Acid–base regulation in cochlear outer hair cells of the guinea-pig

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Summary
The pH regulation of the outer hair cell (OHC) was investigated using fluorescence imaging microscope. The application of NH₄Cl increased intracellular pH in OHC followed by a slow recovery of pH. The subsequent addition of Na-free solution led to the acidification. Moreover, the application of a standard solution gradually increased pH, which was inhibited by amiloride. CO₂-loaded OHC showed a rapid acidification inhibited by acetazolamide. These findings indicate that Na-H exchange and carbonic anhydrase are involved in acid–base regulation in OHC.

Introduction
Acknowledge of biophysical characteristics of hair cells of the cochlea has been accumulated with respect to acoustic transduction and transmission. It is believed that the OHC possessing contractile function contributes to "tuning" by interaction with the inner hair cell. Intracellular pH (pHi) plays important roles in various cellular activities such as solute transport, biochemical function, and cell growth and differentiation. Furthermore, pHi may modulate intracellular Ca²⁺ regulation, which is strongly related to motile response of OHCs.

In the present study, we use the fluorescence imaging microscopy to measure pH in living, isolated outer hair cells to clarify the mechanism of acid–base regulation in this issue.

Materials and methods
The healthy guinea pig weighing 150–300 g was intraperitoneally anesthetized with pentobarbital sodium (35mg/kg) and was sacrificed by decapitation. After the temporal bone was removed, the bony capsule was picked away under the stereoscope to expose the spiral ligament. In the apical to third turns, the lateral wall of the cochlear duct was pulled away from the basilar membrane. Thereafter, the outer hair cells were mechanically isolated.

The specimen in artificial perilymph, which composed of 150mM NaCl, 3.5mM KCl, 1.0mM CaCl₂, 1.0mM MgCl₂, 2.75mM Heps sodium, and 2.25mM Tris (pH 7.4) and the osmolality was adjusted to 300m0sm with D-glucose and supplemented with by 2', 7'-Bis (carboxyethyl) -5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM, 3µM/ml), was incubated at 37°C for 40 to 60 min. The BCECF-loaded outer hair cells were plated on a glass coverslip coated with Cell-Tak®.

Fluorescence measurements were carried out with inverted microscope, image SIT-camera and processor. The ratio of epifluorescence at 530nm emitted with 450nm and 490nm excitation light was used as a measure of cytosolic pH (1).

Results and discussion
Firstly, effects of amiloride, DIDS, and Na-free solution on pHi recovery from acid-load was observed (2). The application of NH₄⁺ containing solution caused a rapid alkalization due to the influx of NH₄⁺, which is immediately protonated to from NH₃ inside the cell. Subsequently, sluggish acidification resulted from the slow influx of NH₄⁺, as a small fraction of NH₄⁺ dissociates intracellularly into H⁺ and NH₃. When external NH₄⁺ and Na⁺ were removed by introducing the N-methyl-D-glucamine (NMDG⁺) solution, as intracellular NH₃ dissociates into H⁺ and NH₄⁺, pHi was promptly decreased showing a large undershoot. Subsequent addition of the standard Na⁺ solution gradually increased pHi. 0.5mM amiloride apparently inhibited this Na⁺-induced increase.
in pH. whereas 0.3mM 4,4'-diisothiocyanostilbene-2,2'-disulphonate (DIDS) did not affect the pH increase. Either amiloride or DIDS had little effect on pH of the steady state. This indicates that Na⁺-H⁺ exchange or Cl⁻-HCO₃⁻ exchange activity in the unstimulated or acid-unloaded condition is too small to be detected. However, acid-loading by the NH₄⁺-prepulse method demonstrated that Na⁺-H⁺ exchange is involved in pH regulation of OHC, but not Cl⁻-HCO₃⁻ exchanges.

Secondly, the intracellular acidifying effect of the weak acid CO₂ was investigated. Successive exposure to the standard solution bubbled with 5% CO₂/95% O₂ and 100% CO₂ gas caused stepwise declines of pH, as CO₂ readily enters the cell, combines with water to form H₂CO₃, and then dissociates to form H⁺ and HCO₃⁻. A pH recovery was observed following removal of 5% CO₂ by substituted with 100% O₂. The slope of this recovery phase was inhibited by 0.5mM acetazolamide. However, the steady-state pH was not affected by application of acetazolamide. These findings suggest that carbonic anhydrase is involved in pH regulation of OHC in the CO₂-induced acidosis.

References