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Brain Research Reviews 32 (2000) 167–171

BRAIN RESEARCH REVIEWS

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Short review

Cell coupling in Corti's organ

J. Santos-Sacchi *

Surgery (Otolaryngology) and Neurobiology, Yale University School of Medicine, BML 244, 333 Cedar St., New Haven, CT 06510, USA

Abstract

The mammalian organ of Corti is responsible for the initial analysis of sound; injury leads to hearing loss. During the last two decades, the characteristics of cellular coupling in this specialized epithelium have been studied. In this review, data on both electrical and mechanical coupling are covered. While electrical coupling likely contributes to homeostasis in the organ, this concept is far from proven.
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Keywords: Coupling; Corti's organ; Cell

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1. Introduction

The organ of Corti is a specialized, avascular sensory epithelium that rests on the basilar membrane of mammals. Within this organ, mechanical energy is transduced into electrical activity. A variety of cell types populate the organ along the whole spiral length of the basilar membrane. The cells can be categorized into two major classes — hair cells and supporting cells. In order to appreciate the consequences of cell coupling in the organ, a brief account of our current concept of mammalian peripheral

* Corresponding author. Fax: +1-203-737-2502; e-mail: joseph.santos-sacchi@yale.edu

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PII: S0165-0173(99)00077-6

auditory physiology is presented (for additional information, see Ruggero and Santos-Sacchi [23]).

2. How the organ works

There are two types of auditory hair cells in mammals — inner hair cells (IHCs) and outer hair cells (OHCs). Each cell type generates receptor potentials when its stereociliar bundle is deflected by sound-induced basilar membrane vibration [5,24]. While OHCs outnumber IHCs by about four to one, it is the IHC type that receives up to 95% of the afferent innervation of the eighth nerve [38]. OHCs are mechanical effectors as well as receptors [1], and are capable of voltage-driven length changes in the acoustic frequency range [3,31,33]. Through their mechanical activity, OHCs provide a boost to basilar membrane motion that ultimately results in enhanced sensitivity and frequency selectivity in the output of IHCs. This physiologically labile enhancement has been termed the ''cochlear amplifier'' [6].

Hair cells are enveloped by supporting cells. IHCs have closely opposed supporting cells that provide a restricted interstitial fluid space. OHCs, on the other hand, lose close appositions with supporting cells during development; in the adult, the OHCs' lateral membranes are exposed to the large fluid spaces of Nuel. However, the supporting Deiters' cells retain close apposition at the apical and basal poles of the OHC.

The mechanism by which hair cells are excited is well characterized (see Pickles and Corey [22]). Bundle deflection modulates a standing cationic current through mechano-sensitive channels located in apical stereocilia tips. The current is predominantly carried by K^+ since the endolymphatic fluid which bathes the bundle is high in potassium (~ 140 mM; Ref. [25]). The driving force for transduction current derives from the voltage drop across the hair cell's apical membrane, i.e., the difference between the positive endolymphatic potential (+80 mV) and the cell's negative resting potential. An accessory epithelium, the stria vascularis, is responsible for the ionic content and potential of the endolymphatic space. The perilymphatic fluid that bathes the basolateral membranes of hair cells is similar to CSF. As a consequence of incessant hair cell and synaptic activity, potassium is continuously released into the extracellular spaces. In the case of the IHC, whose extracellular space is small, even slight increases in potassium may seriously impair hair cell and neural activity. Even though the OHC lateral membrane is exposed to the fluid spaces of Nuel, this membrane lacks voltage-dependent conductances that could contribute to potassium efflux [34]. Instead, voltage-dependent potassium conductances are restricted to the basal pole of the cell — where tight apposition of Deiters' cell cups provide a restricted fluid space with the potential for potassium accumulation. In either hair cell type, potassium-induced depolarization will interfere with sound transduction since the driving force for transducer current will be reduced. Furthermore, normal transmitter release and afferent activity will be disrupted. In the OHC, however, additionally the mechanism responsible for cellular motility is directly susceptible to membrane potential [35]. Thus, the cochlear amplifier may be compromised.

3. Coupling between supporting cells

In the mid-1970's, a few groups characterized the ultrastructural extent of gap junctional contacts in the organ of Corti [11,12,14]. Based on structure, all supporting cells were found to potentially contribute to a syncytium of coupled cells. In 1983, Santos-Sacchi and Dallos [32] electrophysiologically confirmed that functional coupling was present in the organ of Corti. Using high impedance electrodes, they determined, both in vitro and in vivo, that current injection into one supporting cell produced voltage drops in adjacent cells (Fig. 1). Subsequently, it was shown that coupling ratios as high as 0.8 occur between adjacent Hensen cells [26]. Supporting cell coupling is sensitive to the effects of intracellular H^+ and Ca^{2+}, temperature, membrane tension and other uncoupling agents, such as octanol [27,28,31,41]. The sensitivity to intracellular pH is greater than that to intracellular calcium; calcium levels in the millimolar range are required to uncouple Hensen cells [36].

Supporting cells, as evidenced through Hensen cell studies, are so well coupled that voltage-dependent ionic conductances are effectively shared among coupled cells [31]. Outward K currents and inward rectifier currents from adjacent coupled cells are measurable under whole cell voltage clamp in one cell of a cell pair or group (Fig. 2). Uncoupling treatments reduce current magnitudes to single cell levels. This recruitment is believed to be important for the maintenance of the stable supporting cell

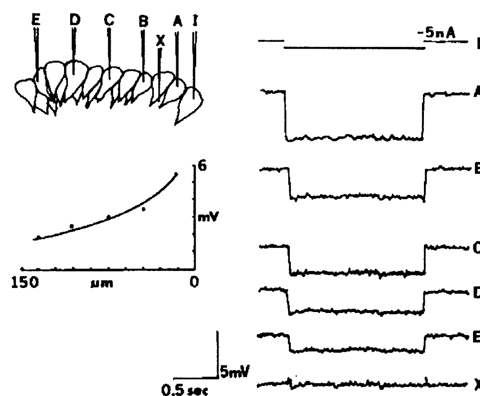

Fig. 1. Voltage responses in the Hensen cells of the isolated organ of Corti as a function of distance from a fixed intracellular current injection electrode. From Ref. [32].

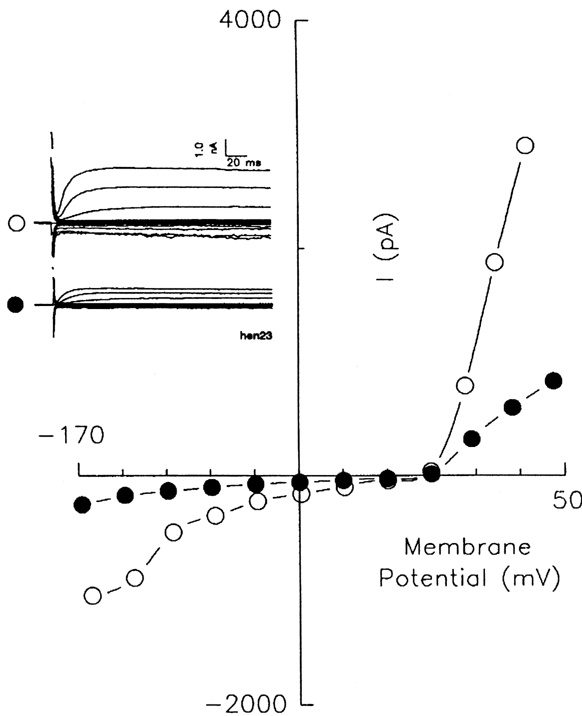

Fig. 2. Whole cell currents obtained under voltage clamp of one cell in a coupled triplet of Hensen cells. Before (open symbols) and after (filled symbols) uncoupling with CO_2 saturated medium. Note the reduction of currents to single cell levels after uncoupling. From Ref. [31].

membrane potentials (∼ −100 mV), since uncoupled single cells, which possess few K channels, are depolarized.

Cell coupling in Hensen and Deiters' cells has been studied with input capacitance measures and the double voltage clamp technique [31,36,41]. Because the supporting cells are so well coupled, input capacitance reflects the parallel combination of the coupled cells' capacitance. For example, visually confirmed single cells, pairs and triplets have average input capacitances of 31.0, 64.8 and 104 pF, respectively [41]. Using capacitance measures, junctional conductance was estimated to be nearly three orders of magnitude greater than single Hensen cell resting nonjunctional conductance [33]. Indeed, direct measurement under double voltage clamp provides an average conductance of about 50 nS, with some pairs exhibiting values above 500 nS (Fig. 3; Ref. [41]).

Although it was found in early studies that low temperature and CO_2 reversibly uncouple the supporting cell syncytium, reversible depolarization invariably accompanied such effects [27,28]. The direct effects of depolarization could not be fully evaluated in that type of preparation since neither the voltage nor the activities of H^+ and Ca^{2+} could be adequately controlled. It is now known that gap junctional conductance in supporting cells is voltage dependent [40,41,43]. In fact, most cell pairs studied with the double voltage clamp technique exhibit transjunctional voltage dependence and/or nonjunctional membrane voltage dependence. This dependence is observable through capacitance measures as well.

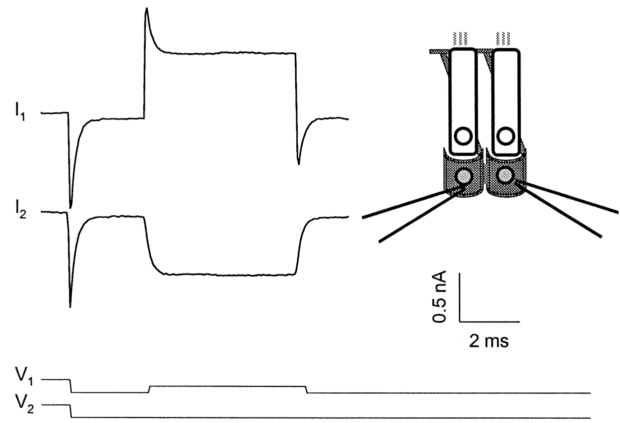

Fig. 3. Double voltage clamp of Deiters' cell pair. Ten millivolts test pulse delivered to cell 1. Junctional currents indicate a conductance, G_j, of about 75 nS. From Ref. [40].

One of the most striking features of the voltage dependence in supporting cell pairs is its diversity. Fig. 4 illustrates two cases of Hensen cell pairs where G_j rectifies to opposite polarities as V_j is altered. In fact, cases are also found that exhibit symmetrical V_j dependence or an absence of voltage dependence [43]. Nonjunctional voltage dependence is just as variable. We have speculated that this variability is due to heterotypic or heteromeric junctional channels [43], since a variety of connexin subtypes, including Cx26, Cx30, Cx43, are localized to the organ of Corti [9,17,18]. Such combinatorial effects may give rise to directional intracellular pathways along the supporting cell syncytium.

Initial studies on dye coupling in supporting cells showed little or no spread of intracellularly injected dyes [32]. Subsequently, it was determined that junctional communication in supporting cells is highly sensitive to photoinactivation occurring during real time viewing of fluorescent dye injections. By using low light level viewing technology, it was possible to demonstrate pronounced

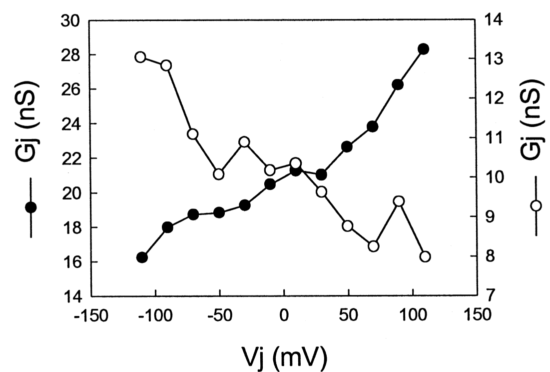

Fig. 4. Double voltage clamp of Hensen cell pairs indicating variability of transjunctional voltage dependence. In one case (open symbols), positive V_j in the voltage-stepped cell, caused a decrease in G_j, whereas in another cell pair (filled symbols) the opposite was found. From Ref. [43].

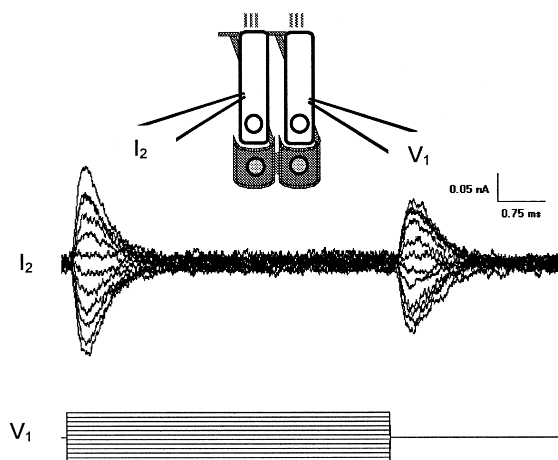

Fig. 5. Double voltage clamp of adjacent OHCs. Voltage steps in one OHC induced transient gating current in the adjacent cell. From Ref. [42].

dye coupling [29]. Recently, very interesting patterns of dye spread have been observed in the living animal, indicating that dye passage may be limited to preferential pathways [19].

4. Coupling between hair cells

In the mammal, there is no morphological evidence for gap junctional communication between hair cells or between supporting cells and hair cells. It is also clear from electrophysiological measures of sound-evoked hair cell activity that coupling is absent in vivo. Dallos and Santos-Sacchi [4] found that intracellularly recorded receptor potential cutoff frequencies for IHCs and OHCs were 340 and 1250 Hz, respectively. These equate to time constants of 0.47 and 0.13 ms, respectively. Considering that the membrane resistance obtained in vivo for either cell type is greater than 10 $M\Omega$, membrane capacitances can be computed to be roughly in the low tens of picofarad range — single cell values. As we have shown before, a coupled cellular syncytium would be predicted to have much greater input capacitance. Additionally, as expected in the absence of hair cell to supporting cell coupling, receptor potentials in the guinea pig are larger in the hair cells and extracellular fluid spaces of the cochlea than in the supporting cells [2,5,20,21]. Contrary results have been obtained in the gerbil [44].

While gap junction-mediated coupling is absent between hair cells, we have recently found a mechanoelectrical coupling between adjacent OHCs [42]. Because of the voltage-dependent mechanical activity of OHCs, voltage drops across the lateral membrane of an OHC will additionally cause deformation of physically adjacent cells. Since the motility voltage sensor in OHCs is sensitive not only to voltage but to membrane tension, gating currents are evoked in the adjacent cell (Fig. 5). The gating currents in adjacent OHCs result from a tension-induced shift in the voltage dependence of gating charge movement [10,13,15]. Interestingly, the resulting charge-induced transient polarization of the adjacent OHC is opposite in polarity to that of the voltage in the stimulated cell — indicative of lateral inhibition. Furthermore, since the voltage dependence of the charge movement and the mechanical response are equivalent, the mechanical activity of any one OHC is directly influenced by that of its neighbors. We believe that sharpening of the basilar membrane response beyond that of Bekesy's passive tuning results from this mechanical coupling.

5. What does it all mean?

Cell coupling in the organ of Corti is pronounced and diverse. It is likely that both the mechanical and electrical interactions reviewed here are important for the organ.

As far as gap junction-mediated coupling is concerned, the truth is that we have no direct indications what function it plays. Since the discovery of junctional coupling in the organ of Corti, we have contended that junctional coupling must be crucial for normal cochlear function [26,33]; however, only recently has this been shown. The occurrence of nonsyndromic deafness associated with connexin gene mutations, such as in Cx26, indicates the physiological requirement for these channels [7,8,16,39]. Yet, while we have argued for a role of coupling in K sinking and metabolic cooperation within the organ [27,29,31,41], there is no reason to believe that sensorineural deafness results solely from the disruption of these homeostatic mechanisms. For example, it is possible that the stria vascularis may be an important target [37]. These answers will only be obtained through further in vivo analysis of junctional coupling within the cochlea [30].

Acknowledgements

We thank the following people whose work has contributed to this review: H.-B. Zhao, Y. Sato, S. Kakehata, M. Wu, S. Takahashi, and M. Mazzucco. This work was supported by NIH-NIDCD grant DC00273.

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