and Technology. Co, Ltd.) was used to deliver current. Before testing, electrodes were calibrated using three Ca^{2+} ion solutions of known concentrations to ensure the electrode test values were within the normal range. When the Ca^{2+} concentration in the test solution was 2 mM, the electrode calibration concentrations were: 0.5 mM, 2.0 mM, 5.0 mM. When the Ca^{2+} concentration in the test solution was 0.2 mM, the electrode calibration concentrations were: 0.1 mM, 0.2 mM, 1 mM. Only electrodes with Nernstian slopes>27 mV/decade were used in our study. The electrode was mounted on a three–dimensional micro–manipulation device on a microscope, with the specimen placed at the center of the stage. Using a microscope computer–based imaging system, the electrode tip was positioned about 2 – 3 m above and perpendicular to the specimen surface, Recording voltages were added at two points near and away from the cell(Excursion 10 m, frequency 0.2–0.3 Hz). By Ficks first law of diffusion equation: $J_0 = -D \cdot dc/dx$, where D is the diffusion coefficient of specific ions or molecules in $cm^{-2}s^{-1}$, dc is the concentration difference between the two points, and dx is the distance between the two points[7], data were processed to obtain the ion mobile rate and unit(pmol/cm^2.s). Test temperature was 20–23℃.

Data were collected through dynamic monitoring of isolated cochlear cells, basilar membrane fragments, cochlear basilar membrane and HEK293T cells. Ca^{2+} ions flow were measured in all the samples in the resting state, and the direction of ion flow was determined. Because OHCs is a special kind of polar cells, Ca^{2+} ion flows were recorded at the head (sterecilia side) and bottom of these cells in pmol/cm^2.s (Figure I A&B).

Results

Ca^{2+} flow in isolated OHCs

Four types of Ca^{2+} ion flow were recorded from isolated OHCs in the resting state: influx at the head of the cell(–1378.30 ± 350.61, n=9) with efflux at the bottom (1095.04 ± 487.68, n=9) (type 1); efflux at the head (558.35 ± 182.34, n=8) with influx at the bottom(–990.22 ± 312.36, n=8) (type 2); influx at the both head (–1454.60 ± 345.05, n= 11) and bottom (–943.26 ± 255.59, n=11) (type 3); and efflux at the both head (851.88 ± 231.71,