Ionic Conductance Change in Supporting Cells of the Organ of Corti Caused by Ca^{2+}

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ABSTRACT. The supporting cells of the organ of Corti are considered to buffer and support the hair cells. Recent technical advances in experiments concerning the inner ear have indicated that these cells play a physiological role in the inner ear. It has also been found that conductance of the gap junction between supporting cells is regulated by Ca^{2+} and H^+. Therefore, it is suggested that Ca^{2+} regulates ionic conductance of the supporting cells. In the present study, 10 μM A23187, 10 μM IP_3 and 10 mM BAPTA, were applied extracellularly and intracellularly. Outward current was increased by 10 μM A23187, 10 μM IP_3, but decreased by 10 mM BAPTA. When Cl^- was replaced with sodium gluconate, the outward current decreased. Therefore, it was concluded that Ca^{2+} regulates the ionic conductance of the supporting cells of the organ of Corti, and it is suggested that Ca^{2+} maintains outward current.

Key words: ionic conductance — Ca^{2+} — supporting cells — organ of Corti

Developments in the study of the supporting cells of the organ of Corti[1,2] and experiments regarding them have indicated that they play a physiological role in the inner ear. The supporting cells are connected with gap junctions.[3,4] The gap junctions of the supporting cells of the organ of Corti are controlled by H^+ and Ca^{2+}.[5-10] At the same concentration, Ca^{2+} has a stronger effect than H^+ on the homeostasis of the human body. Based on measurements of membrane capacitance changes in the supporting cells caused by ryanodine, caffeine and IP_3, it has been concluded that an intracellular calcium release mechanism exists in these cells.[5-10] Furthermore, a difference has been noted in the ionic conductance between Deiters' cells and Hensen's cells and Ca^{2+} has also been found to influence the ionic conductance of supporting cells.[1,2,11] Based on an association between Ca^{2+} and K^+ and Cl^-, Ca^{2+} release is supposed to affect ionic conductance.[12] The supporting cells of the organ of Corti are assumed to be activated to increase the K^+ current and Cl^- current. Then, the concentration of K^+ of the cochlear duct is supposed to increase and the concentration of Cl^- of the cochlear duct is thought to decrease. In this study, we measured the ionic conductance of the cell membrane with voltage clamp techniques.

MATERIALS AND METHODS

Albino guinea pigs were decapitated under halothane anesthesia and their

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cochleas were removed. The top two turns were separated and placed in a calcium-free extracellular solution 136.9 mM NaCl, 5.37 mM KCl, 0.81 mM $MgSO_4$, 0.98 mM $MgCl_2$, 0.44 mM KH_2PO_4, 1.54 mM $NaHPO_4$ and 5 mM Dextrose at pH 7.4 containing 500 μg-1000 μg/ml trypsin. Then, the tissue was agitated gently in a shaker for about 15 min, and isolated cells were allowed to settle onto a coverslip placed at the bottom of a 750 μl perfusion chamber. Next, the cells were continuously perfused in a solution containing 136.9 mM NaCl, 5.37 mM KCl, 1.25 mM $CaCl_2$, 0.81 mM $MgSO_4$, 0.98 mM $MgCl_2$, 0.44 mM KH_2PO_4, 1.54 mM $NaHPO_4$ and 5 mM Dextrose at pH 7.4 Patch pipettes were made from glass capillary tubes with an outer diameter of 1.5 mm using a vertical puller (PB-7, Narishige, Tokyo, Japan). Recordings were made using an Axon-Patch 1D or Axon 200A patch-clamp amplifier. A filter was used to cut noise to less than 2 kHz. Patch electrodes, which contained 140 mM KCl, 10 mM HEPES, 1 or 0.5 mM EGTA, and 2 mM $MgCl_2$ at pH 7.4, were applied intracellularly with the testing chemicals. Intracellular perfusion was performed with BAPTA and IP_3 using a Clark Instrument pippette. A23187 was applied extracellularly. To measure the change in Cl^-, 136.9 mM NaCl was replaced by 140 mM sodium-gluconate. The initial resistance of the patch electrodes was within 10 MΩ. Step pulses were applied at a 10 mV step with a holding potential of -60 mV or -80 mV. Measurements were performed at room temperature (20-25℃). The present experiments were approved by the Animal Research Committee of Kawasaki Medical School (No.96-015, 1996) and conducted according to the "Guide for the Care and Use of Laboratory Animals" of Kawaski Medical School.

RESULTS

Extracellular or intracellular perfusion was performed before chemicals were applied. After the outward current became stable, the chemicals were perfused extracellularly or intracellularly. 10 μM A23187 was applied extracellularly. After 4 min the outward current increased about five times that before perfusion. The changes in outward current are shown in Fig 1.

Intracellular perfusion of 10 μM IP_3 and 2 or 10 mM BAPTA was performed with the control solution and test chemicals. After the outward current became stable, the chemicals were perfused intracellularly. Usually 10 μM IP_3 increased the outward current 2 min after perfusion. The changes in outward current and I-V curves are shown in Fig 2. Outward current increased voltage dependently.

Intracellular application of 10 mM BAPTA decreased the outward current. Even with application by intracellular perfusion, it took about 2 min for the changes shown in Fig 3 to occur. In 2 mM BAPTA, no decrease in outward current was noted, as shown in Fig 4.

To lower the Cl^- concentration, sodium gluconate was used in place of NaCl. When Cl^- was replaced by sodium gluconate extracellularly, the outward current decreased, as shown in Fig 5. Influx of Cl^- into the cell is considered to maintain outward current.

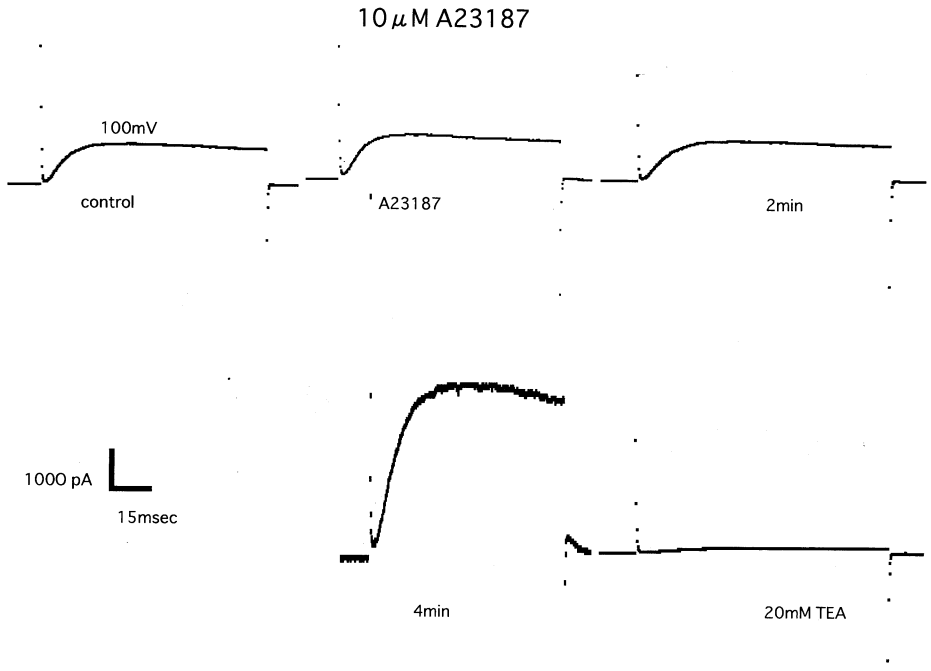

Fig 1. 10 μM A23187 was applied extracellularly, and outward current increased 4 min after extracellular perfusion. After 20 mM tetraethylammonium (TEA) perfusion, outward current was almost abolished.

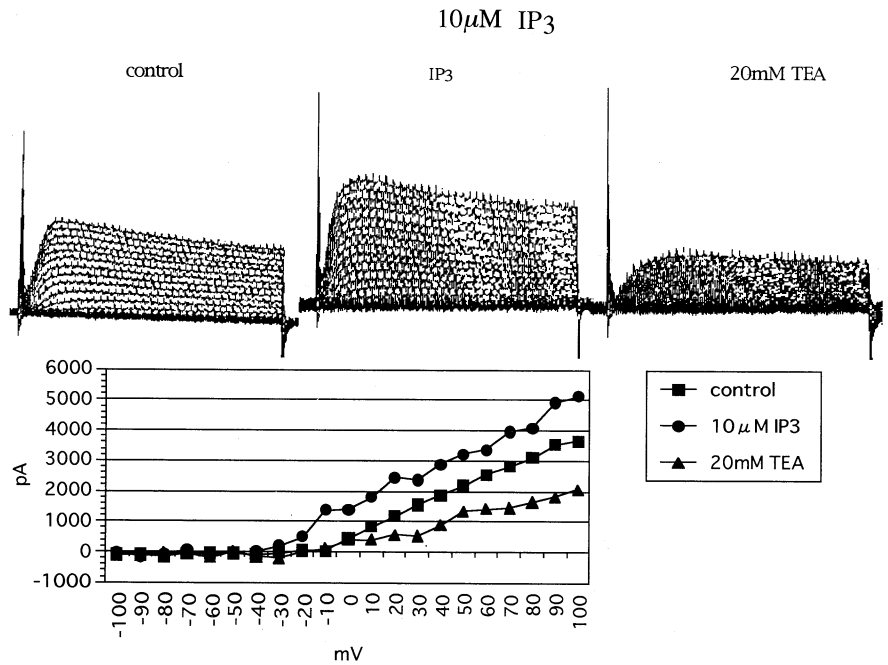

Fig 2. 10 μM IP_3 was applied intracellularly, and outward current increased 2 min after perfusion. The changes in outward current are shown in I-V curves. After 20 mM TEA intracellular perfusion, outward current decreased.

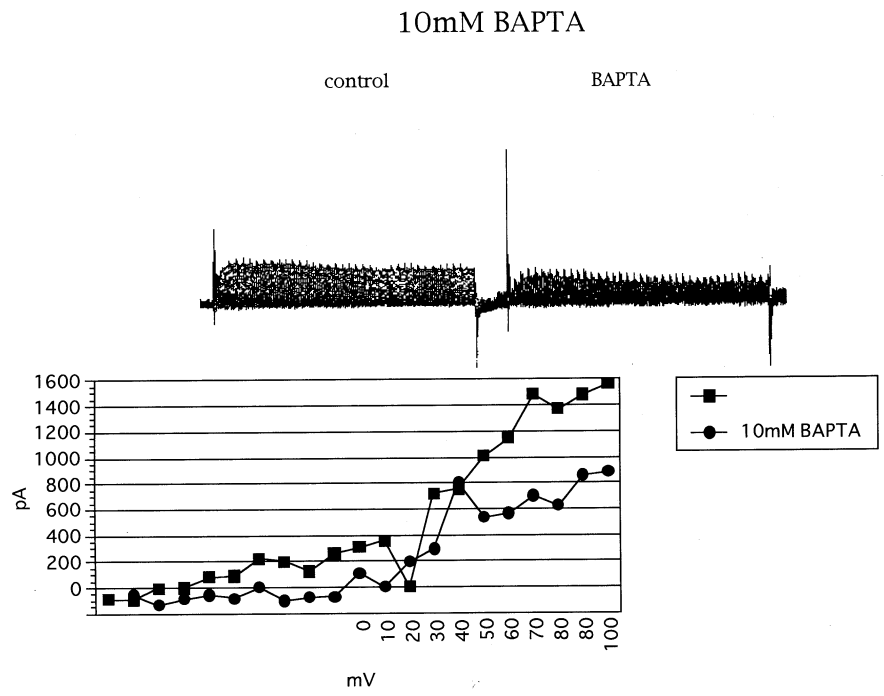

Fig 3. 10 mM BAPTA was applied intracellularly, and outward current decreased 2 min after intracellular perfusion.

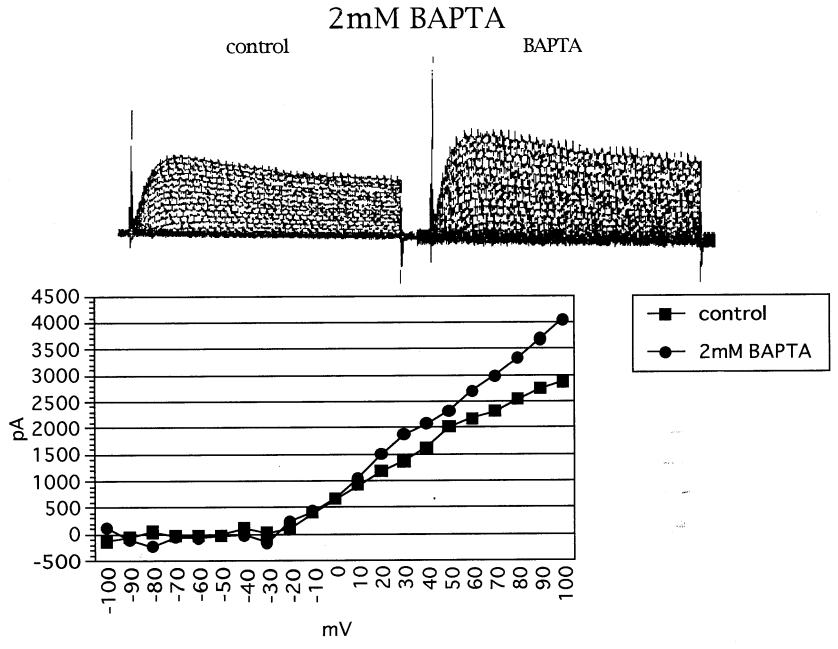

Fig 4. 2 mM BAPTA was applied intracellularly, and outward current did not decrease.

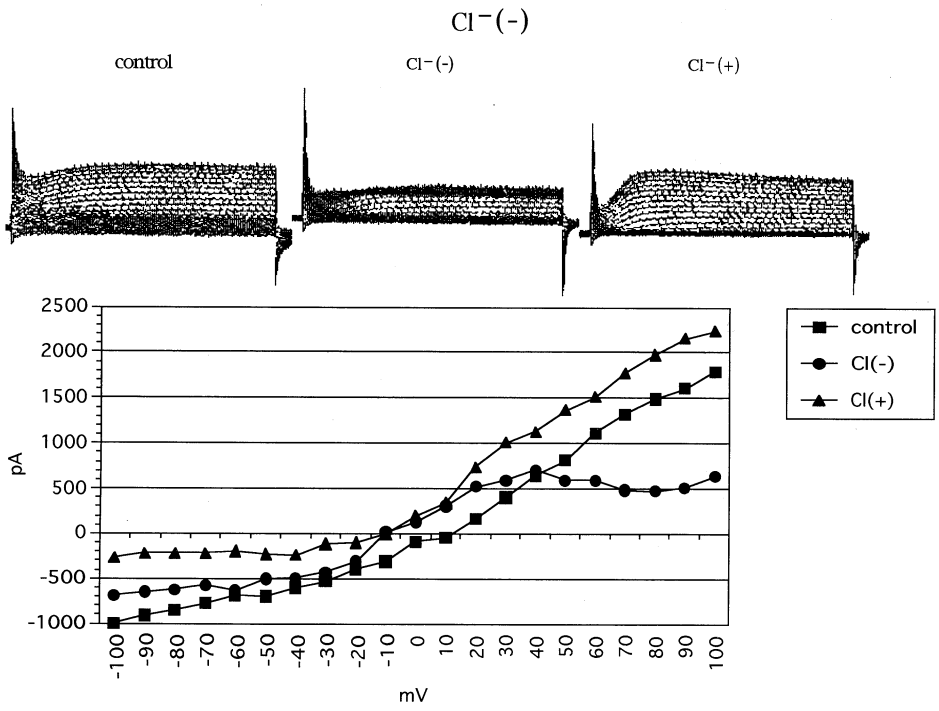

Fig 5. Sodium gluconate, which replaced NaCl, was applied extracellularly and outward current decreased.

DISCUSSION

From the results of experiments with the applied calcium-related chemicals, A23187, ryanodine, caffeine and IP_3, it has been considered that Ca^{2+} could control the gap junction of the supporting cells.[5-10] In addition, it is suggested that there is an intracellular Ca^{2+} store in the supporting cells and the release of Ca^{2+} from the store increases the Ca^{2+} concentration in the supporting cells. Although details of the calcium store are unclear, electron microscopic observations indicate that the endoplasmic reticulum exists in the cells and stores Ca^{2+} in its organelles.[13] However, low concentrations of calcium chelating chemicals did not cause any outward current change. Such low concentrations of calcium-chelating chemicals are thought to be insufficient to chelate Ca^{2+} in the supporting cells. Ca^{2+} is thought to change ionic conductance in the important ions like K^+, and Cl^-. These ions are supposed to change ionic conductance with gap junction conductance. K^+ is an important ion in the regulation of cell function. In the fast motility of hair cells, a change in ionic conductance is not considered to be predominant. However, K^+ is also important in the regulation of the slow motility. K^+ is regulated through Ca^{2+} and Cl^-, which is important in the regulation of osmolarity, is also regulated through Ca^{2+}. When Ca^{2+} increases, the efflux of K^+ and the influx of Cl^- are supposed to increase. As a result of ionic conductance change, the slow motility of hair cells will increase.[14] Therefore,

Ca^{2+} is important not only for regulation of the ionic conductance of the cell membrane of the supporting cells but also in hearing mechanics.

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