Connexin-mediated coupling in non-sensory cells of the developing cochlea: a biophysical study

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Hearing loss is the most common form of sensory impairment, with approximately one infant/1000 born with profound congenital deafness and over 50% of cases attributable to a genetic cause [1]. Nonsyndromic hearing loss and deafness (DFNB1) is an inherited condition with a mild to severe deafness phenotype caused by mutations in GJB2 (which encodes the protein connexin26) and GJB6 (which encodes connexin30) [2]. Gap junction channels formed primarily by these two connexin protein subunits couple non-sensory cells (supporting and epithelial cells) of the mammalian cochlea, forming vast functional syncytia. Previous work has shown that electrical and metabolic coupling mediated by gap junction channels is fundamental for the development and maintenance of the hearing function [3,4,5]. However, precise estimates of the degree of coupling and its alterations under DFNB1 conditions are lacking, notwithstanding the vast body of studies conducted in recombinant expression systems.

In this work, we combined large scale optical recordings, single cell electrophysiology and computer simulations to elucidate the mechanisms that underlie intercellular communication in cochlear non-sensory cells from juvenile mice (first postnatal week). First, we developed a novel technique based on voltage imaging to map the extent and the degree of electrical coupling in non-sensory cell networks of the developing mouse cochlea. Our method exploits a newly synthesized photo-induced electron transfer-based voltage sensitive dye, Vf2.1.Cl [6], that allows the simultaneous mapping of electrical activity of multiple cells using fluorescence imaging. We quantified precisely the reduction of electrical coupling in cochlear organotypic cultures from transgenic mice with hearing defects due to absence or mutation of connexin30 compared to wild type animals. By comparing our experimental results with numerical simulations, we estimated that cochlear supporting cells in the mouse are already well coupled in the first postnatal week by as many as 1500 channels per cell pair. In age-matched cultures from connexin30(T5M/T5M) and connexin30(−/−) mice, junctional conductance was reduced respectively by 14% and 91%, and these data account for the increased hearing thresholds exhibited by these animals in the adult stage [4,7].

Besides electrical coupling, inner ear gap junction channels and hemichannels have been shown to participate in ATP- and IP_3- dependent intercellular Ca^{2+} signalling [3,4,5]. We thus performed Ca^{2+} imaging experiments aimed at elucidating the mechanisms underlying the generation and intercellular propagation of ATP-mediated Ca^{2+} signals in cochlear non-sensory cells. We determined that ATP- and IP_3- dependent Ca^{2+} oscillations in cochlear non-sensory cells can occur at constant intracellular IP_3 concentration. We finally combined the information gathered from the different experimental approaches in a mathematical model that (i) correctly reproduces the range and propagation speed of intercellular Ca^{2+} waves, (ii) indicates that inception and culmination of self-sustained Ca^{2+} oscillations are marked by two supercritical Hopf bifurcations at ATP concentrations of ~100 nM and ~1 µM, respectively and (iii) predicts that ATP release through connexin hemichannels is the primary mechanism responsible for the long range propagation of Ca^{2+} signals in the developing mouse cochlea.

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