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Short Communication

A re-evaluation of cell coupling in the organ of Corti

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Intercellular electrical coupling was assessed in an in vitro organ of Corti preparation using separate electrodes to inject current and record voltage drops in Hensen's cells. The results demonstrate much better coupling among these supporting cells than previously thought. Coupling ratios between adjacent Hensen's cells are greater than 0.6.

electrical coupling, organ of Corti, gap junction

All the supporting cells of the organ of Corti are structurally joined to each other by communicating or gap junctions [2,3,4]. Recently, Santos-Sacchi and Dallos [5], demonstrated that these junctions permit the passage of electrical currents between Hensen's cells. In that study, the degree of electrical coupling between cells was difficult to assess accurately since a single electrode-bridge balance system was used to inject current and record voltage simultaneously. The coupling ratio is defined as the voltage drop in one cell (V_2) divided by the voltage drop due to current injections in a neighboring cell (V_1). Thus, if an inaccurate measure of the voltage drop, V_1 , is obtained due to bridge unbalancing as a result of electrode resistance change, the degree of coupling between cells is misjudged. A decrease of the electrode resistance occurring upon entry into a cell will produce overestimates of coupling ratios; an increase in resistance will produce underestimates. There is evidence that electrode resistance increases intracellularly [6]. This possibility led us to speculate from indirect measures (spatial spread of current studies) that the coupling between supporting cells was greater than that suggested by the

data (see footnote in [5]). This report corroborates that initial speculation.

Cochleas were removed after decapitation of anesthetized guinea pigs. The apical and third turns of the cochlea, lateral wall removed, were placed in one piece into a microscope perfusion chamber. The organ was perfused at a rate of 0.8 ml/min with artificial perilymph, bubbled with O_2 (tissue culture medium M199 with Hank's salts (1.26 mM $CaCl_2$, 1.7 μM $Fe(NO_3)_3$, 5.36 mM KCl, 0.44 mM KH_2PO_4 , 0.81 mM $MgSO_4$, 137 mM NaCl, 4.16 mM $NaHCO_3$, 0.33 mM Na_2HPO_4), pH 7.2, Gibco, Grand Island, N.Y.) and the temperature was maintained near 37°C. Membrane potentials and coupling responses were measured with high-input impedance devices using KCl (3 M) electrodes. Coupling measures were performed by injecting current pulses into one cell through one barrel of a double-barreled electrode, while measuring voltage drops in that same cell with the other barrel and in a neighboring cell with a single electrode.

Immediately after dissection, membrane potentials of the Hensen's cells are low (ca. -20 mV); however, over the next 2–3 h the potentials gradually recover and reach levels which are recorded in vivo (-60 to -80 mV [1,5]). Cell-to-cell coupling occurs throughout this recovery period. Fig. 1 demonstrates electrical coupling between two adjacent Hensen's cells, both with good membrane

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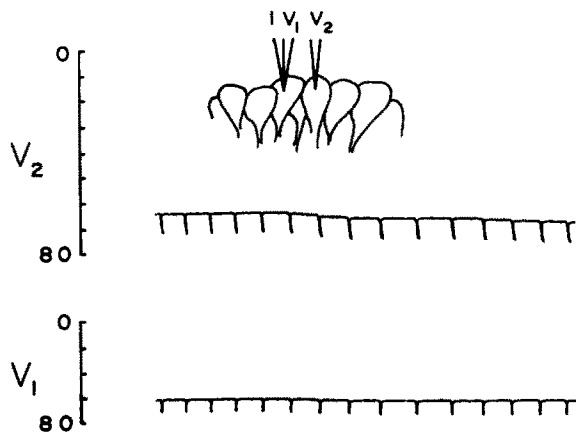


Fig. 1. Under visual control, adjacent Hensen's cells were impaled with 3 M KCl glass microelectrodes. Constant current pulses (-11 nA, 0.5 s) were delivered every 5 s through one barrel of the double-barreled microelectrode. Voltage drops in that same cell and the adjacent cell were measured with voltage recording electrodes. The coupling ratio (V_2/V_1) for this preparation was 0.77 . The organ had been in culture for 2 h when the measure was taken.

potentials. The coupling ratio in this case is 0.77 . Although coupling can be variable, it is usually greater than 0.6 for adjacent cells (5 animals) and is much greater than the estimates previously obtained with the single electrode technique [5]. Input resistance of Hensen's cells in this in vitro preparation is $1-3$ M Ω , one order of magnitude less than previous measures using the single electrode technique.

These results clearly show that electrical coupling among the supporting cells of the organ of Corti is much better than previously thought. It is not clear, however, whether these results are indicative of the in vivo situation. Considering the harsh treatment to the organ necessitated by the in vitro setup, if a difference in coupling did exist, one might expect coupling to be poorer in vitro than in vivo (M.V.L. Bennett, pers. commun.). The fact that membrane potentials are similar in both preparations lends credence to the notion that coupling is also similar. Nevertheless, other factors (e.g. EP, endolymph-perilymph compartmentalization) may be influential in affecting in vivo coupling. In vivo re-evaluations are planned.

The fact that coupling (and space constant) is now known to be substantial provides impetus to

re-evaluate the absence of dye coupling previously reported [5]. It had been suggested then that dye coupling might exist, but that because of slow transfer between cells and dilution within the cytoplasm the dye was impossible to visualize with the experimental setup available. Image enhancement techniques may be necessary to fully evaluate this issue.

The role that such electrical communication may play in an organ whose electrical activity is of paramount functional importance remains unclear. There can be no doubt, however, that supporting cells play a crucial role in inner ear function – perhaps even in sensory transduction. That is, it is inconceivable that the supporting cells do not influence cochlear micromechanics. Supporting cells, as do hair cells, contain contractile and structural proteins [7], which may be modified by ionic conditions. Substantial electrical/ionic communication might provide a means to effectively influence organ of Corti structure and function.

Acknowledgement

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