Chlorpromazine Alters Cochlear Mechanics and Amplification: In Vivo Evidence for a Role of Stiffness Modulation in the Organ of Corti

Jiefu Zheng,1 Niranjan Deo,2 Yuan Zou,1 Karl Grosh,2 and Alfred L. Nuttall1,3,4,5

1Oregon Hearing Research Center, Department of Otolaryngology/Head and Neck Surgery, Oregon Health and Science University, Portland, Oregon; 2Department of Mechanical Engineering and 3Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan; 4Department of Otolaryngology, Renji Hospital, Shanghai Jiao Tong University, Shanghai, China; and 5Department of Biomedical Engineering, Oregon Health & Science University, Beaverton, Oregon

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Zheng J, Deo N, Zou Y, Grosh K, Nuttall AL. Chlorpromazine alters cochlear mechanics and amplification: in vivo evidence for a role of stiffness modulation in the organ of Corti. J Neurophysiol 97: 994–1004, 2007. First published November 22, 2006; doi:10.1152/jn.00774.2006. Although prestin-mediated outer hair cell (OHC) electromotility provides mechanical force for sound amplification in the mammalian cochlea, proper OHC stiffness is required to maintain normal electromotility and to transmit mechanical force to the basilar membrane (BM). To investigate the in vivo role of OHC stiffness in cochlear amplification, chlorpromazine (CPZ), an antipsychotic drug that alters OHC lateral wall biophysics, was infused into the cochleae in living guinea pigs. The effects of CPZ on cochlear amplification and OHC electromotility were observed by measuring the acoustically and electrically evoked BM motions. CPZ significantly reduced cochlear amplification as measured by a decline of the acoustically evoked BM motion near the best frequency (BF) accompanied by a loss of nonlinearity and broadened tuning. It also substantially reduced electrically evoked BM vibration near the BF and at frequencies above BF (≥80 kHz). The high-frequency notch (near 50 kHz) in the electrically evoked BM response shifted toward higher frequency in a CPZ concentration-dependent manner with a corresponding phase change. In contrast, salicylate resulted in a shift in this notch toward lower frequency. These results indicate that CPZ reduces OHC-mediated cochlear amplification probably via its effects on the mechanics of the OHC plasma membrane rather than via a direct effect on the OHC motor, prestin. Through modeling, we propose that with a combined OHC somatic and hair bundle forcing, the upward-shift of the ~50-kHz notch in the electrically-evoked BM motion may indicate stiffness increase of the OHCs that is responsible for the reduced cochlear amplification.

Introduction

In the mammalian cochlea, outer hair cells (OHCs) possess a unique motor capability termed “electromotility” whereby they change the somatic length in a voltage-dependent manner (Brownell et al. 1985; Santos-Sacchi 1991). The electromotility is assumed to provide mechanical force to the basilar membrane (BM), therefore locally amplifying the sound-evoked traveling wave in the cochlea to ensure normal cochlear sensitivity (Dallos 1992, 1996). Although OHC electromotility depends on the motor protein prestin (Dallos and Fakler 2002; Liberman et al. 2002; Zheng et al. 2000), proper stiffness of OHCs is also essential to the electromotile capability (Kakehata and Santos-Sacchi 1995; Santos-Sacchi et al. 2001). OHC stiffness is largely dependent on the biophysical properties of the basolateral wall, which has a unique nanoscale organization of three layers: the plasma membrane, the cortical cytoskeleton, and the subsurface cisterna (Brownell et al. 2001; Dallos 1992). The static axial stiffness of isolated OHCs has been intensively investigated (e.g., Hallworth 1995; Holley and Ashmore 1988; Iwasa and Adachi 1997; Ulfendahl et al. 1998; Zenner et al. 1992), and in vitro data have associated OHC stiffness with OHC electromotility (e.g., Batta et al. 2003; Borko et al. 2005; Chan et al. 1998; Dallos et al. 1997; Hallworth 1997; He and Dallos 1999; He et al. 2003; Lue and Brownell 1999; Oghalai et al. 2000; Russell and Schauz 1995). However, the in vivo role of OHC stiffness in electromotility and cochlear amplification in the intact organ of Corti (OOC) has remained unclear.

Manipulation of OHC stiffness in the in vivo experiments is difficult, but data from in vitro work suggest that at least three lines of experiments could be considered for in vivo study: applying electrical stimulation to the cochlea to modulate OHC stiffness, manipulating OHC turgor by changing perilymph osmolality, and applying pharmacological agents into the cochlea that could alter OHC stiffness. Modulation of BM mechanical tuning and amplification by DC current has been observed (Parthasarathi et al. 2003). However, the putative stiffness changes were not verified. Manipulation of perilymph osmolality can modulate cochlear sensitivity (Oghalai et al. 2006), but this method is not specific to the target (OHCs), and many other variables (e.g., mass, viscosity, and damping) are affected, which complicates the analysis. The effects of several pharmacological agents (i.e., salicylate, diamide, ocdacic acid, and chlorpromazine) on OHC lateral wall mechanics have been reported (Adachi and Iwasa 1997; Borko et al. 2005; Dieler et al. 1991; Russell and Schauz 1995; Hallworth 1997; Lue and Brownell 1999; Lue et al. 2001; Oghalai et al. 2000). In particular, chlorpromazine (CPZ), an antipsychotic drug that affects plasma membrane biophysics, alters OHC lateral wall micromechanics and electromotility without a known direct action on prestin (Lue et al. 2001; Oghalai et al. 2000). CPZ preferentially intercalates into inner leaflet of the phospholipid bilayer (Lue et al. 2001; Sheetz and Singer 1974), causing an inward bend of the plasma membrane. This curvature alteration results in decrease of the fluidity of the plasma membrane, which is associated with the tension or stiffness of the OHC lateral wall (Oghalai et al. 2000). Unlike salicylate (SAL), which also causes a curvature change (but outward bend) of the
plasma membrane and reduces OHC electromotility by a direct inhibition effect on prestin (Kakehata and Santos-Sacchi 1996; McLaughlin 1973; Oliver et al. 2001) and has been suggested to reduce the stiffness of the cochlear partition (Murugasu and Russell, 1995), CPZ changes the OHC length-velocity relation without an effect on the absolute value of OHC length change, and this effect is likely solely through an effect on the OHC plasma membrane biophysics (Lue et al. 2001; Morimoto et al. 2002; Oliver et al., 2000, 2001). Therefore CPZ appears a relatively ideal chemical for investigation of the in vivo role of OHC stiffness modulation.

To test the hypothesis that in vivo OHC electromotility is related to OHC stiffness, we investigated the effects of CPZ on both acoustically and electrically evoked BM motions. Whereas the acoustically evoked BM motion provides a direct measure of the cochlear amplification (Robles and Ruggiero 2001), the electrically evoked BM motion represents the OHC-mediated electro-mechanical transduction process, thus providing a tool for investigation of the in vivo OHC electromotility (Grosh et al. 2004; Nuttall and Ren 1995). In addition, the high-frequency (i.e., ∼100 kHz) electromotile responses of BM evoked by bipolar current stimulation across the cochlear partition in the basal turn is likely an asymmetrical local resonance associated with OHCs (Scherer and Gummer 2004; Grosh et al. 2004) which could have a mode of piezoelectric resonance (Spector et al. 2003; Weitzel et al. 2003). Thus investigation of the electrically evoked high-frequency responses of BM may inform about the cochlear electromotile processes and mechanical properties that cannot be obtained with acoustic stimulation (Grosh et al. 2004). In this report, we present data from living guinea pigs showing CPZ-induced alterations of cochlear mechanics along with evidence that stiffness changes in the organ of Corti play a role in modulation of cochlear amplification.

METHODS

Animal preparation

Pigmented guinea pigs (strain 2NCR, obtained from Charles River Laboratory) weighing 250–350 g were used (n = 32). The animals were housed in facilities approved by the American Association for Accreditation of Laboratory Animal Care. Experimental protocols were approved by the Institutional Animal Care and Use Committee, Oregon Health and Science University. The animals were anesthetized using both ketamine (40 mg/kg im) and xylazine (10 mg/kg im). Supplemental doses of both anesthetics were given on a schedule or as needed, judging by leg withdrawal to a toe pinch.

Rectal temperature of the animals was maintained at 38 ± 1°C with a servo-regulated heating blanket. Cochlear temperature was additionally controlled by supplemental heat to the head from a lamp and a heated head-holder. The guinea pig’s head was firmly fixed in the head-holder, which was mounted on a custom-made manipulator and was electrically isolated from the operation table. A tracheotomy was performed, and a ventilation tube was inserted into the trachea to ensure free breathing. A ventral and postauricular combined approach was used to open the left auditory bulla and expose the cochlea. The middle ear muscle tendons were carefully sectioned.

Cochlear sensitivity was monitored throughout the experiment by recording the evoked compound action potential (CAP) of the auditory nerve. For this purpose, a ball electrode made of Teflon-coated silver wire (75 μm diam) was placed in the round window niche. An Ag/AgCl wire was inserted into neck soft tissue to serve as the ground electrode. A plastic coupler with two speakers (made of ½-in B&K microphones) mounted inside was fitted to the ear canal to deliver acoustical stimuli. Tone bursts (10-ms duration, 1-ms rise/fall, 2–36 kHz) were delivered to the ear canal to evoke the CAP. The round window signal was amplified 1,000 times and displayed on an oscilloscope for CAP threshold assessment. In addition, the quadratic distortion signal at 900 Hz from the round window evoked by two tones at 18 and 18.9 kHz was repeatedly checked during the surgery on the cochlea. This distortion product is highly sensitive to surgical trauma at the location corresponding to 18 kHz.

Measurement of basilar membrane velocity

The magnitude and phase of BM transverse velocity were measured at the location corresponding to the 18 kHz best frequency (BF) using a laser interferometer (Polytec OFV 1102). To measure BM motion, a small fenestra was created in the first-turn scala tympani bony wall of the cochlea. Gold-coated glass beads (10–30 μm in diameter) were put onto the BM at the appropriate location in the OHC region to serve as reflective objects that track the motion of the BM. A coverslip was used to close the hole to prevent perilymph surface vibration and to correct the optical distortion of imaging through a fluid meniscus. A laser beam from the laser interferometer was focused on a bead with the aid of a compound microscope (Nuttall et al. 1991). Velocity of BM motion was measured in the end of some experiments by measuring the position of a gold-coated glass bead (150 μm diam) on the footplate of stapes. The data of BM velocity were used to normalize the BM motion.

For measurement of acoustically evoked BM motion, acoustic stimuli (3–24 kHz, 10–100 dB SPL) were generated by a Tucker-Davis Technologies (TDT) System II and presented to the external ear canal through a speaker in the acoustic speculum. The output signal of the interferometer is proportional to the velocity of the targeted bead. This signal was low-pass filtered by an 8-pole filter with a 40-kHz cut-off frequency digitized by a 16-bit A/D converter. BM velocities were determined after fast Fourier transform (FFT) of the Hann-windowed responses from the interferometer. Phases of BM motion in reference to TDT output signal were also measured. The reference phase was that of the electrical output to the speaker. The data were stored on a PC hard disk for off-line analysis.

Electrically evoked BM responses provide indirect measures of in vivo OHC electromotility (Grosh et al. 2004; Nuttall and Ren 1995). Techniques for measurement of electrically evoked BM velocity responses have been reported previously (Grosh et al. 2004). Briefly, as illustrated in Fig. 1, two wire electrodes (Pt–Ir, 50 μm diam) were inserted into the scala vestibuli (SV) and scala tympani (ST), respectively, in the basal turn, forming a bipolar pair at the BM measurement location across the cochlear duct. Sinusoidal current from a constant-current stimulator was delivered through the electrodes. The BM velocity in response to sinusoidal current stimuli was measured with
the same laser interferometer as described in the preceding text. Voltage control to the constant-current stimulator was from the oscillator output of a digital lock-in amplifier (Stanford Research Systems SR830). The current intensity was 100 μA rms, and the frequency range was 5–80 kHz. Signals from the laser interferometer were recorded by the same lock-in amplifier and displayed in terms of amplitude and phase.

Perilymphatic perfusion of the scala tympani

Perilymphatic perfusion was performed to deliver pharmacological agents into the scala tympani of the cochlea. An inlet hole (diameter: ~70 μm) was made in the scala tympani close to the round window niche and the hole for BM measurement served as fluid outlet (Fig. 1). A three-way perfusion device that allows solution substitution was used for scala tympani perfusion. A polyethylene tube was connected to this device, and its fine tip (diameter: ~60 μm) was inserted into the inlet hole of the cochlea. Tissue glue was applied to seal the inlet hole and fasten the tube in position. Agents in artificial perilymph were infused into the scala tympani at a rate of 2 μl/min using a syringe pump (WPI, SP 210iw). The normal artificial perilymph composition is (in mM) 132 NaCl, 3.5 KCl, 25 NaHCO3, 1.3 CaCl2, 1.14 MgCl2·6H2O, 0.51 NaH2PO4·H2O, 5.0 Tris, 3.3 glucose, and 2.1 urea. The pH of all solutions was adjusted to ~7.4, and the osmolarity was 300 ± 10 mOsm. Artificial perilymph perfusion using the technique described here did not affect the BM response. However, because the artificial perilymph was used to carry the agents for intra-cochlear perfusion and also for drug washout, BM measurement after artificial perilymph perfusion was always performed in each experiment as control, although we also measured the BM motion before perfusion. The duration of perfusion for each agent was usually 10 min but was prolonged to as long as 30 min in certain cases as needed. The duration for drug washout was usually 30 min. Measurement of BM motion was usually performed at least five minutes after the end of each perfusion. Effluent from the outlet hole was absorbed within the bulla using cotton wicks.

Statistical analysis

An analysis of variance (ANOVA) was utilized to determine significant difference across treatment groups. A probability of <0.05 is considered a statistically significant difference.

RESULTS

Effects of CPZ on acoustically evoked BM vibration

In cochlear mechanics research, the BM responses are usually presented in the form of “mechanical tuning curve” by plotting out the BM vibration magnitude in response to a certain stimulus level as a function of frequencies. A whole set of normal BM mechanical tuning curves to acoustic stimuli ranging from 3 to 24 kHz at 10–100 dB SPL (10 dB/step) is presented in Fig. 2A. Also calculation of the gain of BM motion from the ratio of BM velocity/stapes velocity provides information of cochlear amplification. Figure 2B shows an example of the transfer functions of the BM velocity gain (for
sound levels 10–100 dB SPL) in a sensitive ear. The tuning of BM responses is sharp and the gain of BM is large at low sound levels. They become less sharp and smaller, respectively, when the sound level is increased, exhibiting a nonlinear “compressive growth” of the tuning curves (Robles and Ruggero 2001). Scala tympani perfusion of CPZ substantially affects both the mechanical tuning and the gain of BM motion. As shown in a typical animal in Fig. 2, when 5 mM CPZ was administered, the magnitude of BM motion in response to low-level acoustic stimulation near the best frequency (BF, i.e., the frequency where the BM exhibits the highest response) was greatly reduced (Fig. 2C). The mechanical tuning was broadened with a shift of the BF toward lower frequency. The compressive growth of responses to higher-level sound was less obvious compared with normal responses (compare Fig. 2, A and C). A loss of the gain of BM motion by ≥30 dB was observed in this example (Fig. 2D). These changes demonstrate a loss of cochlear amplification under CPZ administration.

More details of alterations in BM motion induced by CPZ are shown in Fig. 3. In an example shown in Fig. 3A, significant reduction in the peak magnitude of the mechanical tuning curve (evoked by 40 dB SPL tones) indicates a loss of sensitivity near BF, whereas a broadened tuning indicates a loss of frequency specificity. A shift of BF toward lower frequency is also obvious; this is a feature of sensitivity loss. Figure 3B shows the phase change of BM motion as a function of frequency in the same animal. With CPZ perfusion, the phase lags below BF but leads above BF compared with the phase in control. To learn whether or not CPZ also affects the passive mechanics of BM, we compared the BM responses to very high sound level (100 dB SPL) in conditions with and without CPZ. As shown in another example in Fig. 3, C and D, 10 mM CPZ perfusion significantly reduced the magnitude of BM response evoked by acoustic stimulation at 100 dB SPL. Phase changes similar to that with low level sound stimulation were also observed (compare Fig. 3, B and D).

The sensitivity loss induced by 5 mM CPZ was ~20 dB (Fig. 3E, data from 6 animals). Calculation of the Q_{10 dB} value (the BF divided by the bandwidth of the tuning curve 10 dB from the tip, a measure of the sharpness of tuning) shows significant difference in tuning (P < 0.01) between control (5.69 ± 1.34, mean ± SD, n = 6) and 5 mM CPZ (3.29 ± 1.03, n = 6). These results indicate a worse tuning in BM motion due to CPZ. Loss of nonlinearity of BM motion was also seen as shown by BM velocity magnitude input-output functions (Fig. 3E).

In pilot experiments, different concentrations of CPZ (i.e., 0.1–50 mM) were used, and concentration-dependent effects of CPZ on BM motion were observed (data not shown). However, CPZ at 0.1 mM had a very minimal effect. At 1.0 mM, a clear effect could be seen, yet the reduction of BM vibration at BF was still very small (~5 dB). A significant effect on BM motion was observed when CPZ concentrations were 5 and 10 mM, and its effects were reversible after washout with artificial perilymph (see an example in Fig. 3F). The reversible effects verified that alterations in BM responses we observed were due to the effects of CPZ. At levels >15 mM, CPZ severely suppressed cochlear sensitivity, which could not recover. For this reason, we used 5 and 10 mM on most of the animals in this study. In addition, because of the concern that CPZ could act on OHC via an effect on cholinergic receptors (Park et al. 2001), strychnine (100 μM), a potent cholinergic receptor antagonist, was infused into the scala tympani before the application of CPZ. The effect of CPZ was not blocked by

![FIG. 3. Acoustically evoked BM vibration altered by CPZ. A and B: BM velocity magnitude and phase evoked by 40 dB SPL pure tones. C and D: BM velocity magnitude and phase evoked by 100 dB SPL pure tones. E: averaged input-output functions (n = 6) of BM velocity at BF. F: example of input-output functions of BM velocity at BF showing the reversible effect of CPZ. The legend “control” in all panels stands for the condition with artificial perilymph perfusion.](http://jn.physiology.org/)

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strychnine, ruling out the possibility of an action on OHC cholinergic receptors (data not shown).

**Effects of CPZ on electrically evoked BM vibration**

In sensitive cochleae, electrically evoked BM responses in the OHC region near the 18-kHz location had sharply tuned multiple peaks <20 kHz (“below-BF-part”) that corresponded to traveling waves (see the phase change with frequency in Fig. 4B), and poorly tuned responses >20 kHz (“above-BF-part”) with a notch near 50 kHz (Fig. 4A). This notch was always accompanied by a phase change (Fig. 4B) as we reported previously (Grosh et al. 2004). CPZ affects the electrically evoked BM responses in a concentration-dependent manner. The effects on the below-BF-part were similar to those of acoustically evoked BM vibration (Fig. 4, A and C). In the above-BF-part, a reduction in the magnitude of BM vibration was also seen. But the more interesting change was the frequency shift of the −50-kHz notch toward higher frequency when CPZ was applied (Fig. 4A). The amount of frequency shift was CPZ concentration dependent and was as large as several kilohertz (in Fig. 4A, it was 3.8 kHz with 5 mM CPZ and 5.4 kHz with 10 mM CPZ). The frequency of phase change corresponding to the notch systematically shifted accordingly (Fig. 4B). The change in the phase at the notch frequency indicates a likely change in the mode of vibration of the OoC (Grosh et al. 2004), and thus these data suggest that the OoC vibration mode at this frequency is altered by CPZ. With CPZ concentrations ≤10 mM, the effects of CPZ on the electrically evoked BM responses were reversible (see Fig. 4C for a recovery in BM vibration magnitude in the below-BF-part and the return of the high-frequency notch after washout).

Because the phase changes due to CPZ in the acoustically evoked BM motion (Fig. 3) are in accordance with the stiffness increase as proposed by other’s work (Cooper and Guinan 2003), the frequency shift of the −50-kHz notch toward higher frequency in the electrically evoked BM responses with CPZ perfusion (Fig. 4) may imply stiffness increase of OHCs by the action of CPZ on the plasma membrane. To test this hypothesis, we applied SAL, a chemical that blocks the motor protein of OHCs and has been shown to reduce the in vitro OHC stiffness (Hallowsworth 1997; Kakehata and Santos-Sacchi 1996; Lue and Brownell 1999; Oliver et al. 2001; Shehata et al. 1991) and likely also the stiffness of the cochlear partition (Murugasu and Russell, 1995) with its effects on the plasma membrane biomechanics resulting in an outward bend in the curvature of plasma membrane, a change being opposite to that of CPZ (Gutknecht and Tosteson 1973; McLaughlin 1973). SAL caused a shift of the high-frequency notch toward lower frequency, which is opposite to the shift caused by CPZ. This “downward” shift was small in degree but unambiguous and was reversible with artificial perilymph washout (Fig. 5, A and B).

![FIG. 4. CPZ altered electrically evoked BM vibration. Sinusoidal current (5–70 kHz, 100 µA rms) was injected into the cochlea with scala vestibuli to scala tympani configuration. A and B: magnitude and phase of BM velocity evoked by electrical stimulation with 5 and 10 mM CPZ perfusion. Arrows in A indicate the notch frequency. C and D: magnitude and phase of BM velocity evoked by electrical stimulation with 5 mM CPZ perfusion and with artificial perilymph washout. In control conditions, artificial perilymph was infused.](http://jn.physiology.org/lookup/doi/10.1152/jn.00351.2006)
BF, the spring-like impedance of the HB connecting the cochlear fluid-structure waves arising from this local excitation were well above the BF at this location, we assumed that the Piezoelectric model of the OHC is used to model its electromotile behavior. 3) The connection between the basal pole of the OHC and the BM through the Deiters' cell (DC) is achieved via a spring and damper. 4) The BM is modeled as a spring-mass-damper system.

With these assumptions the OoC model reduces to a 3 degree of freedom system as described in Fig. 6. The definitions of the parameters are given in the figure caption. The force arising from the electromotility of the OHC is given by $F_{ohc}$. This force is applied to mass $M_1$ and $M_2$, whereas the HB force $F_{hb}$ is applied to mass $M_3$ and the TM as shown in the Fig. 6. Assuming time harmonic motion of the form $\exp(j\omega t)$, where $j = \sqrt{-1}$ and $\omega$ is the radian frequency of vibration, the system of equations for the model takes the following form

$$
\begin{pmatrix}
-M_1\omega^2 + j\omega C_1 + C_3 \\
+K_1 + K_3 \\
-K_3 - j\omega C_2
\end{pmatrix} - \begin{pmatrix}
-K_1 - j\omega C_2 \\
-K_2 - j\omega C_3 \\
0
\end{pmatrix} - \begin{pmatrix}
-M_2\omega^2 + j\omega C_1 + C_3 \\
+K_1 + K_3 \\
-K_3 - j\omega C_2
\end{pmatrix} - \begin{pmatrix}
-K_1 - j\omega C_2 \\
-K_2 - j\omega C_3 \\
0
\end{pmatrix} \times \begin{pmatrix}
u_1 \\
u_2 \\
u_3
\end{pmatrix} = \begin{pmatrix} 0 \\
-F_{ohc} \\
-F_{hb}
\end{pmatrix}
$$

Here $u_1$, $u_2$, and $u_3$ represent the displacements of masses $M_1$ (BM + 1/2 DC), $M_2$ (1/2 DC + 1/2 OHC), and $M_3$ (1/2 OHC + RL), respectively. The motion of different structures is simplified to be colinear, which is not really the case in the apical attachment of the OHC, including the hair bundle (HB), RL and other cell structures supporting the RL. $F_{ohc}$ represents equal and opposite active forcing from OHCs due to their piezoelectric-like behavior. $F_{hb}$ is active forcing from the HBs. $u_1$, $u_2$, and $u_3$ represent displacement of masses $M_1$, $M_2$, and $M_3$, respectively.
We assume the HB force takes the form current source excitation. Electro-mechanical coupling coefficient, and Stiffness of OHC can solve amplitude of the current from the constant current source. We calculate the OHC basolateral wall resistance, $R_m$, which is the basolateral capacitance, $C_m$, is the basolateral voltage relative to the resting value. Consequently the force that the OHC applies to the mass due to electrical excitation is $F_{ohc} = -e\Delta \phi$. Solving Eqs. 2 and 3 yields

$$F_{ohc} = -eZ_{ohc}[I_{ohc} + j\omega(u_i - u_3)]$$

thus the OHC force adds terms to the left-hand side of the equation as well as to the right-hand side (current dependent terms) in Eq. 1. We make the assumption that the current passing through the OHC is equal to that provided by the constant current source, in this case a 100-μA rms sinusoidal term. Therefore the known forcing term in Eq. 1 is $I_{ohc} = I_0 \exp(j\omega t)$, where $I_0$ is the amplitude of the current from the constant current source. We can solve Eq. 1 for the mechanical response to a constant current source excitation.

The HB force acts at the apical pole of the OHC (see Fig. 6). We assume the HB force takes the form $F_{hb} = BF_{ohc}$, where $B$ is an unknown proportionality constant. One can also assume that the HB force is proportional to $I_{ohc}$ with the same resulting conclusions (results not shown). Parameters used in the model are detailed in Table 1.

### Model results

Predictions from the model are made by solving Eq. 1 using the parameters given in Table 1 subject to a time harmonic oscillatory current, $I_0$. As in the experiments, the driving force is the applied current. In addition to solving for the displacement, we solve Eq. 1 in closed form for the frequencies corresponding to zeros of the BM displacement of mass 1 ($u_1$). In the limit of very small damping the “notch” frequencies is given by

$$\omega_{notch} = \sqrt{\frac{K_4 - \beta K_5}{M_3}}.$$

Considering the case $\beta = 0$ (i.e., OHC somatic forcing only), we have $\omega_{notch} = \sqrt{K_4/M_3}$. The notch frequency only depends on the HB-RL stiffness (embodied by $K_4$) and mass loading the apex of the OHC ($M_3$), but not the OHC stiffness ($K_5$). Embellishing the model with further details (for instance, adding a stiffness coupling between the BM and the RL) does not change this conclusion. Figure 7, A and B, shows model response predictions of amplitude and phase with different OHC stiffness values numerically demonstrating the theoretical result of Eq. 5. If this model is a faithful representation of the mechanics in vivo, then it implies that the stiffness of the structures on the apical end of OHC, such as the HB and/or the RL, is being affected by CPZ, rather than the stiffness of the OHC soma (see Fig. 7, A and B).

If force production by the HB is involved, this force is applied to the cuticular plate and RL as reflected in Eq. 1. The notch frequency, $\omega_{notch}$, is computed from Eq. 5, recalling that $\beta$ is the ratio of HB forcing to OHC somatic forcing in the direction perpendicular to the BM. The notch frequency here is sensitive to OHC stiffness manipulation as embodied by the dependence on $K_5$. Simulations of this model with an assumed HB forcing using $\beta = 0.05$ are shown in Fig. 7, C and D. The model shows a shift in the notch frequency when OHC stiffness is changed, in which upward notch-frequency shift occurs with increased OHC stiffness and vice versa. With higher HB forcing (increasing $\beta$), the shift in frequency of the notch is

### Table 1. Parameters used in the model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius of OHC at 5 mm from base ($r$)</td>
<td>5 μm</td>
<td>Estimated from Dallos (1996)</td>
</tr>
<tr>
<td>Length of OHC at 5 mm from base ($l$)</td>
<td>35 μm</td>
<td>Estimated from Dallos (1996)</td>
</tr>
<tr>
<td>Mass of OHC</td>
<td>0.172 ng</td>
<td>Estimated from Dallos (1996)</td>
</tr>
<tr>
<td>Stiffness of OHC $K_4$</td>
<td>12 mN/m (at rest)</td>
<td>Mass = $\rho\pi r^4 l/16$; $\rho = 1000$ (density of water), factor of 16 is used to fit Frank et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>20 mN/m (with CPZ)</td>
<td>Rest value from Frank et al. (1999); CPZ value estimated from He and Dallos (1999) data for percentage stiffness change at extreme hyperpolarized voltages</td>
</tr>
<tr>
<td>Electromechanical coupling $e$</td>
<td>98.8 nN/V (at rest)</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td></td>
<td>49.4 nN/V (with CPZ)</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td>OHC resistance $R_m$</td>
<td>25 Mohms</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td>OHC capacitance $C_m$</td>
<td>23.28 pF (at rest)</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td></td>
<td>12.64 pF (with CPZ)</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td>BM stiffness $K_1$</td>
<td>1 N/m</td>
<td>Deo and Grosh (2005)</td>
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<tr>
<td>BM mass + Fluid loading</td>
<td>78.1 ng</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td>RL/HB stiffness $K_4$ (Free parameter)</td>
<td>9 mN/m</td>
<td>Deo and Grosh (2005)</td>
</tr>
<tr>
<td>Deiters’ cell stiffness $K_2$</td>
<td>1 N/m</td>
<td>Deo and Grosh (2005)</td>
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<tr>
<td>Deiters’ cell mass (free parameter)</td>
<td>21.99 ng</td>
<td>Deo and Grosh (2005)</td>
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<td>HB forcing factor $\beta$</td>
<td>0.05</td>
<td>Deo and Grosh (2005)</td>
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<td>Damping, $C_1$, $C_2$, $C_3$</td>
<td>17.6 μNs/m, 3.3 μNs/m, 32.1 nNs/m</td>
<td>Deo and Grosh (2005)</td>
</tr>
</tbody>
</table>

OHC, outer hair cell; BM, basilar membrane; RL, reticular lamina; HB, hair bundle.
greater, but the notch becomes less prominent. Further, with HB forcing, the notch is not as deep as for the case of OHC somatic forcing alone. As shown in Fig. 7C, an alteration in the stiffness of HB-RL also results in a shift in the notch frequency, just as in the somatic only forcing case.

**DISCUSSION**

**Cochlear amplification and OHC electromotility altered by CPZ**

We demonstrate here that CPZ reduces in vivo OHC electromotility and thus affects cochlear amplification. A previous in vitro study (Lue et al. 2001) has shown that CPZ alters isolated OHC electromotility as measured by nonlinear capacitance (NLC) and voltage-dependent OHC length change. In that study, the peak capacitance (Cmpk) and OHC length-voltage transfer function shifted in a depolarizing direction, indicating an inhibitive effect of CPZ on OHC motility. In accordance with this implication, CPZ-induced elevations in CAP and DPOAE thresholds have been reported (Oghalai 2004). Observation of the BM motion enabled us to gain better insight into the cochlear mechanics and the effectiveness of the cochlear amplifier. In this study, 5 mM CPZ substantially reduced cochlear sensitivity as evidenced by a reduction in velocity magnitude of acoustically evoked BM motion, and a loss of gain by 20–30 dB near BF (Fig. 2); a broadening of tuning (Figs. 2, C and D, and 3A); and a reduction of nonlinearity (Fig. 3, E and F). These changes are consistent with the aforementioned reduction in electromotility of isolated OHCs (Lue et al. 2001) and alterations in CAP and DPOAEs (Oghalai 2004). In addition, a reduction in electrically evoked BM vibration magnitude both below and above BF (Fig. 4) confirms the decrease of OHC electromotility that could account for the decline of cochlear amplification.

We considered, but discounted, the following two mechanisms that could underlie the actions of CPZ on OHC electromotility.

First, an action via anti-dopaminergic receptor effect as summarized by Awad and Voruganti (2005). Evidently, the OHCs were not affected by this mechanism because dopamine was found to act only on the lateral efferents innervating the inner hair cells but not on the OHCs (Eybalin 1993). In addition, the endocochlear potential (EP) was not affected by CPZ (Oghalai 2004), ruling out an EP-related OHC driving force reduction via an effect on cochlear blood flow that could be modulated through a dopamine receptor-mediated mechanism (Ernster and Meyers 1986; Zeng et al. 2004).

Second, actions on cholinergic receptors on the OHCs. Cholinergic receptor-mediated OHC electromotility has been well elucidated (e.g., Dallos et al. 1997; Erostegui et al. 1994; Housley...
and Ashmore 1991; Kalinec et al. 2000). CPZ could act on certain subtypes of cholinergic receptors (Park et al. 2001) so that the observed CPZ effects could be mediated by these receptors. In the control experiment, the effect of CPZ on cochlear sensitivity was not affected by strychnine, a potent antagonist of cholinergic receptor. This possibility was thus ruled out.

The following two mechanisms for CPZ’s action are better supported by our model and data as well as data in the literature.

First, alterations in the voltage-motility relationship of the OHCs. Unlike SAL (a prestin inhibitor), CPZ does not reduce the \( C_m \) or the absolute value of voltage-dependent OHC length change. However, CPZ shifts the voltage location of the \( C_m \) and OHC length-voltage transfer function in a depolarizing direction (Lue et al. 2001). These alterations could be due to a change in surface charge of the plasma membrane (Zhang et al. 2001). The OHCs thus may not operate at their optimal electromotility due to the change of the motor status. Reduced OHC motility will directly lead to reduced cochlear amplification.

Second, alterations in biomechanics of the OHC lateral wall. In vitro data suggest that CPZ acts mainly on the plasma membrane of OHCs (e.g., causing inward bend, altering the tension and reducing the fluidity of the plasma membrane) (see Leu et al. 2001; Murdock et al. 2005; Oghalai et al. 2000; Sheetz and Singer 1974) without any evidence for a direct effect on the motor protein (Lue et al. 2001; Morimoto et al. 2002; Oghalai et al. 2000). The experimental data and modeling work presented here suggest that an increase in stiffness of the OoC may occur due to CPZ (see detailed discussion in the following text). The model results (Fig. 7, A and C) also show that increasing OHC stiffness reduces BM response to bipolar electric stimulation. Thus reduced motility by CPZ could be attributable to increased stiffness of the OHCs, which is consistent with the observation of OHC motility and stiffness in an in vitro study (Borko et al. 2005). In addition, CPZ-induced shift of the voltage for \( C_m \) could result in an OHC axial stiffness change (e.g., He and Dallos 1999; Iwasa 2000), which in turn will affect the motility. However, this effect leads to a reduction in OHC stiffness and thus an increase of motility. We assume this effect is only a minor one which is dominated by the stiffness increase effect of CPZ as shown by our data.

**OHC stiffness increase by CPZ: experimental data and model prediction**

The most interesting and novel finding in this study is the shift of the ~50-kHz notch toward higher frequency in the electrically evoked BM vibration when CPZ was applied (Fig. 4). In acoustically evoked BM motion, CPZ-induced phase lead above BF (Fig. 3) has suggested an increase in stiffness of the cochlear partition (Cooper and Guinan 2003). The ~50-kHz notch shift induced by CPZ thus may be associated with the stiffness change. Indeed, whereas a shift of this notch toward higher frequency occurred with administration of CPZ, a shift toward lower frequency was observed when SAL, a drug known to decrease the OHC axial stiffness (Hallworth 1997; Kakehata and Santos-Sacchi 1996; Lue and Brownell 1999; Shehata et al. 1991) and probably the stiffness of the cochlear partition (Murugasu and Russell, 1995), was applied (Fig. 5). The experimental data therefore suggested that a change in the stiffness of OHCs and probably some other structures in the OoC was involved in controlling the shift of this high-frequency notch. Opposite directions of this notch shift by SAL and CPZ suggest opposite actions on biomechanics (e.g., plasma membrane curvature and stiffness) of the OHCs, which are in line with previous in vitro studies (Lue et al. 2001; Morimoto et al. 2002; Oliver et al. 2000, 2001) as well as an in vivo study on BM responses (Murugasu and Russell, 1995). Thus a shift of the ~50-kHz notch toward higher frequency may indicate an increase of the stiffness of the OoC and vice versa.

It is noticed that SAL has less effect on the degree of ~50-kHz notch shift than CPZ, whereas their effects on the magnitude of BM motion are similar. These may suggest different mechanisms of their actions on the OHC electromotility. We speculate that SAL affects OHC electromotility dominantly by a direct effect on the motor protein along with a relatively minor effect on OHC lateral wall biophysics, whereas CPZ probably affects OHC electromotility more indirectly through effects on the voltage-motility relationship as well as on the stiffness of OHC lateral wall.

The modeling work presented here supports the CPZ-induced stiffness modulation of cellular components in the OoC. Our simple model shows that if OHC somatic forcing is the only electromotile forcing present in the cochlea at these high frequencies, then CPZ can cause a shift in the notch frequency by affecting the stiffness of hair bundle (HB) and/or reticular lamina (RL) as shown in Fig. 7. A and B, although stiffness change of OHC soma has no effect on the notch frequency shift in this case. Access to these structures is possible for CPZ, which could be through an intra-cellular pathway to HB (because CPZ can penetrate the OHC plasma membrane and intercalate into the inner leaflet of this membrane) (see Leu et al. 2001; Sheetz and Singer 1974) and via extracellular fluid in the OoC and probably also intracellular pathway to RL.

Intriguingly, if HB motility is introduced (as proposed by Kennedy et al. 2005), our model predicts that the OHC stiffness will play a role in setting the frequency of the notch. Then CPZ-induced OHC stiffness change can cause a shift of the notch towards higher frequency as shown by predictions of the notch shift by the combined HB-OHC somatic forcing model in Eq. 5. This is consistent with the proposal by He and Dallos (1999) that relatively modest stiffness changes of OHCs might have significant influence on cochlear mechanics. Importantly, this could be in vivo evidence that OHC stiffness plays a role in determining the mode of organ of Corti motion in the face of HB motility. Again, stiffness changes of HB and RL also play a role in this case (Fig. 7, C and D).

It should be pointed out that the effect of CPZ on the plasma membrane is nonspecific and thus CPZ-induced changes in the micromechanics of supporting cells in the OoC should also be taken into account. Especially the stiffness change of Deiters’ cells should not be ignored because a prominent role for Deiters’ cells in cochlear sensitivity has been reported (Flock et al. 1999). However, the stiffness of the rigid pillar cells and Deiters’ cells is largely determined by the intracellular filaments and tubules that span the distance between the RL and BM (Slepecky 1996). Changes in plasma membrane stiffness of these cells may not significantly affect the overall stiffness of them. Indeed, our model does not show involvement of the
stiffness of supporting cells (K_s) in the shift of the high-frequency notch.

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