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Mammalian outer hair cells (OHCs) convert electrical energy into mechanical energy. The significance of this electromotility rests in the ability of the OHCs to modulate the vibrations of the cochlear partition *in vivo*. While high-frequency electromotility of isolated OHCs has been demonstrated at frequencies up to 100 kHz, a similar measure of the effect of OHC electromotility on motion of the sensory epithelium has not been made *in vivo*. In this study, *in vivo* electrical stimulation of the guinea pig cochlea is found to induce a mechanical response of the basilar membrane for frequencies to at least 100 kHz, nearly twice the upper limit of hearing for the guinea pig. The perfusion of salicylate in the cochlea reversibly reduces the electromotile response, indicating that an OHC-mediated process is the key contributor. © *2004 Acoustical Society of America.* [DOI: 10.1121/1.1695431]

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I. INTRODUCTION

The outer hair cells (OHCs) of the mammalian cochlea are biologically unique because they are both sensors and actuators. In other words, OHCs function as mechano-electrical and electro-mechanical transducers. *In vitro* studies have shown that isolated OHCs are capable of mechanical oscillations at frequencies at least as high as 100 kHz in response to transmembrane sinusoidal electrical stimulation (Frank *et al.*, 1999). Electrical stimulation applied to the intracochlear fluids is known to elicit basilar-membrane motion (Xue *et al.*, 1995). Extra- and intracochlear electrical stimulation has been found to produce sound emissions measured in the external ear (Hubbard and Mountain, 1983; Nuttall, 1995; Xue, 1996) known as electrically evoked otoacoustic emissions (EEOAE). The upper-frequency limit of these emissions is about equal to the upper limit of hearing for that animal. In this paper, we investigate the frequency characteristics and test the upper-frequency limit of basilar-membrane (BM) velocity in response to sinusoidal intra- and extracochlear electrical stimulation *in vivo* and compare these results to the *in vitro* limits of isolated OHCs. We present results from control and postmortem experiments. These high-frequency results are used to determine mechanisms of electromotility in the cochlea. We discuss how these measurements could be used to estimate parameters in a mathematical model.

The precise micromechanical mechanisms for the *in vivo* OHC electromotility remain unknown, but their potential significance for hearing requires that they be able to modulate the vibrations of the cochlear partition *in vivo*. The present view is that the OHC mediates a cycle-by-cycle sound amplification in the following manner: (1) sound energy causes a traveling wave along the basilar membrane, displacing the components of the organ of Corti; (2) that this displacement leads to the deflection of OHC stereocilia, gating an ionic current that causes an oscillatory OHC membrane potential at the frequency of the stimulus; and (3) that OHCs convert the electrical energy into mechanical energy that feeds back with the correct phase to enhance vibration. This is the so-called active process of cochlear amplification (Dallos, 1992). The high-frequency motility of outer hair cells, encompassing (and surpassing) the highest physiologically relevant frequencies, provides for the frequency selectivity and sensitivity of mammalian hearing (see, e.g., Liberman *et al.*, 2002). While *in vitro* studies of high-frequency isolated OHC electromotility are available, there are no data on the high-frequency limit of electrical stimulation *in vivo*.

II. METHODS

A. Surgical preparation

We summarize the surgical preparation and animal handling used for this study, which are discussed in more detail

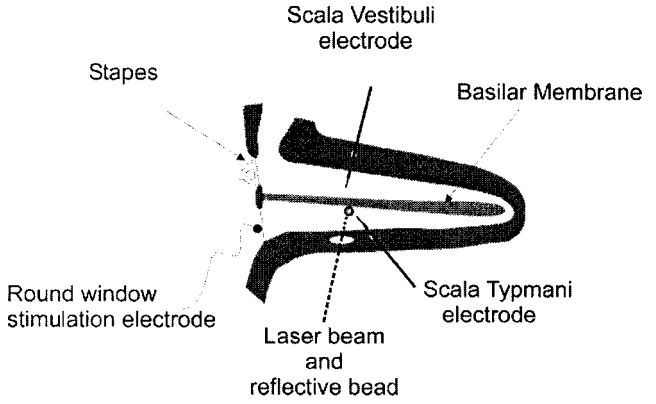

FIG. 1. Simplified (uncoiled) rendition of the cochlea showing the configuration for local or RW electrical stimulation. The local BM velocity was measured using a laser Doppler velocimeter. The scala tympani opening for the laser was also used to introduce the sodium salicylate.

in Parthasarathi *et al.*, 2003. Healthy young pigmented guinea pigs (250–400 g) were used in this study. The animals were anesthetized. A tracheotomy was performed after the animal's head was fixed to a headholder, and a ventilating tube inserted to ensure free breathing. The experimental protocols used were in accordance with the rules established by the Committee on Use and Care of Animals at the Oregon Health and Science University. After the bulla was opened to expose the cochlea, a silver wire (75-μm diameter) electrode was placed on the round window (RW).

A total of three openings was made in the cochlea [two in the *scala tympani* (ST) and one in the *scala vestibuli* (SV)]; see Fig. 1. An opening approximately 300 μm wide was made on the ST side of the cochlear basal turn to allow for measurement of BM velocity. The other two openings were approximately 75 μm in diameter and were made to insert the SV and ST electrodes into the perilymph.

The compound action potential (CAP) measured from the RW electrode, with reference to an Ag–AgCl ground electrode in the soft tissues of the neck, was used to obtain information on the N_1 detection threshold of the CAP at a given acoustic frequency. The threshold was used as an indicator of the cochlear sensitivity. For animals used in this study, the CAP loss at the first-turn best frequency (BF) was less than 20 dB. The high-frequency electromotility (above BF) seen in the present study was not critically impacted by a depressed CAP threshold. However, the response below and near to BF is reduced for animals whose CAP threshold was depressed after surgery.

B. Excitation and response measurement

Acoustic stimuli were delivered through a 1/2-in. Bruel & Kjaer condenser microphone coupled to the external ear through a speculum. Electrical stimuli were delivered to the cochlea from a custom-designed constant current unit (CCU). Electrical stimulation was delivered at two locations, at the RW and across the first cochlear turn. The wire electrode (Pt–Ir 75-μm diameter) cemented in position at the RW membrane was used to deliver the current. The return electrode for the RW electrode was a chlorided silver wire in the soft tissue of the neck. The electrical stimulation from the

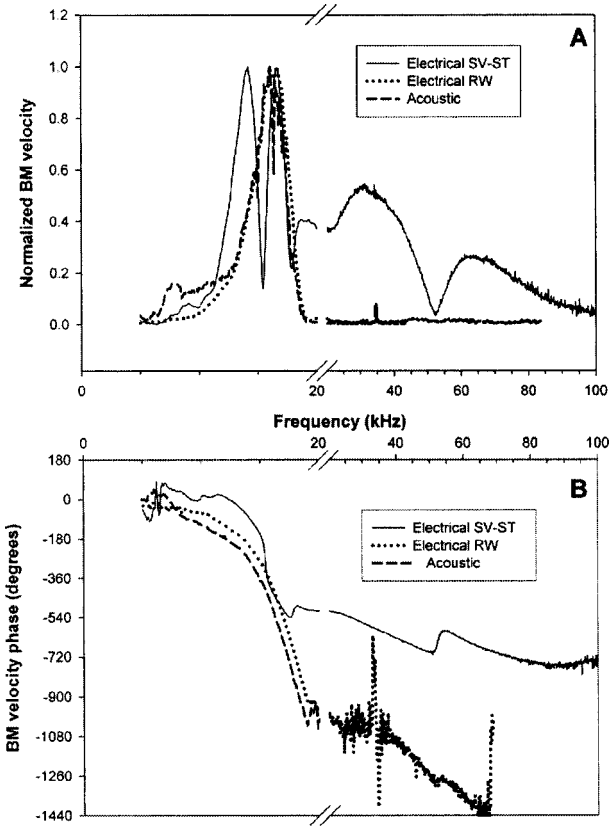

FIG. 2. Basilar-membrane velocity amplitude [panel (A)], normalized to maximum response in each case to acoustic, RW electrical, and local electrical (SV–ST) stimulation. In the case of electrical stimulation, the voltage sent to the constant current generator serves as the reference for the phase and the velocity was normalized to the applied current (GP 483). Phase relative to the voltage input to the excitation source is shown in panel (B). In response to bipolar electrical stimulation, the basilar-membrane response is seen to extend up to 100 kHz. An expanded scale of 5 kHz per tick mark is used for frequencies below 20 kHz.

RW–neck electrode pair is denoted as RW electrical stimulation in the sequel. Pt–Ir wires 50 μm in diameter were inserted into holes made in the bony cochlear wall of the first cochlear turn forming a bipolar pair across the cochlear duct from the SV to ST (see Fig. 1). We will denote this type of excitation as local bipolar stimulation. Voltage control to CCU and the speaker (via an amplifier) was from the oscillator output of a Stanford Research Systems (SR830) lock-in amplifier. BM velocity measurement was accomplished by directing the laser beam of a Polytec OFV 1102 through a compound microscope at a glass bead (20- or 3-μm diameter) placed onto the basilar membrane (see Fig. 1). The voltage output of the velocimeter was directed to the input of the lock-in amplifier.

III. RESULTS AND DISCUSSION

A. High-frequency basilar-membrane velocity response

Figure 2 shows the BM velocity response to three conditions (1) acoustic stimulation in the ear canal; (2) 35-μAmp rms electrical stimulation at the round window (RW); and (3) 100-μAmp rms local bipolar electrical stimulation. For acoustic stimulation (case 1), the mechanical response of

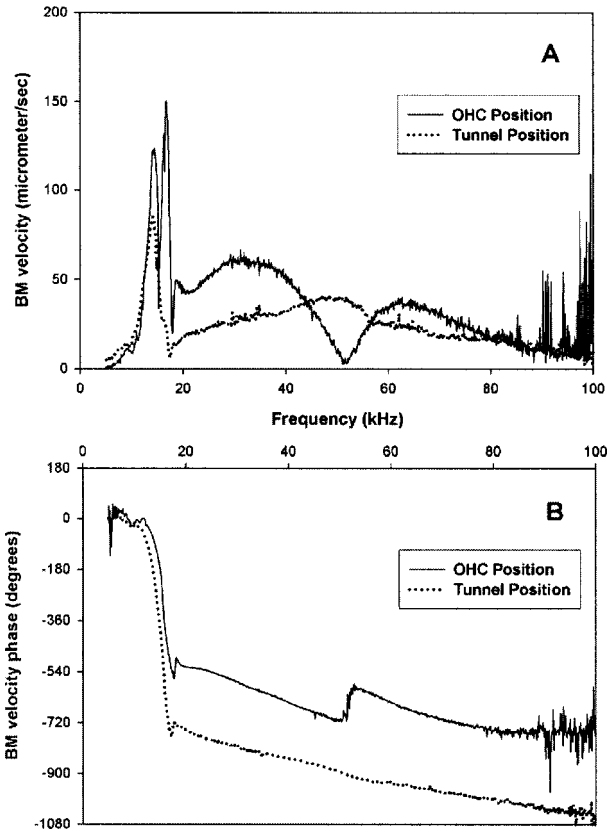

FIG. 3. Non-normalized basilar-membrane velocity amplitude [panel (A)] and phase [panel (B)] in response to local electrical stimulation (bipolar electrode placed across the SV/ST). These data are from the tunnel and OHC radial locations on the BM of GP 483. The BM velocity is seen to depend on the radial location of the bead.

the BM is tuned and has a peak response at about 17 kHz, the BF for this location. For frequencies above the BF the response falls to the noise floor [panel (A), dashed curve]. The corresponding phase curve [panel (B)] shows a pattern of increasing phase lag that is characteristic of a traveling wave. For electrical stimulation from the RW near the base of the cochlea (case 2), the pattern of response is similar to that from acoustic stimulation. This confirms earlier findings that electrical stimulation near the base of the cochlea results in forward-propagating traveling waves on the BM (Kirk and Yates, 1996; Nuttall *et al.*, 2001). In contrast, local bipolar electrical stimulation (case 3) produces a markedly different response (solid curves). This frequency response curve has both a more complex multipeaked pattern near the BF and the response extends to 100 kHz [panel (A)].

The high-frequency (above BF) response of the BM velocity was consistently measured in over 20 guinea pigs and showed remarkably little animal-to-animal variation. The amplitude response at this radial location (over the third row of OHCs) and longitudinal location shows a dip near 50 kHz and an approximate 180-deg phase reversal. This minimum in the response and the roughly 180-deg phase shift are indicative of a mechanical vibration mode, likely due to a resonance in the organ of Corti structures. The electrically evoked high-frequency response is present on all flexible portions in the radial direction of the BM. For instance, in Fig. 3, the BM velocity over the third row of OHCs is compared to the velocity measured over the tunnel region at the 17-kHz place (using two beads on the BM of the same animal). In both locations the high-frequency BM response to electrical stimulation is evident. The local electrical stimulation excites a radially varying response pattern that is most prominent above the BF of this location. The 50-kHz dip in the response seen over the OHCs is not present when measured at the tunnel region. Instead, a local maximum is seen at 50 kHz, and only a small phase shift is seen in these tunnel velocity data at 50 kHz. Due to the asymmetrical radial location of the OHC, actuation of the OHC will excite an asymmetrical structural mode. Since the asymmetrical BM mode has a higher resonance frequency than the more symmetric first mode, the excitation of this second structural mode will be more clearly seen at higher frequencies. For acoustic input, the asymmetric mode is not as prominent for two reasons. First, the force of the fluid pressure on the BM due to acoustic input has a spatial pattern that is more uniform in the radial direction than for excitation from OHCs, and therefore will more preferentially excite a symmetric BM mode. Second, since the acoustic pressure excites this region BM at frequencies at and below BF, the antisymmetric modal response is low as there is very little acoustical energy input to this mode near its resonance frequency (which is presumably above BF).

For frequencies less than the BF of the measurement location, bipolar electrical stimulation causes energy to be propagated basally to the stapes, setting up standing waves in the fluid between the excitation location and the base. The multiple peaks can be interpreted as resulting from interference among multiple traveling waves created by local forced stimulation of the BM by the OHCs. The smaller phase accumulation of the response evoked by local electrical stimulation [Fig. 2, panel (B)] is indicative of the presence forward and reverse wave propagation and inhomogeneous forcing (i.e., a combination of standing waves, traveling waves, and local forced responses) resulting in a lower phase shift than for a forward-traveling wave alone, such as is launched by acoustical and RW electrical excitation.

The constructive and destructive interference due to the wave propagation complicates our interpretation of the local mechanics at the measurement location. In a temporal bone preparation, Gummer *et al.* (1996) used electrical and acoustical excitation in much the same way as we have in the *in vivo* situation. There, they interpret the local amplitude minima and phase shifts around the BF as resonances in the TM and BM. This is one possible interpretation. There is no doubt that the local structural modal nature of the organ of Corti (OoC) and TM are involved. Over the third row of OHC, we see a beautiful antiresonance precisely between two peaks, one at the acoustical BF and another at a frequency nearly 1/2 octave below the BF (see Fig. 2). Some sort of dip at this frequency (between the BF and 1/2 octave below BF) is commonly seen in animals during electrical stimulation. A model that treats the BM, OoC, and TM structure as a locally reacting (i.e., no longitudinal stiffness) two degree of freedom oscillator, predicts that OHC forcing of the TM and BM, such as would occur from the electrical stimulation of the OHC, will cause a zero in the BM re-

sponse at the uncoupled resonance frequency of the TM (effective mass of TM combined with the effective stiffness of the TM and organ of Corti). If the cochlea were that simple, then the zero in the BM response would precisely locate the resonance of the TM. However, there is longitudinal stiffness in the cochlear structures, along with fluid–structure wave interference which, in our view, renders that interpretation ambiguous but worth further pursuit as a tool for identifying the *in vivo* properties of the OoC. While the dynamical system that is being excited by the bipolar stimulation is the same as that excited by the acoustical source, the location and type (electrical versus mechanical) are different. Hence, when estimating parameters of a model (e.g., Dimitriadis and Chadwick, 1999), these two experiments will provide independent data sets for such parameter estimation.

In a healthy cochlea, the BM response due to electrical or low-level acoustic input from the ear canal will be the sum fluid pressure plus OHC activity and other forces from the OoC unto the BM. We conjecture that at low input sound levels, the forcing from the OHCs would be more pronounced than for higher input acoustic excitation amplitudes, as they would account for a larger portion of the total response. One would then expect greatest asymmetry in the radial dependence of the BM velocity due to acoustic input to be seen in the most sensitive animals at low input levels, as is indicated in the results of Nilsen and Russell (2000). Electrical stimulation allows for the analysis of high-frequency spatial responses on the BM that are impossible with ear canal insonification.

It has been shown that the EEOAE response measured in the ear canal resulting from local, intracochlear electrical stimulation is bandlimited (Kirk and Yates, 1996; Nuttall *et al.*, 2001). These experiments show that the high-frequency cutoff of the EEOAE is correlated to the tonotopic location of the local electrical stimulation. For the guinea pig the frequency content of the EEOAE for RW electrical stimulation extends to 40 kHz, for first-turn excitation the limit is roughly 20 kHz, and for a turn 3 location the limit is 10 kHz (e.g., Nuttall *et al.*, 2001). This indicates that the electrical excitation from the bipolar electrodes is confined to a region close to the electrodes. If the electrical stimulation were to spread basally from the electrode location, evoked emissions would extend to higher frequencies by exciting the high-frequency, more basal OHCs. The EEOAE data from the literature are recounted because the velocity data due to local electrical stimulation in Fig. 2 show a local BM velocity response that is not bandlimited. Hence, the local mechanical response due to the putative OHC motor is active above BF, but those high-frequency waves are evanescent and do not reach the ear canal at measurable levels.

At ultrasonic frequencies above the BF, the response is dictated by local mechanical, electrical, and fluidic effects, rather than global wave propagation. The fluid loading at ultrasonic frequencies is mainly a local effect, since significant energy does not propagate away from the electrically excited OHC at ultrasonic frequencies. As such, this high-frequency excitation may provide a means of interrogating local health of a region of the cochlea as well as identifying parameters of a cochlear model that would be difficult to identify without high-frequency information. While the wave propagation effects in the cochlea are apparently simplified, there are other complications associated with using data from the high-frequency regime. The inertia of supporting cells becomes non-negligible and the structure of the organ of Corti will add additional loading. Electrically isolated OHCs will exhibit resonance-like behavior with resonance occurring at the 40–70-kHz region, as shown in the groundbreaking experiments of Frank *et al.* (1999) and in a recent model of OHC behavior (Weitzel *et al.*, 2003). The *in vivo* configuration is more complicated than the isolated hair cell configuration. The OHCs will, of course, become a component of the larger *in vivo* system which will possess its own dynamic characteristics. The details of some of the complicating factors (local mass, for example) are precisely what we may be interested in identifying.

B. Control experiments

We tested whether the measured velocity pattern is a result of a tracking problem between the BM vibration and the bead reflector (which is on the BM) (Nuttall and Dolan, 1996). The vibrations of 3- and 20-μm diameter beads were compared and no difference was found (3-μm bead data not shown). For one animal we successfully recorded BM vibration over the OHC without a reflective bead and also found a similar frequency response pattern, including the ''notch'' at 50 kHz (data not shown). The latter data support the earlier finding that beads can properly track BM motion (Cooper, 1999), although one other study concluded otherwise (Khanna *et al.*, 1998). The notch was a constant feature of the high-frequency portion of the spectrum for beads located over the rows of OHCs.

To demonstrate that the BM response is due to OHC electromotility, we applied sodium salicylate to the intracochlear fluids. Salicylate reduces OHC electromotility (Kakehata and Santos-Sacchi, 1996; Tunstall *et al.*, 1995). In Fig. 4, the perfusion of 20-μl of artificial perilymph containing 10-mM salicylate is shown to cause almost complete elimination of the electromotile response above 20 kHz. This effect was reversible. The BM velocity measurements shown in Fig. 4 are close to the tunnel region; hence, the response at 50 kHz has a peak consistent with Fig. 3. Salicylate reversibly reduces the high-frequency electromotility of the basilar membrane at concentrations that reversibly reduce isolated OHC motility. This implies that the OHCs are the main contributor to the high-frequency electromotility seen in this experiment.

While the data are consistent with OHC motility due to the presence of a basolateraltransmembrane protein, there may be a different mechanism at work. However, there is no experimental evidence to support significant electromotility in any of the other mammalian cochlear structures much above 1 kHz. Some limited electromotility at frequencies up to 20 kHz is evidenced in some cells, such as Chinese hamster ovary (CHO) cells (Ludwig *et al.*, 2001), although the force applied to an atomic force lever is 0.5 times that for prestin-transfected CHO cells, a factor of 10 less than for prestin-transfected human embryonic kidney cells, and there is a 180-deg phase difference between native and transfected

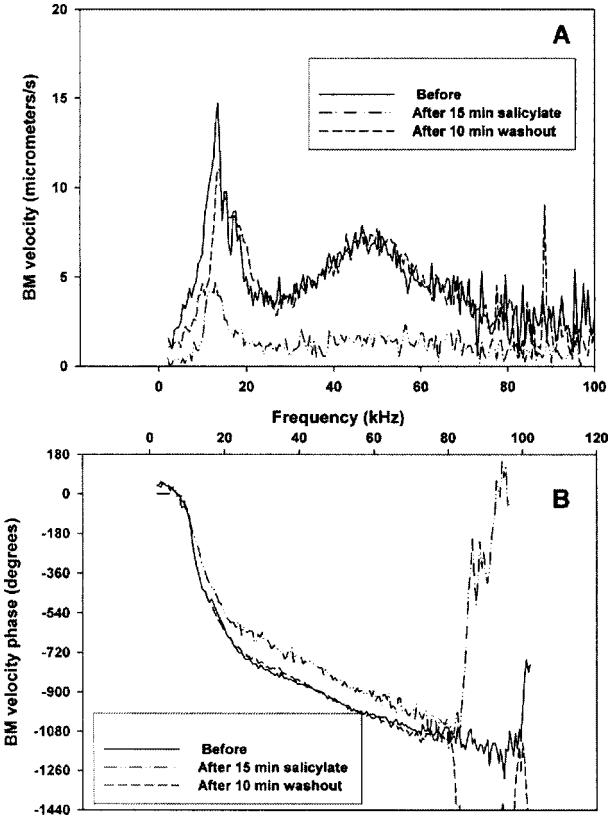

FIG. 4. BM velocity amplitude [panel (A)] and phase [panel (B)] before, during, and following washout of sodium salicylate from the cochlea (GP 1-06-03). Salicylate reversibly reduces the motility in response to electrical stimulation.

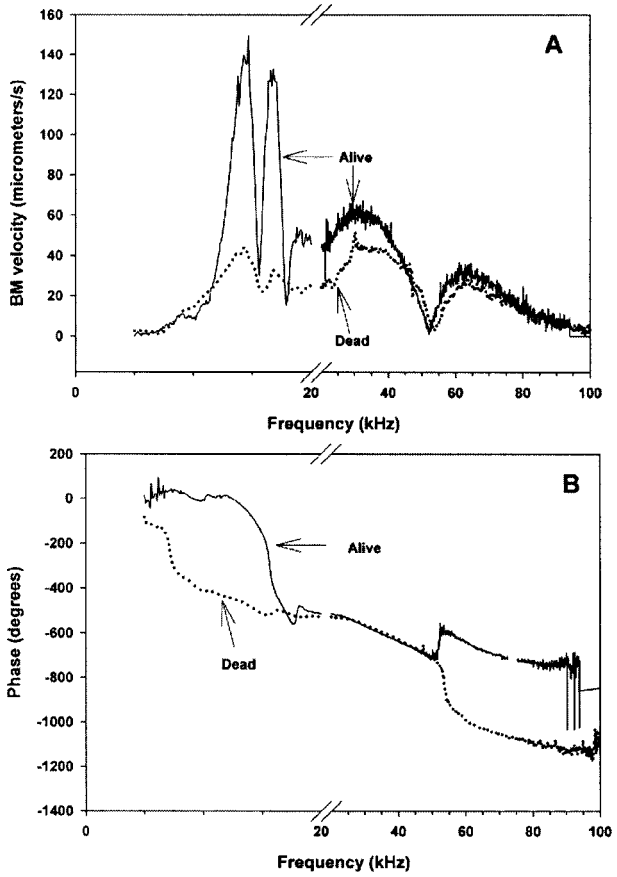

FIG. 5. Postmortem basilar-membrane velocity amplitude [panel (A)] and phase [panel (B)] compared to live data for the same animal (GP 483). Results are for local electrical bipolar stimulation. An expanded scale of 5 kHz per tick mark is used for frequencies below 20 kHz.

CHO cells. Another source of motility may come from the stereocilia. For instance, the hair bundles from the bullfrog sacculus evidence motility due to transepithelial electrical stimulation up to 100 Hz (Bozovic and Hudspeth, 2003). Calcium binding in a mechanically gated stereocilia channel has been postulated as a fast electromotile mechanism (possibly to the kHz range, e.g., Fettiplace *et al.*, 2001). Another possibility is that motility may arise from electrical stimulation of the lipid bilayer electric dipole in the cylindrical OHC (Petrov and Sachs, 2002), although this has not yet been confirmed in cochlear structures. OHC lipid motility (Petrov and Sachs, 2002) is partially blocked by salicylate. Note that spontaneous emissions at frequencies as high as 4 kHz from a gecko are suppressed by salicylate (Stewart and Hudpeth, 2000). Since there are no OHCs in the gecko, the implication is that salicylate also might inhibit hair-bundle motility. Therefore, we may be eliciting such a response from an unknown source in the cochlea via our electrical stimulations, effects that might be reduced by salicylate.

Electromotility of the cells is expected to be present postmortem as OHCs exhibit electromotility *in vitro*. Indeed, this is the case as shown in Fig. 5, where the BM velocity in response to bipolar stimulation is plotted before and immediately postmortem. For frequencies below the BF, the postmortem BM vibrations resemble the salicylate intoxicated frequency response. The reduction of BM velocity seen postmortem can be attributed to a disruption of the resting electrical and mechanical state of the cochlea by death. Because the endocochlear potential is nearly extinguished postmortem and hair cell active ionic pumps are nonfunctional, it is quite likely that the cell will be depolarized. Because of the change in polarization of the OHC, the mechanical state of the cell will be changed. The mechanical state characteristics include the OHC turgor pressure, the position of the stereocilia, and any tension/compression acting on the cell. Such an alteration of the transmembrane potential-to-mechanical length operating point of the OHCs could be postulated to either decrease or increase their overall effectiveness (or ''gain''). However, we argue that the *in vivo* resting configuration is somehow optimal for enhancing the response of the BM velocity in the normal physiological range (i.e., frequencies at and below BF). The response measured at this location for frequencies at and below BF involves, to some extent, all OHCs basal to this location. Hence, disrupting the *in vivo* operating condition is deleterious to global wave propagation and to the response near the BF, as seen in Fig. 5.

The *in vivo* and postmortem BM velocity response patterns above 40 kHz differ by only a small amount (Fig. 5). The notch at 50 kHz is retained in the postmortem velocity response. The smaller reduction is consistent with the view that very little energy propagates away from the locally excited OHCs at frequencies higher than BF in either the normal or postmortem case. Hence, this ultrasonic electromotility is less affected by alterations in the operating point of the

OHC as fewer OHCs are involved in this active process, which does not involve interaction with the traveling wave (and hence the basally located hair cells). The postmortem OHC evoked response depends on the slope of the motility versus voltage dependence. The postmortem OHC response might have shown an increase in electromotility (if moved to a more favorable point on the operating characteristic). However, we never saw an increase in the response postmortem.

IV. CONCLUSION

Our data show that sufficient force is produced *in vivo* to evoke vibrations at frequencies to 100 kHz and that a salicylate-sensitive process is involved. The dynamics of the fluid and mechanical environment surrounding the OHCs is likely modally rich at the ultrasonic frequencies measured in this study. Resonance structures, including a radially varying spatial dependence of the response and phase shift in the frequency response, are seen in the BM velocity. The ultrasonic response was found to show very little variability from animal to animal. Intracochlear bipolar electrical stimulation is a means of locally interrogating the *in vivo* OHC response, providing a complimentary alternative to *in vitro* preparations for determining electromotile processes and properties. In order to conclusively identify the physiological mechanism of electromotility in the cochlea, we expect that we will need to reconcile the results of *in vivo*, various types of *in vitro* experiments, and theoretical predictions.

Why perform high-frequency electrical stimulation? One goal is to identify the sources of electromotility in the cochlea. As of yet, only the somatic motility of the OHC has been demonstrated at these frequencies (Frank *et al.*, 1999). Other sources of electromotility are possible (such as the stereocilia), but in order for a convincing argument to be made for hair-bundle vibration as a main contributor, *in vitro* measurements on isolated cells showing high-frequency motility are needed. As mentioned by Fettiplace *et al.* (2001), there is no evidence *yet* of hair-bundle electromotility in mammalian OHCs. Once technical difficulties in such measurements are overcome, high-frequency bundle motility in mammalian OHCs might be seen. Arguing from the standpoint of conservation of genetic mechanisms, since activity is seen in lower vertebrates without OHCs, it might also be used by mammals in conjunction with somatic OHC motility. The argument for a combination of OHC somatic motility with some form of hair-bundle motility is still tenable. No matter the source of the electromotility, intracochlear electrical stimulation serves as a mechanically noninvasive way to excite the cochlea that is complimentary to acoustical excitation. The localized electrical stimulation is different spatially and temporally from acoustic stimulation directed through the ear canal. The frequencies of excitation are not limited by the best frequency at the measurement location, and the point of excitation is now localized along the BM. These high-frequency probes are used to examine high-frequency modal structures in the cochlea, which may be difficult to study using acoustical excitation (Nilsen and Russell, 2000) but might be important for cochlear sensitivity. Considering the response of the BM as a combination (albeit nonlinear) of forcing from the fluid pressure and active processes (e.g., from OHC forcing), bipolar electrical excitation is one means of teasing out the electrical and mechanical aspects of active processes. We note here that our bipolar stimulation is clearly different than electrical excitation that occurs normally in the cochlea. The intracochlear injection of current artificially fluctuates the local cochlear potentials. Finally, the local electrical excitation serves as an independent test of a cochlear model. For instance, one could fit a mathematical model to match standard acoustical excitation, then test the ability of the model to replicate electrically induced motion.

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