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Cholesterol influences potassium currents in inner hair cells isolated from guinea pig cochlea

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ABSTRACT

Objective: There is a correlation between serum hyperlipidemia and hearing loss. Cholesterol is an integral component of the cell membrane and regulates the activity of ion channels in the lipid bilayer. The aim of this study was to investigate the effects of cholesterol on the potassium currents in IHCs by using the cholesterol-depleting drug, MβCD, and water-soluble cholesterol.
Methods: IHCs were acutely isolated from a mature guinea-pig cochlea and potassium currents were recorded. MβCD and water-soluble cholesterol were applied to IHCs under pressure puff pipettes.
Results: IHCs showed outwardly rectifying currents ($I_{K,f}$ and $I_{K,s}$) in response to depolarizing voltage pulses, with only a slight inward current ($I_{K,n}$) when hyperpolarized. In 10 mM MβCD solutions, the amplitude of outward K currents reversely decreased; however, fast activation kinetics was preserved. In contrast, in solution of 1 mM water-soluble cholesterol, the amplitude of outward K currents reversely increased. At the membrane potential of +110 mV, relative conductances were 0.87 ± 0.07 and 1.18 ± 0.11 in MβCD solutions and cholesterol solutions, respectively.
Conclusion: The amplitude of K currents in isolated IHCs was reversely changed by cholesterol-depleting drug and water-soluble cholesterol. These results demonstrated the possibility of the involvement of IHC function in hyperlipidemia-induced inner ear disorders.

1. Introduction

Recent reports suggest that there is a correlation between serum dyslipidemia/hyperlipidemia and hearing loss [1–3]. The reduction of cholesterol by fibrinogen and LDL apheresis was associated with more rapid recovery after the onset of sudden hearing loss [4]. Hearing problems associated with age or resulting from lipid challenges are ameliorated by statins, drugs used to lower the cholesterol level [5,6]. In animal models, high serum cholesterol showed associations with auditory dysfunction and morphological changes of the cochlea [7]. In addition, loading cholesterol in mice at a concentration of 1 mM was paralleled by an initial increase of DPOAE amplitudes followed by a decrease of up to 20 dB [8].

In the mammalian cochlea, there are two types of hair cell that subserve distinct functions and receive characteristic patterns of innervations. Inner hair cells (IHCs) receive nearly all afferent innervations and are primary acoustic transducers. The three IHC potassium currents are distinguishable by their pharmacology and their activation kinetics [9–11]. The fast activating current, $I_{K,f}$, is blocked by tetraethylammonium (TEA) but is resistant to 4-aminopyridine (4-AP). $I_{K,s}$ is activated more slowly on depolarization and is blocked by 4-AP but not by TEA. Another potassium current, $I_{K,n}$, is already activated at the resting potential and thus determines the resting membrane potential and membrane constant. Potassium currents are known to participate in the repolarization and discharge behaviors of action potentials in neurons [12,13];

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therefore, changes in IHC potassium currents may affect the IHC presynaptic function. Thus, potassium currents are crucial for maintaining the cell's physiological functions.

The plasma membrane consists of ordered microdomains, which are rich in cholesterol, sphingolipids, and saturated phospholipids [14,15]. Several ion channels gravitate toward the cholesterol-rich plasma membrane, so lipids regulate channel protein functions [16,17]. Indeed, cholesterol modulates calcium-sensitive potassium currents (BK currents) in smooth muscle, glioma, neuronal, and endothelial cells [18–20]. In cochlear hair cells, the plasma membrane contains lipid microdomains [21]. Cholesterol depletion by a cholesterol-depleting agent (methyl-β-cyclodextrin, MβCD) was also shown to reduce BK currents by 50% in chick cochlear tall hair cells [22]; however, a functional role in mammalian hair cells has not been reported.

In the present study, in order to evaluate the role of the local lipid environment in coordinating ion channel physiology in mammalian auditory hair cells, we acutely isolated the IHCs from guinea-pig cochlea and identified potassium currents. The effects of a cholesterol-depleting drug, MβCD, and water-soluble cholesterol on potassium currents were investigated.

2. Materials and methods

2.1. Preparation of isolated IHCs

An adult albino guinea pig (200–350 g) was killed by rapid cervical dislocation, both bullae were removed, and the cochlea was exposed. The cochlea, fused to the bulla, was placed in Ca^{2+}-free external solution (mM: 142 NaCl, 4 KCl, 3 $MgCl_2$, 2 NaH_2PO_4, 8 Na_2HPO_4, adjusted to pH 7.4 with NaOH). The otic capsule was opened, allowing removal of the organ of Corti attached to the modiolus. The organ of Corti was treated with trypsin (0.5 mg/ml, T-4665; Sigma–Aldrich, St. Louis, MO) for 12 min, and gentle mechanical trituration was carried out. Trypsin was rinsed from the specimen by perfusing with a standard external solution (mM: 142 NaCl, 4 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 2 NaH_2PO_4, 8 Na_2HPO_4, adjusted to pH 7.4 with NaOH) for at least 10 min before starting any experiments. The osmolalities of all solution were maintained at 320 ± 5 mmol/kg by adding glucose to the solutions (OM-806; Bio Medical Science, Tokyo, Japan). The most important landmarks for identifying IHCs are a tight neck and the angle between the cuticular plate and the axis of the cell.

2.2. Recording procedures

Membrane currents were measured by conventional whole-cell voltage-clamp recording using an EPC-10 (HEKA, Lambrecht, Germany). Data acquisition was controlled by using the software PatchMaster (HEKA, Lambrecht, Germany). Recording electrodes were pulled with a two-stage vertical puller (PP830; Narishige, Tokyo, Japan) using 1.5 mm outside-diameter borosilicate glass (GC-1.5; Narishige, Tokyo, Japan) filled with an internal solution (mM: 144 KCl, 2 $MgCl_2$, 1 NaH_2PO_4, 8 Na_2HPO_4, 2 ATP, 3 D-glucose, 0.5 EGTA, adjusted to pH 7.4 with KOH). Pipettes showed resistance of 4–8 MΩ in the bath and were coated with ski wax (Tour-DIA; DIAWax, Otaru, Japan) to minimize capacitance. Automatic adjustment of capacitance transient cancelation and series resistance compensation were applied during each whole-cell experiment. Cell capacitance was 12.0 ± 4.0 pF (mean ± s.d.) and the series resistance was 14.4 ± 2.1 MΩ (n = 14). Methyl-β-cyclodextrin (MβCD, C4555; Sigma–Aldrich, St. Louis, MO) and water-soluble cholesterol (C4951; Sigma–Aldrich, St. Louis, MO) were applied under pressure (Pressure microinjector: PMI-200; Dagan, Minneapolis, MN) using pipettes with a tip diameter of 2–4 μm positioned around 50 μm from the IHCs. Currents were recorded within 30 s after applying MβCD or water-soluble cholesterol solutions. Cells were continuously perfused with external saline and all experiments were performed at room temperature (20–25 °C).

2.3. Animal care

The experimental design was reviewed and approved (Accession No. A25-003-0) by the Animal Care and Use Committee, Kyushu University. All procedures were conducted in accordance with the Guidelines for Animal Care and Use Committee, Kyushu University.

3. Results

3.1. MβCD suppressed potassium currents in IHCs

Membrane currents in response to hyperpolarizing and depolarizing voltage steps from a holding potential of −60 mV were recorded from IHCs. In standard control solutions, IHCs showed outwardly rectifying K currents ($I_{K,f}$ and $I_{K,s}$) in response to depolarizing voltage pulses, with only a slight inward current ($I_{K,n}$) when hyperpolarized (Fig. 1A, left). To examine the roles of cholesterol, MβCD was applied directly to the isolated IHCs. In solution of 10 mM MβCD, the amplitude of outward K currents decreased by 24% (Fig. 1A, middle), and the reduction induced by MβCD was reversed by washing with control solutions (Fig. 1A, right). A total of 12 of 14 cells showed reduction with MβCD and recovery by washing. In the MβCD solutions, fast activation kinetics was preserved, that is, displaying a rapid rising phase in the outward currents (Fig. 1B). Fig. 2 represents the steady-state current–voltage (I–V) relationship measured at the end of each 100 ms command step for all 14 cells in control solutions and 10 mM MβCD solutions. Both curves showed pronounced outward rectification with maximal slope conductances of 71.2 nS and 57.7 nS in control solutions and MβCD solutions, respectively.

3.2. Cholesterol potentiated potassium currents in IHCs

In the solution of 1 mM water-soluble cholesterol, the amplitude of outward K currents increased by 11% (Fig. 3, upper-middle), and the potentiation induced by cholesterol was reversed by washing with control solutions (Fig. 3, upper-right). A total of 11 of 13 cells showed potentiation with cholesterol and recovery by washing. In the cholesterol solutions, the fast

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