Reduced Electromotility of Outer Hair Cells Associated with Connexin-Related Forms of Deafness: An In silico Study of a Cochlear Network Mechanism

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ABSTRACT

Mutations in the GJB2 gene encoding for the connexin 26 (Cx26) protein are the most common source of non-syndromic forms of deafness. Cx26 is a building block of gap junctions (GJs) which establish electrical connectivity in distinct cochlear compartments by allowing intercellular ionic (and metabolic) exchange. Animal models of the Cx26 deficiency in the organ of Corti seem to suggest that the hearing loss and the degeneration of outer hair cells (OHCs) and inner hair cells is due to failed K⁺ and metabolite homeostasis. However, OHCs can develop normally in some mutants, suggesting that the hair cells death is not the universal mechanism. In search for alternatives, we have developed an in silico large scale three-dimensional model of electrical current flow in the cochlea in the small signal, linearised, regime. The effect of mutations was analysed by varying the magnitude of resistive components representing the GJ network in the organ of Corti. The simulations indeed show that reduced GJ conductivity increases the attenuation of the OHC transmembrane potential at frequencies above 5 kHz from 6.1 dB/decade in the wild-type to 14.2 dB/decade. As a consequence of increased GJ electrical filtering, the OHC transmembrane potential is reduced by up to 35 dB at frequencies >10 kHz. OHC electromotility, driven by this potential, is crucial for sound amplification, cochlear sensitivity and frequency selectivity. Therefore, we conclude that

reduced OHC electromotility may represent an additional mechanism underlying deafness in the presence of Cx26 mutations and may explain lowered OHC functionality in particular reported Cx26 mutants.

Keywords: cochlea, gap junction, outer hair cell, modelling, cochlear mechanics

INTRODUCTION

Deafness exists in a variety of inherited nonsyndromic forms, with more than 70 loci identified so far (Petit 2006). Mutations in a single gene, the gap junction beta 2 (GJB2) gene, account for nearly 50% of cases. The GJB2 gene encodes for the protein connexin 26 (Cx26), which together with other connexins such as Cx30, forms the gap junctions between non-sensory cells in the cochlea (for review, see Nickel and Forge 2008; Rabionet et al. 2000). The most common GJB2 mutations are single nucleotide deletions and frame shifts (such as 35delG, 167delT, and 235delC). These are well documented as leading to deafness in human populations. Missense mutations such as M34T or R75W have a dominant negative effect on hearing (Rabionet et al. 2000) and functional analysis of such mutations have identified reduced ionic conductivity of the gap junction (GJ) as the associated defect (Bicego et al. 2006; Bruzzone et al. 2003). It has also been proposed that mutations such as V84L, with reduced permeability to metabolites such as IP₃ (Beltramello et al. 2005) may exert their effect by interfering with the development and maintenance of the organ of Corti.

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FIG. 1. The three-dimensional model of current flow in the cochlea. **A** Schematic representation of *radial* current flow between different cochlear compartments in a single cross-section with stria vascularis (StV), inner/outer hair cells (IHC/OHC), Deiters' (DC), Hensen (HC), Claudius (CC) cells and Reissner's membrane (RM). **B** An equivalent electrical circuit of a cochlear cross-section, Z_{IHC} and Z_{OHC} , the effective IHC and OHC impedances, respectively. The circuit implements the detailed current flow around the OHC seen in **(C)**. R_{DC} and C_{DC} , effective resistor and capacitor of Deiters' cells membrane; R_{HC} and C_{CC} for Claudius cells; V_{SV} , R_{SV} and C_{SV} , the effective battery, resistance and capacitance of stria vascularis; R_{SL} and R_{LS} , resistors of spiral limbus; R_{GJ} , *radial* GJ connection between Deiters' and Claudius cells. Nodes 8, 9 and 10 are coupled between adjacent cross-sections and represent *longitudinal* intercellular GJ connections in StV, organ of Corti and spiral limbus. Nodes 11 in adjacent sections are connected by the resistor R_{SN} representing the space of Nuel (extracellular space surrounding OHCs). **C** Electrical coupling between an outer hair cell and a supporting cell (the OHC impedance in detail). R_{AO} , C_{AO} resistance and capacitance of the OHC apical membrane (R_{AO} is modulated by sound input); R_{BO} , C_{BO} resistance and capacitance of OHC basolateral membrane; V_{OHC} the OHC electrochemical battery, EP endocochlear potential. The IHC impedance is treated similarly, but R_{BI} and C_{BI} , the resistor and capacitor representing IHC basolateral membrane, are in parallel. Nodes 3 and 5 of IHC correspond to 2 and 4 of OHC (Mistik et al. 2009). See Table 1 for values. **D** View of the circuit along the tonotopic axis at the level of OHCs and showing *longitudinal* current flow extracellularly through the space of Nuel (node 11 and R_{SN}) and intercellularly through organ of Corti (node 9, R_{GJ}).

Mutations in GJs can lead to the apoptosis of hair cells. This has been observed in an animal model with a cochlea-specific GJB2 ablation (Cohen-Salmon et al. 2002). The observation is compatible with GJs serving to buffer extracellular K^+ by supporting cells, as occurs in astrocytes in the central nervous system (Kikuchi et al. 2000; Santos-Sacchi 1985). Nevertheless, cell degeneration is not inevitable, for it has been shown in an animal model expressing the human dominant-negative Cx26 mutation R75W that there may be significant anatomical deformations of the organ of Corti (Kudo et al. 2003), but that the outer hair cells (OHCs) develop normally (Minekawa et al. 2009).

Audiological examination of patients carrying GJB2 mutations exhibit reduced or missing distortion product oto-acoustic emissions (DPOAEs; Engel-Yeger et al. 2002; Engel-Yeger et al. 2003; Medica et al. 2005; Morell et al. 1998) although not in every case (Cheng et al. 2005; Santarelli et al. 2008). As reduction of the DPOAE is a

sign of non-functional OHCs, such finding suggests that the underlying pathology in this case might be reduced OHC electromotility, essential for the active amplification of sound as shown by animal experiments where the 'motor' protein has been deleted (Liberman et al. 2002) or reduced (Cheatham et al. 2009).

Gap junctions constitute two major systems of intercellular communication in the cochlea: the *epithelial cell gap junction system* coupling supporting cells in the organ of Corti and the *connective tissue gap junction system* connecting the type 2 fibrocytes in the spiral ligament with basal and intermediate cells in the stria vascularis (Nickel and Forge 2008; Zhao et al. 2006). It is widely held that the passage of ions, mainly K^+ from scala media, generates electrical currents in the cochlear partition upon the activation by sound of the mechanotransducer (MET) channels in hair cells (Figure 1A). This ion flow constitutes an electrical current pathway in the cochlea.

We have recently developed a large-scale *in silico* model to describe current flow in the cochlea (Mistrik et al. 2009). Such large-scale 3D models have been developed previously to study cochlear potentials (Strelhoff 1973) and *in vivo* cochlear stimulation (Suesserman and Spelman 1993). The model used here differs in several important aspects: (1) it is finer grained (the cochlea is divided into 300 cross-sections instead of 90 or 51 sections as previously used); (2) it uses a more accurate algorithm for the calculation of basilar membrane (BM) displacements (Mammano and Nobili 1993); and (3) the organisation of the equivalent electrical circuit reflects the advances in characterization of the molecular components of the cochlea and their electrical properties.

Such a model represents a prototype of an *in silico* diagnostic tool to assess the effect on system cochlear potentials of constituent molecular mutations. Previously, we used the model to analyse the role of lateral current spread through GJs in controlling extracellular field potentials and electrical filtering in the cochlear network (Mistrik and Ashmore 2009, Mistrik et al. 2009). The essential demonstration in such a model is that the extracellular fields around the OHCs can drive the cell's electromotility largely independently of the cell's time constant, and that these fields are modulated by current spreading through *the gap junction systems* in the cochlea. Such simulations show that the GJ systems are critical for keeping the anticipated frequency-dependent roll-off of the OHC transmembrane potential relatively small (6 dB/decade). For comparison, the potential of an isolated cell, outside a coupled network, would be reduced by 21 dB/decade or 6 dB/octave once above its corner frequency.

In this paper, we describe the method of implementing in the model the effects of different types of Cx mutations and the consequences for cochlear tuning and amplification. The simulations suggest that Cx26 mutations could increase the attenuation of the OHC transmembrane potential from 6 dB/decade to 14.2 dB/decade and reduce the electromotile forces by up to 35 dB at frequencies greater than 10 kHz. Such a decrease could nearly completely eliminate the 40 dB gain due to cochlear amplification associated with normal hearing. Such a mechanism might explain the impaired DPOAEs in spite of normal OHC non-linear capacitance in the R75W mutant mouse (Minekawa et al. 2009) and the absence of DPOAEs in subjects carrying the 35delG (Engel-Yeger et al. 2002) or 167delT (Morell et al. 1998) mutations.

METHODS

An equivalent electrical circuit of the cochlea

The elementary circuit describing the *radial* current flow in a single cross-section is shown in Figure 1B. It is derived from a two-dimensional equivalent electrical model of the organ

of Corti (Dallos 1984). Here, effective IHCs and OHCs are treated separately and assigned impedances Z_{IHC} and Z_{OHC} , respectively (Jagger and Forge 2006). The three rows of OHCs (and Deiters' cells) were simulated as one row and the membrane parameters adjusted accordingly. The OHC is connected to a Deiters' cell through a pathway expected to pass K^+ ions during sound stimulation (Boettger et al. 2002; Johnstone et al. 1989; Santos-Sacchi 1985). The circuitry between an OHC and a supporting cell is shown in detail in Figure 1C. In contrast to previous models (Dallos 1984; Mountain and Cody 1999; O'Beirne and Patuzzi 2007; Ospeck et al. 2003), the resistors and capacitor describing the OHC baso-lateral membrane are distributed. This is to accommodate the observed spatial separation of K^+ conductances (R_{BO}) in the basal pole buried in the interface with a Deiters' cell (node 7) and the lateral membrane with prestin (C_{BO}) exposed to perilymph (node 11) of the space of Nuel (Boettger et al. 2002; Winter et al. 2007; Yu et al. 2006). A resistive pathway between the space of Nuel (node 11) and the perilymph (node 0) suggested by *in vivo* measurements of cochlear receptor potentials (Dallos et al. 1982) is facilitated in the model by current flowing through the capacitance divider C_{BO} - C_{DC} at the acoustic frequencies explored here. Simulations with an additional resistive element directly connecting these two nodes did not affect the gain functions calculated below and were not considered further. The additive effect of the OHC nonlinear capacitance (Spector et al. 2003) was included in the C_{BO} value and its voltage dependence was not considered in the model as only small signals are being considered. To keep within reasonable computational times without resorting to high performance computing clusters, the complex cellular structure of stria vascularis, important for the generation of the endocochlear potential (Nin et al. 2008; Quraishi and Raphael 2008), was represented only as a single compartment and its electrical properties characterised by an effective resistor and capacitor (R_{SV} and C_{SV} , respectively). A battery, V_{SV} , represents the mechanism for generating endocochlear potential, essentially at a constant value in this simulation.

The *radial* GJ conductance between supporting cells in the organ of Corti was implemented explicitly by the resistor R_{GJ} connecting "uncoupled" Deiters' (R_{DC} and C_{DC}) and cells lining the basilar membrane. These cells consist of Hensen's cells, Claudius cells and Boettchers' cells (at cochlear basal turns). The circuit connections were simplified to a single-lumped element consisting of a parallel R_{CC} and C_{CC} as done previously (Lagostena et al. 2001; Santos-Sacchi 1991; Santos-Sacchi and Dallos 1983). The main effect of this pathway is to allow a pathway to perilymph at high frequencies. The use of a single resistance/capacitor for these sets of cells in each of 300 cross-sections ensured that the computational load was kept at reasonable levels although the intracellular potential of supporting cells was not defined in the circuit.

The *longitudinal* GJ conductance between adjacent cross-sections was implemented by three resistive connections by

R_{GJL} (see Figure 1D). The connection through the node 9 represents intercellular coupling in the organ of Corti; through the node 8, the coupling in stria vascularis and through the node 10 in the spiral limbus. An additional *longitudinal* coupling by the resistance R_{SN} through the node 11 represents a low-impedance pathway at all frequencies through the extracellular space surrounding OHCs in the organ of Corti (Mountain and Cody 1999).

The acoustic stimulus was implemented by modulation of resistances R_{AO} and R_{AI} , which represent the MET channels in apical stereocilial membrane of the OHC and IHC respectively (Figure 1C shows the case of R_{AO}). The MET conductances were modulated around their steady state values in proportion to the basilar membrane displacement computed by a model of BM mechanics (Mammano and Nobili 1993). This model gives realistic BM tuning, with the result that R_{AI} and R_{AO} were effectively modulated in 10-20% of the 300 cross-sections (see below). The critical OHC receptor potential, generated by such sound stimuli, is the voltage difference between nodes 3 and 11 (Figure 1C). This is the OHC lateral transmembrane potential which drives conformational changes in the prestin molecule.

The values of circuit parameters are summarised in Table 1. For both hair cells and supporting cells, these represent effective values at each of 300 cross-sections derived from single cells characteristics (multiple data sources) by appropriate scaling: taking the length of the guinea pig cochlea as 18 mm and the distance between the hair cells rows as 10 μ m, each cross-section contains six IHCs, 18 OHCs and 18 Deiters', Hensen, Claudius and Boettcher's cells (each of them in parallel). Batteries V_{IHC} and V_{OHC} represent K^+ electrochemical gradient for IHC and OHC, respectively (Ospeck et al. 2003). The effective resistance values for spiral limbus and stria vascularis were chosen to provide the known intracellular IHC and OHC steady-state potentials in the absence of sound stimulus of -45 and -70 mV, respectively (Cody and Russell 1987; Russell and Sellick 1978). Similarly, the effective cross-section capacitance of stria vascularis was used to obtain known values of cochlear microphonics at node 1 (Misrahy et al. 1958; Tasaki 1957) as analysed previously (Mistrik et al. 2009). Finally, the longitudinal GJ coupling between cross-sections in this coarse-grained model (300 cross-sections in the model represent 3,000 rows of hair cells in the cochlea) was chosen to obtain the observed frequency tuning of the extracellular receptor potential in the organ of Corti (Fridberger et al. 2004). In this configuration, the effective values of GJ resistors in the organ of Corti (radial and longitudinal) are 10 $\times$ smaller than those of supporting cells, in a reasonable agreement with experimental observations (Lagostena et al. 2001; Santos-Sacchi 1991). There are relatively few free parameters in this model: the major uncertainties which are not constrained by experimental data are the values of the GJ resistances between cochlear compartments.

Computational model of current flow in the cochlea

The described circuit with 12 nodes in each of 300 cross-sections is very complex. This makes the determination of the 3,600 nodal voltages computationally very demanding. Their time dependence is given by the matrix equation

$$CdV/dt = -GV + I_{ind} \quad (1)$$

where V is the vector of nodal voltages and C and G are the matrices of known capacitances and conductances (including the contributions from the MET channels), respectively. I_{ind} is the vector of known independent current and voltage sources used for self-consistent construction of equations (see Mistrik et al. 2009) and including the endocochlear potential (V_{SV}) and inner and outer hair cell electrochemical potassium potentials (V_{IHC} and V_{OHC} , respectively) at each cross-section.

The analysis of the effect of reduced GJ conductivity on the OHC transmembrane potential required to calculate the voltages at nodes 3 and 11 (Figure 1C) at all 300 cross-sections. To reduce the computational load associated with a large size of the C , G matrices ($3,600 \times 3,600$), Eq. 1 was solved in the frequency domain rather than in the time domain. The maximal amplitude and phase, instead of the full time course of nodal voltages were calculate using two simplifying assumptions: (1) that the sound stimulus was sinusoidal (single-tone with frequency $f = \omega/2\pi$); and (2) that the evoked potential $\Delta V (= V - V_0)$ results from a small perturbation of the steady-state potential V_0 (in the absence of a sound stimulus). As a result, the equations are tractable, linearised equations. The sound-evoked nodal potentials $\Delta V(x, \omega)$ at every frequency and distance x from the stapes is then given by the matrix equation

$$\Delta V(x, \omega) = (-j\omega C + G_o)^{-1} \gamma e^{-j\theta} G_0^{-1} I \quad (2)$$

where G_o is the matrix of steady-state conductances, G_o^{-1} signifies matrix inversion, $\gamma = \gamma(x, \omega)$ and $\theta = \theta(x, \omega)$ represent the amplitude and phase perturbation of the steady state apical transducer conductance produced by the BM displacement at a distance x from the stapes. In this small signal approximation, γ at the best position was taken to be 10% of the resting transducer conductance. The excursion of potential across the OHC membrane, the perturbed electric field ΔE_{OHC} , which prestin in the membrane experiences, was then determined as the difference between the ΔV at nodes 3 and 11

$$\Delta E_{OHC}(x, \omega) = \Delta V_{node3}(x, \omega) - \Delta V_{node11}(x, \omega) \quad (3)$$

for every section at distance x from the stapes. The large matrices were generated from a connectivity Table 1 automatically using the modified nodal analysis approach.

TABLE 1

Parameters used in simulations						
Circuit element	Description	Node 1	Node 2	Value	Connectivity	Data source
R_{AI}	IHC apical resistance	1	2	1.2 M Ω	-	Calculated from R_{BI} (Lopez-Poveda and Eustaquio-Martin 2006)
C_{AI}	IHC apical capacitance	1	2	6 pF	-	
R_{BI}	IHC basal resistance	2	4	5.5 M Ω	-	
C_{BI}	IHC basal capacitance	2	6	60 pF	-	(Lopez-Poveda and Eustaquio-Martin 2006) (Raybould et al. 2001)
V_{IHC}	IHC ionic potential	4	6	-100 mV	-	
R_{SL}	Resistance of spiral lamina	6	10	25 M Ω	-	
R_{LS}	Resistance of scala tympani	10	0	25 M Ω	25 M Ω	
R_{AO}	OHC apical resistance	1	3	1.4-70 M Ω		Calculated from R_{BO} Calculated from C_{BO} (Housley and Ashmore 1992; Mammano and Ashmore 1996; Preyer et al. 1996)
C_{AO}	OHC apical capacitance	1	3	10.5 pF	-	
R_{BO}	OHC basal resistance	3	5	0.6-30 M Ω	-	
R_{CD}	OHC lateral resistance	3	11	100 M Ω		Lower bound from leak resistance of OHCs (Housley and Ashmore 1992)
C_{BO}	OHC lateral capacitance	3	11	176 pF	-	
V_{OHC}	OHC ionic potential	5	7	-100 mV	-	Estimate from geometry
R_{SN}	Longitudinal resistance in space of Nuel	11	11		0.05 M Ω	
C_{DC}	Capacitance of Deiter's cell	9	11	180 pF		(Lagostena et al. 2001; Santos-Sacchi 1991)
R_{DC}	Resistance of Deiters' cell	7	9	0.1 M Ω		
R_{GJ}, R_{GJL}	Resistance of gap junction	9	12	0.01 M Ω (for R_{GJ})	0.01 M Ω (for R_{GJL})	"
R_{CC}	Lumped resistance of Claudius cells	12	0	0.1 M Ω		
C_{CC}	Lumped capacitance of Claudius cells	12	0	150 pF		Estimated from Hensen cell values (Lagostena et al. 2001; Santos-Sacchi 1991)
V_{SV}	Endocochlear potential in stria vascularis	1	8	80 mV	-	
C_{SV}	Stria vascularis capacitance	1	0	5,000 pF	-	(Mistrik et al. 2009)
R_{SV}	Stria vascularis resistance	8	0	0.1 M Ω	0.1 M Ω	

"Node 1" and "Node 2" indicate the location of element in the circuits in figure 1 and "Value" its electrical magnitude; "Connectivity" indicates whether the node 1 is coupled longitudinally in the adjacent cross-sections, and if so, a value of the element if appropriate is given. The data sources are given in the last column

The full derivation has been described previously (Mistik et al. 2009). The computations were carried out in Matlab (ver. 7.04, The Mathworks, Natick, MA, USA) using conventional matrix methods and provided stable and unique solutions.

RESULTS

Generation of OHC gain functions

Figure 2 shows the generation of the input-output functions in the model. First, the network response to a

1 kHz tone was considered and the amplitude and phase, calculated according to Eqs. 2 and 3, are shown in Figure 2A, B. The dotted line represents the input BM envelope used to control the hair cell transducer conductances and the continuous line shows the OHC transmembrane potential, ΔE_{OHC} , at all positions along the cochlear partition. In Figure 2A, ΔE_{OHC} is plotted normalised to unity for comparison with the BM amplitude.

The OHC transmembrane potential, ΔE_{OHC} , indicates broader electrical tuning than its mechanical input. The broadening can be traced to longitudinal coupling between

FIG. 2. The generation of cochlear gain functions for the OHC transmembrane potential, $\Delta E_{\text{OHC,max}}$. **A** and **B** An overlay of normalised BM travelling wave (broken line) and ΔE_{OHC} tonotopic pattern (solid line) produced by a 1 kHz tone (**A** amplitude, **B** phase). $\Delta E_{\text{OHC}}(x)$ is the OHC receptor potential between nodes 3 and 11 at the BM position x ($0 < x < 1$), relative to the stapes. **C** and **D** An overlay of eight BM travelling waves corresponding to frequencies of 0.25, 0.5, 1, 2, 4, 8, 16 and 32 kHz (**C** amplitude, **D** phase). For comparison, the travelling waves evoked in the tuned (solid line) and detuned (broken line) cochlea are both plotted. The amplitude of the detuned BM travelling wave is 20 dB smaller than in the tuned case. **E** and **F** An overlay of eight tonotopic patterns of ΔE_{OHC} generated using the tuned BM travelling waves from panels **C** and **D**. For each tone, the maximal amplitude (**E**) as well as the phase accumulated in the corresponding best position (**F**) are both indicated (asterisk). **G** The frequency dependence of the maximum OHC transmembrane potential, $\Delta E_{\text{OHC,max}}$, for each tone (asterisk) derived from **E**. The case of an isolated OHC is also shown (broken line), where the external potential would be 0 mV. **H** The frequency dependence of the phase accumulated at the best position. The responses in this and subsequent figures correspond to low level acoustic stimuli (20–40 dB SPL), permitting computations to be made from a linearised model (Eq. 2).

the sections. In addition, the phase of the electrical response is delayed by approximately 90° relative to the mechanical input as would be expected for a voltage generated across the capacitance of the OHC membrane (Figure 1B).

The amplitude and phase of the BM travelling waves, generated by single tones with a variety of logarithmically spaced best frequencies between 0.25 and 30 kHz, are shown in Figure 2C, D. The corresponding OHC transmembrane potentials, $\Delta E_{\text{OHC}}(x, \omega)$, are shown in Figure 2E, F. The maximal values as well as the accumulated phases at corresponding best position for each tone are indicated by an asterisk (*). Finally, $\Delta E_{\text{OHC,max}}(\omega)$, the *maximum* value of $\Delta E_{\text{OHC}}(x, \omega)$ at each stimulus frequency ω is shown in Figure 2G and the corresponding accumulated phase in Figure 2H. Such

plots, connecting the mechanical input and electrical output, represent the gain functions (amplitude and accumulated phase) for the cochlea as a whole.

An examination of the frequency dependence of the OHC amplitude in Figure 2G reveals an attenuation of the response by 6 dB/decade with increasing frequency. For comparison, the frequency dependence of an isolated OHC is shown on the same plot, where the DC asymptote has been scaled to the same point. In this case, the external potential is 0 mV, whereas for cell coupled into a network, the external potential will depend on all the other elements in the circuit, and $\Delta E_{\text{OHC,max}}$ will be modified accordingly. The isolated cell exhibits a roll off as $1/f$, characteristic of a cell with a single RC membrane time constant with an attenuation of 21 dB/decade (or equivalently 6 dB/octave). As

FIG. 3. OHC gain function and the effect of the apex base gradient in the OHC membrane conductance. **A** The frequency dependence of $\Delta E_{\text{OHC, max}}$ for different apex-base gradients (—, fivefold increase between base and apex conductance; ---, 20-fold; —·—, 50-fold; ···, 100-fold). **B** The dependence of the $\Delta E_{\text{OHC, max}}$ attenuation in the 0.25–30 kHz range on the tonotopic conductance gradient. The slope is computed from the results in **A**. **C** The frequency dependence of the phase accumulated at the best BM position for different apex base gradients.

argued previously (Mistrik et al., 2009) the reduced attenuation of $\Delta E_{\text{OHC, max}}$ in a cochlear network arises from the gradient in OHC conductance which increases from cochlear apex to cochlear base and the GJ coupling which allows the extracellular currents to build up non-zero extracellular potentials around the OHCs.

The effect of the OHC conductance gradient on the attenuation of the OHC transmembrane potential is shown in Figure 3A. It demonstrates that a gradient exponentially increasing the conductance by fivefold from apex to base would reduce the OHC attenuation from 21 dB/decade (the isolated cell case) to 11.6 dB/decade. A 100-fold apex-base conductance increase would reduce the attenuation to only 3 dB/decade (Figure 3B), even though the phase accumulated at the best frequency is affected only to small extent by such a gradient (Figure 3C). In subsequent calculations, a 50-fold apex-base conductance increase was used as it corresponds to the experimentally observed tonotopic variation in OHC conductances (Housley and Ashmore 1992; Mammano and Ashmore 1996; Preyer et al. 1996). The OHC membrane capacitances were kept constant along the cochlea because, experimentally, OHC capacitances vary only threefold along the longitudinal tonotopic axis, and therefore by a relatively minor factor compared to the conductance changes.

Cx26 mutations reducing intercellular connectivity (35delG)

The OHC gain functions from Figure 2 (the frequency dependence of the OHC transmembrane potential $\Delta E_{\text{OHC, max}}$) were used to assess the effect of Cx26 mutations. The changes in specific conductance of GJ were taken from the literature. For example, the residual permeability of the Cx26 mutant F83L for monovalent ions such as K^+ is reported to be 72% of the wild-type (Bruzzone et al. 2003) and that of M34T 11% of the wild-type (Bicego et al. 2006). Furthermore, mutations such as 35delG, V37I, W77R, L90P, S113R, M163V, 167delT, and R184P result in a complete loss of function with residual conductivity $\leq 1\%$ of the wild-type values (Bicego et al. 2006; Bruzzone et al. 2003). Other mutations, such as V84L, although reducing the conductivity for metabolites (Beltramello et al. 2005), were not considered here.

Reduced GJ conductivity was simulated in the model by increasing the value of corresponding resistors R_{GJ} and R_{GJL} representing, respectively, *radial* and *longitudinal* intercellular conductance between supporting cells in the organ of Corti. A 10% increase in either or both resistance values (to $R_{\text{GJ}}, R_{\text{GJL}} = 0.1 \text{ M}\Omega$) had little effect on the frequency dependence of $\Delta E_{\text{OHC, max}}$ amplitude and accumulated phase (Figure 4A and B, respectively). Therefore, we conclude that mutations such as M34T (with 11% residual conductance) or F83L (with 72% residual conductance) are unlikely to reduce $\Delta E_{\text{OHC, max}}$ and, by implication, would not compromise OHC electromotility.

On the other hand, mutations such as 35delG, V37I, W77R, L90P, S113R, delE120, M163V, R184P, and 235delC with a complete loss of permeability (conductance decreasing to 0.1% of wild-type value or, equivalently, setting $R_{\text{GJ}}, R_{\text{GJL}} = 10 \text{ M}\Omega$) significantly reduced the

FIG. 4. The effect of 30delG-like mutations on the OHC gain functions. **A** and **B** The frequency dependence of the amplitude and accumulated phase of $\Delta E_{\text{OHC}, \text{max}}$ for different residual GJ conductance (100%, —; 10%, -.-; 1%, - · -; 0.1% ...). The GJ conductance was decreased by increasing the value of R_{GJ} and R_{GJL} . This represents the situation of 30delG-like mutations reducing intercellular conductivity between supporting cells in the organ of Corti. Tuned BM travelling waves were used as the input for the calculation of ΔE_{OHC} . **C** The dependence of the attenuation of amplitude in the 0.25–30 kHz frequency range on the GJ conductivity. **D** and **E** Like **A** and **B** but with detuned BM travelling wave as input (and as a result the amplitude is 20 dB smaller than in Figure 2C). **F** The dependence of the attenuation on the GJ conductivity (a detuned BM was used as input).

$\Delta E_{\text{OHC}, \text{max}}$ amplitude at frequencies above 1 kHz (Figure 4A). The corresponding increase in the attenuation from 6.1 to 10.7 dB/decade over the 0.25–30 kHz range (Figure 4C) can be explained by high frequency currents shunting to the ground (the perilymph) through the membrane capacitance of the supporting cells. It should be pointed out that this calculation is semiquantitative only. A larger value of R_{GJ} is required to compensate for the supporting cell values chosen here: the precise network values are unknown experimentally. Qualitatively, at frequencies above those corresponding to the characteristic membrane time constant of the supporting cell network (here formed by R_{CC} and C_{CC}) most of the current gated by the transducer will pass to ground through the membrane capacitances.

A reduced $\Delta E_{\text{OHC}, \text{max}}$ amplitude implies that the electromotile forces amplifying initial mechanical BM displacements are therefore likely to be much smaller. The simplest

assumption is that the feedback forces are reduced in proportion. Nevertheless, implementing a stable feedback to the cochlear mechanics means significantly increasing the complexity of a cochlear mechanical model. For this reason, the implied reduced cochlear amplification was modelled by using a detuned rather than a sharply tuned BM displacement as the input for electrical calculations (Figure 2C, dashed lines). The BM input in this case corresponded to a quasi-passive cochlea model where the OHC feedback parameter, λ , used in Mammano-Nobili model is reduced from 1.2 to 0.05 (Mammano and Nobili 1993). The BM displacement with $\lambda = 0.05$ will be referred to below as a ‘detuned’ input. The BM envelope still exhibited phase accumulation and frequency dependence, but the feedback was chosen to reduce the amplitude by 20 dB.

The gain functions (amplitude and accumulated phase) obtained for reduced conductances with such

detuned BM input are shown in Figure 4D, E. The derived dependence of the amplitude attenuation on the GJ conductance (Figure 4F) indicated a maximal value of 12.3 dB/decade over the 0.25–30 kHz range for the mutations with 0.1% conductivity of wild-type. It should be noted that the phase accumulated at the best frequencies >300 Hz was increased (Figure 4E). Such an increase in phase accumulation arises from a parallel current injection into larger number of cross-sections as a result of a detuned mechanical input, and hence an increased filtering due the capacitance currents delaying responses in the circuit. It might be anticipated that such OHC receptor potentials would be further attenuated due to the cancelling effect of neighbouring OHC

rows (Dallos and Cheatham 1976), but this would have required a finer grain model to investigate the point.

Cx26 mutations which reduce intercellular and extracellular conductivity (R75W)

It is well known that a functional *epithelial cell gap junction system* is essential for normal development of the inner ear as it facilitates necessary intercellular signalling (Nickel and Forge 2008). It is therefore not surprising that some GJ mutations lead to developmental malformations of the organ of Corti. For example, R75W significantly reduces the space of Nuel, the extracellular fluid-filled compartment surrounding OHCs (Kudo et al. 2003). Such defect could easily affect both the

FIG. 5. The effect of R75W-like mutations on the OHC gain functions. The same as in Figure 4 but in addition to R_{GJ} and R_{GIL} , R_{SN} was also increased in these simulations to mimic reduced extracellular conductivity through the space of Nuel (0.1%).

micromechanics of the cochlear partition and extracellular conductivity in the organ of Corti. In what follows, we shall explore the electrical consequences of the mutation.

Such additional (indirect) effect of changed extracellular conductivity was investigated by increasing the value of the R_{SN} resistor connecting the node 11 between adjacent cross-sections (Figure 1D) and representing *longitudinal* extracellular conductivity through the space of Nuel. The gain functions (amplitude and accumulated phase) obtained with conductances reduced to 0.1% of wild-type values (R_{GJ} , R_{GJL} =10 M Ω , R_{SN} =50 M Ω) and a sharply tuned mechanical input are shown in Figure 5A, B, respectively. Figure 5C shows that the attenuation at frequencies above 5 kHz is increased relative to the wild-type (9.6 dB/decade for the mutant against 6.1 dB/decade for the wild-type over a 0.25–30 kHz range). Such attenuation is less than that obtained for GJ mutations producing only reduced intercellular conductivity (e.g. Figure 4C, showing an attenuation of 10.7 dB/decade). The explanation is that more current will always flow through the extracellular path through the space of Nuel at high frequencies since R_{SN} is small. However, if R_{SN} is increased as the space fails to open, longitudinal current is shunted to earth across RDC and the attenuation of the potential across the OHC membrane is decreased.

When reduced electro-mechanical feedback was implemented using a detuned BM input (the case of R75W), the OHC attenuation was increased to 14.2 dB/decade over the 0.25–30 kHz range (Figure 5F). This value is larger than that obtained when only intercellular conductivity was reduced (Figure 4F, 12.3 dB/decade). Such *in silico* findings emphasises the importance of the low-resistance pathway through the space of Nuel in the maintenance of electromotile forces.

Cx26 and Cx30 mutations and endocochlear potential

In addition to a reduction of the gap junction conductivity in the organ of Corti, it is known that the targeted deletion of Cx26 can lead to a reduction of endocochlear potential (EP) from +80 to +40 mV, and to a reduction in endolymph potassium concentrations (Cohen-Salmon et al. 2002). It is also known that Cx30 deletions in stria vascularis can reduce EP to 0 mV (Teubner et al. 2003).

The effect of reducing EP on $\Delta E_{OHC, max}$ was simulated most simply by reducing the value of the battery V_{SV} and changing V_{OHC} , V_{IHC} in order to maintain intracellular resting potentials in the hair cells. The results for both Cx26 and Cx30-type deletions with tuned and detuned mechanical input are shown in Figure 6A and B, respectively. The amplitude reduction was uniform across the whole frequency range and no increase in the attenuation was observed even in the case of a tuned mechanical input. The decrease was relatively

small for the Cx26 simulated deletion (4.3 dB) but reached 10.2 dB for the simulated Cx30 deletion (Figure 6C). There was no effect on the accumulated phase (data not shown).

FIG. 6. The effect of reducing endocochlear potential. Non-functional connexins are associated with a decrease of the endocochlear potential battery (V_{SV}) and of K^+ electrochemical potentials (V_{OHC} and V_{IHC} for OHC and IHC, respectively), see Table 1. In the case of Cx26^{-/-}, V_{SV} was reduced from 80 to 40 mV and both V_{OHC} and V_{IHC} from -100 to -70 mV to maintain stable intracellular resting potentials. Similarly, in the case of Cx30^{-/-}, V_{SV} was reduced to 0 mV and V_{OHC} and V_{IHC} to -55 mV. The frequency dependence of the OHC receptor potential with **A** tuned and **B** detuned mechanical input in the presence (wt) or absence of any of the two connexins. **C** The amplitude reduction due to a deletion of Cx26 or Cx30. Whether the BM was sharply tuned or detuned had no effect.

FIG. 7. Connexin mutations can reduce the OHC receptor potential. **A** and **B** The frequency dependence of the amplitude (**A**) and accumulated phase (**B**) for different Cx26 mutations. The curve for M43T was obtained with a tuned BM input and 10% residual GJ conductivity ($R_{GJ}=R_{GJL}=1\text{ M}\Omega$); delG with a detuned BM input, 0.1% residual GJ conductivity ($R_{GJ}=R_{GJL}=100\text{ M}\Omega$) and reduced circuit batteries as in Figure 6; R75W with a detuned BM input, 0.1% conductance ($R_{GJ}=R_{GJL}=100\text{ M}\Omega$), reduced extracellular conductivity in space of Nuel ($R_{SN}=50\text{ M}\Omega$) and reduced circuit batteries as in Figure 6. **C** The amplitude attenuation for the OHC transmembrane receptor potential $\Delta E_{OHC, \max}$ at the 0.25–30 kHz range for different Cx26 mutations. The attenuation was calculated from the data in panel A. **D** The difference (shift) between the $\Delta E_{OHC, \max}$ amplitude generated by a 30 kHz tone and a wt reference (250 Hz) for different Cx26 mutants. *GJ* the contribution purely due to electrical filtering through the cochlear network; *BM+GJ* the case with detuned mechanical input.

Global effects of Cx26 mutations on the OHC receptor potential

The effect of different classes of Cx26 mutations is presented in Figure 7. Here, the curves for wild-type and M43T were obtained with tuned BM input since the analysis in Figure 4 indicated that 10% residual GJ conductance (M43T) does not interfere with the feedback mechanism. The curve for delG (35delG-like mutations) was obtained with a 0.1% residual GJ conductance, a detuned BM and reduced circuit batteries (Figures 4 and 6). The same settings were used also for R75W, but in addition with a residual 0.1% conductance through the space of Nuel, to mimic the absence of the extracellular current flow in the organ of Corti (Figures 5 and 6).

The examination of the frequency dependence of the amplitude and phase indicates a large variation in the effect of Cx26 mutations on the OHC receptor potential (Figure 7A, B). On the one hand, the responses for the M34T mutation is indistinguishable from the wild-type (the M34T result superimposes directly on top of the wild-type response). Such finding suggests that even a 11% residual GJ conductance in the *in silico* model of the organ of Corti is sufficient to maintain normal current flow and frequency dependence of the OHC receptor potential. Therefore, mutations such as M34T or F83L (72% residual GJ conductance) are unlikely to reduce the OHC electromotility.

On the other hand, Cx26 mutations resulting in a *complete* loss of function (35delG, V37I, W77R, L90P, S113R, M163V, 167delT, R184P) and residual conductivity of 0.1–1% lead to a strong increase in the attenuation at frequencies above 5 kHz. The largest effect is found for the R75W mutation where increased attenuation is apparent already for frequencies >1 kHz. This demonstrates the importance of the current flow through the space of Nuel, which is abolished by mutations resulting in a volume that does not open up during development.

The attenuation for different mutations is compared quantitatively in Figure 7C. It indicates that 35delG-like mutations double and R75W nearly triple the attenuation over the studied frequency range of 0.25–30 kHz. As the effect is largest at frequencies above 10 kHz, the effect of mutations at high frequencies is shown separately in Figure 7D. The figure compares the relative amplitude of the OHC receptor potential $\Delta E_{OHC, \max}$ at 30 kHz for wild-type and all mutants with respect to the wild-type amplitude at 250 Hz. In the case of the wild-type, the expected reduction would be 12.8 dB. On the other hand, the reduction of 26 and 35 dB was found for 35delG and R75W mutations, respectively, even before the effects of reduced electromotile feedback were included (and of 45 and 56 dB, respectively, when included). Such a reduction indicates that electrical filtering due to Cx26 mutations could, at least at frequencies ~ 30 kHz, nearly abolish the 40–60 dB gain provided by the OHC somatic amplifica-

tion under normal physiological conditions (Ruggero and Rich 1991).

DISCUSSION

A computational laboratory for studying connexin mutations

The large-scale computational model of electrical currents used here provides an insight into the effect of circuit perturbations due to connexin mutations through their effects on ionic conductance. It offers, in addition, an opportunity to see how cells perform when coupled into a network, based on the data from single or small cell populations.

In a cochlear network circuit without any perturbations, the OHC receptor potential would be expected to show an attenuation of 6 dB/decade over the 0.25–30 kHz range. Such a low value (in a comparison to 21 dB/decade for an isolated OHC), results from the network distribution of current flow. The attenuation of 6 dB/decade is a quite conservative estimate, because the BM wave envelopes used as the input for the calculation of the frequency dependence of the potentials were all normalised to the same peak amplitude. However, there may be significant boosting of the BM amplitudes towards the cochlear base: as much as a 30 dB increase is predicted for some cochlear models (Ramamoorthy et al. 2007). This would provide a further ‘flattening’ of the OHC gain function. Thus, this minimal attenuation of the OHC transmembrane potential predicted from such cochlear models suggests that the OHC somatic electromotility can operate effectively in the whole auditory frequency range and not necessarily just in the low frequency range as originally predicted from measurements on isolated outer hair cells (Mammano and Ashmore 1996).

Effect of Cx26 mutations

The computational analysis of GJ mutation phenotypes performed here has revealed several indicative features of Cx26 mutations on current flow: (1) only a complete loss of function (0.1–1% residual GJ conductance) significantly reduces the OHC receptor potential; (2) a reduction in the OHC receptor potential requires detuning of mechanical input, presumably due to weaker electro-mechanical feedback into the system; (3) Cx26 mutations can be simulated so that some affect intercellular while others affect also extracellular connectivity; and (4) the loss of Cx26 function (uniform reduction of GJ conductivity) associated with reduced endocochlear and electrochemical potentials can also be modelled.

The computations show how Cx26 mutations could have a significant effect on the potentials developed across the basolateral membrane of OHCs. The computed

reduction was largest for the Cx26 mutations such as R75W and 35delG where the potentials $\Delta E_{\text{OHC,max}}$ can be decreased by up to 35 dB at 30 kHz relative to wild-type. As such potentials are thought to be the driver of the OHC somatic electromotility, we suggest that these types of mutation could result in hearing loss due to reduced OHC electromotility in addition to any hair cell loss resulting from failed ionic homeostasis in the organ of Corti. Accordingly, in the R75W animal model of Cx26-related deafness, there is an absence of oto-acoustic emissions despite OHCs apparently being unaffected (Minekawa et al. 2009). A reduced input for OHC electromotility could also explain the deafness phenotype of 35delG or 167delT human carriers, who also lack DPOAEs in the absence of any other audiological defects (Engel-Yeger et al. 2002; Morell et al. 1998).

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