Published in final edited form as:

Hear Res. 2009 April ; 250(1-2): 63–75. doi:10.1016/j.heares.2009.02.001.

Displacements of the organ of Corti by gel injections into the cochlear apex

Alec N. Salt¹, Daniel J. Brown¹, Jared J. Hartsock¹, and Stefan K. Plontke²

¹Department of Otolaryngology, Washington University School of Medicine, St. Louis, MO, 63110, USA

² Department of Otorhinolaryngology, Head and Neck Surgery and Tübingen Hearing Research Center (THRC), University of Tübingen, D-72076, Germany

Abstract

In order to transduce sounds efficiently, the stereocilia of hair cells in the organ of Corti must be positioned optimally. Mechanical displacements, such as pressure differentials across the organ caused by endolymphatic hydrops, may impair sensitivity. Studying this phenomenon has been limited by the technical difficulty of inducing sustained displacements of stereocilia *in vivo*. We have found that small injections (0.5 to 2 μ L) of Healon gel into the cochlear apex of guinea pigs produced sustained changes of endocochlear potential (EP), summing potential (SP) and transducer operating point (OP) in a manner consistent with a mechanically-induced position change of the organ of Corti in the basal turn. Induced changes immediately recovered when injection ceased. In addition, effects of low-frequency bias tones on EP, SP and OP were enhanced during the injection of gel and remained hypersensitive after injection ceased. This is thought to result from the viscous gel mechanically limiting pressure shunting through the helicotrema. Cochlear microphonics measured as frequency was varied showed enhancement below 100 Hz but most notably in the sub-auditory range. Sensitivity to low-frequency biasing was also enhanced in animals with surgically-induced endolymphatic hydrops, suggesting that obstruction of the perilymphatic space by hydrops could contribute to the pathophysiology of this condition.

Keywords

transduction; operating point; cochlea; helicotrema

1. Introduction

While the mechanical and electrical responses of the organ of Corti to stimuli of acoustic frequencies are well documented, our understanding of how the static position of the organ of Corti is regulated is rather limited. If cochlear transduction were linear, the resting position of the organ of Corti would be inconsequential, as static displacements would have little influence on responses to stimulation. It is well established however, that cochlear outer hair cells (OHCs) exhibit saturating nonlinear transduction characteristics (Russell et al., 1986; Kros et al., 1992), which are reflected in the generation of otoacoustic emissions (Bian et al., 2002; Bian

Corresponding Author: Dr. Alec N. Salt, Department of Otolaryngology, Box 8115, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO, 63110, e-mail: salt@msnotes.wustl.edu, Phone: (314) - 362-7560, FAX: (314) - 362-1618.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 2004), distortions of cochlear microphonic (CM) waveforms (Patuzzi and Moleirinho, 1998), and the generation of SPs (Choi et al., 2004). The resting position of the transducer, or its status at zero crossings of an applied stimulus, termed the “operating point” (OP), has a marked influence on the transducer output. Displacements of OP strongly influence the generation of even-order distortions ($2f_1$, f_2-f_1), and to a lesser degree odd-order distortions ($3f_1$, $2f_1-f_2$; Frank and Kössl, 1996; Sirjani et al., 2004). Furthermore, it is commonly believed that displacements of OP are directly linked to changes in cochlear sensitivity (Patuzzi et al., 1984a, 1984b; Dallos, 1992). In order to maintain the optimal resting position of the organ of Corti for sensitivity to sounds in the auditory range the effects of infrasonic hydrostatic pressure fluctuations, such as respiratory- and cardiac-induced pulsations of cerebrospinal fluid pressure, must be minimized. This is predominantly achieved by pressure shunting between scala vestibuli and scala tympani through the helicotrema (Dallos, 1970). In addition, endolymph and perilymph pressures are maintained equal in the normal cochlea (Long and Morizono, 1987; Takeuchi et al., 1990) by highly compliant membranes bounding the endolymphatic space, (Wit et al., 2000). These mechanisms combine to reduce the influence of static and infrasonic pressures occurring between the endolymphatic space and scala tympani, i.e. across the organ of Corti, in the normal cochlea. Within the organ of Corti there are additional mechanisms that could allow the functional resting position of the transducer to be altered, such as mechanical contractions of the OHCs (Zenner, 1986), or adaptive processes at the tip links on hair cell stereocilia that are present in non-mammalian hair cells (Fettiplace and Ricci, 2003) but are apparently not prominent in the mammal (Kros et al., 1992).

To study the auto-regulation properties of OHCs that might effect transduction, previous studies have performed *in vitro* experiments in which hair cell stereocilia were mechanically displaced while the magnitude and time constants of adaptive processes are monitored (Assad and Corey, 1992; Eatock, 2000). In the intact cochlea, some investigators have used endolymphatic injections in an attempt to manipulate transducer position (Sirjani et al., 2004; Valk et al., 2006). However, due to the highly compliant membranes bounding the endolymphatic space, the pressure changes occurring during such injections result in only small pressure differentials between endolymph and perilymph (Wit et al., 2000). As a result, induced OP changes by volume injections into scala media of the normal cochlea are unstable and rapidly return to the pre-injection state within a matter of minutes (Sirjani et al., 2004).

Larger pressure differentials between endolymph and perilymph have been observed in some animals with chronically induced endolymphatic hydrops (Böhmer, 1993). However, the correlation of organ of Corti displacement with hearing sensitivity changes in the hydropic ear is confounded by secondary changes that occur, including EP changes (Cohen and Morizono, 1984; Salt et al., 1995), endolymph Ca^{++} changes (Ninoyu and Meyer zum Gottesberge, 1986; Salt and DeMott, 1994) and morphologic changes in stereocilia (Rydmarker and Horner, 1991; Horner, 1992; Dunnebier et al., 2001) whose effects may exceed the mechanically-induced changes. In this respect, chronically-induced endolymphatic hydrops is a poor model of mechanically-induced change in function.

Only one prior study has reported the effects of plugging the helicotrema (Konishi and Nielson, 1978). They used soft bone wax to fill the helicotrema and closed the defect in the bony otic capsule with a glass cover slip. They demonstrated that after the helicotrema was occluded, mechanically-driven displacements of the round window (RW) membrane produced sustained voltage changes in the scalae in accordance with the direction of basilar membrane displacement. They also reported that while a minority of afferent fibers increased firing rates with displacements of the basilar membrane towards scala vestibuli, the majority increased firing rate with displacements towards scala tympani, when EP was elevated.

In the present study, a novel method has been used to manipulate the organ of Corti position and helicotrema properties mechanically. A small volume (0.5 to 2 μL) of viscous hyaluronate gel was injected at precisely controlled rates from a glass pipette sealed into the bony otic capsule at the apex. Functional changes were monitored during injections of gel, and during withdrawals after sufficient volume of gel had been injected to plug the helicotrema.

2. Methods

2.1 Animal Preparation

Experiments used 20 NIH strain pigmented guinea pigs of both sexes. Animals were anesthetized with an initial dose of 100 mg/kg sodium thiobarbital (Inactin, Sigma, Saint Louis) and deep anesthesia was maintained by periodic supplements given via an intravenous cannula in the external jugular vein. Animals were artificially ventilated through a tracheal cannula, with end-tidal CO_2 maintained near 38 mm Hg (5%). Heart rate and vascular pO_2 were monitored with a pulse-oximeter (Surgivet, Waukesha, WI). Rectal temperature was maintained at 38 °C with a DC-powered, thermistor-controlled heating blanket. Animals were mounted in a head-holder and the auditory bulla was exposed by a ventral approach, allowing access to the cochlea. The external ear canal was transected to allow placement of a hollow earbar, through which acoustic signals were delivered. Prior to acoustic and electrical recordings, pancuronium bromide (2 mg/mL) was given as a muscle relaxant to reduce myogenic artifacts and to eliminate middle ear muscle contractions.

The experimental protocols for this study were approved by the Animal Studies Committee of Washington University (Protocols 20040209 and 20070147).

2.2 Gel Injections into the Cochlear Apex

Small volumes (0.5 to 2 μL) of 1% sodium hyaluronate gel (Healon, Advanced Medical Optics, Santa Ana, CA) were injected into scala vestibuli at the cochlear apex via a glass pipette sealed into the bony scala. The pipette was a glass electrode, broken to an outer diameter of 20 – 30 μm that was filled with Healon gel. The pipette was mounted on a 10 μL micro-syringe with a MPH6S10 pipette holder (World Precision Instruments, Sarasota, FL). The pipette holder was glued onto the glass micro-syringe with cyanoacrylate adhesive to ensure that no leak could occur at this junction. The syringe and pipette holder were both filled with artificial perilymph, although the volume of the injection pipette ensured that only Healon was injected into the cochlea. The syringe and pipette were mounted onto a WPI Ultrapump unit, which allowed injections and withdrawals at rates from 20 to 250 nL/min. The pump system was mounted on a micromanipulator on a magnetic base to permit accurate positioning of the pipette tip.

Prior to placing the injection pipette, the mucosa covering the cochlea at the apex was removed and the bone dried with absorbent tissue. A layer of thin cyanoacrylate glue was spread over the apex, followed by a thin layer of Kwik-Cast two-part silicone adhesive (World Precision Instruments, Sarasota, FL) to provide a hydrophobic coating on the bone. A small, 30 – 50 μm diameter perforation was made through the adhesive and bone at the apex and the pipette tip was inserted. Fluid emerging from the apex formed a bead on the silicone surface, which was removed with a wick just before applying a droplet of cyanoacrylate adhesive to seal the electrode in place. This technique allowed the pipette to be sealed into the cochlea without any perilymph leakage following the procedure.

The likely mechanical influence of gel injection into scala vestibuli at the apex is shown schematically in Figure 1. The 3D representation of the cochlear apex shows that the helicotrema simply represents the opening from scala tympani into scala vestibuli that occurs

near the middle of the cochlear spiral. Scala tympani is narrow throughout the apical turns of the guinea pig and the reconstruction shows that there is no specific narrowing of the fluid space in the region of the helicotrema. An even better appreciation of helicotrema anatomy is provided by movies showing “fly-throughs” past the structures, available on our website at <http://oto.wustl.edu/cochlea/>. When non-viscous fluids are injected from pipettes sealed into the apex, they displace perilymph, which exits the cochlea through the cochlear aqueduct at the base of scala tympani (Salt et al., 2006). As gel is injected, we expect the narrow ST to become filled with gel, resulting in increased pressure in both scala vestibuli and the endolymphatic space (by pushing on Reissner's membrane), as shown in Figure 1. As the injected volumes (0.5 to 2 μL) were far lower than the cochlear perilymph volume of the guinea pig, reported to be 8.6 – 8.8 μL (Thorne et al., 1999; Shinomori et al., 2001), the injected gel will remain localized in the apical turns. Since the capacity for fluid outflow through the endolymphatic duct in response to static pressure increase in the vestibule is limited by the membrane of the endolymphatic sinus (Salt and Rask-Anderson, 2004), a small static pressure between the endolymph and ST will be induced, potentially producing a sustained displacement of the organ of Corti throughout the cochlea.

2.3 EP recording

EP was recorded in the basal turn using glass pipettes, beveled to an external tip diameter of 3–5 μm and filled with 500 mM KCl. They were connected through teflon-coated Ag wires, chlorided at the tip, to a high-impedance electrometer with 10X gain. The ground connection was made through an Ag/AgCl sintered pellet cell (RC1- World Precision Instruments, Sarasota, FL) connected to the muscles of the animal's neck with a 3 cm long electrolyte-filled salt bridge. Endolymph was accessed through the bony wall of the mid-basal turn by first thinning the bone with a flap knife (Mueller AU13400) and then making a 30–50 μm fenestra with a 30 degree pick (Storz N1705 80). Both the DC voltage (EP) and sound-evoked cochlear responses were recorded from the pipette. EP measurements were not corrected for liquid junction potential changes between perilymph and endolymph. In some experiments, similar pipettes filled with 500 mM NaCl were inserted into perilymph of the mid-basal turn of scala tympani.

2.4 Acoustic Stimulus Generation, Calibration and Acoustic Emissions Recording

Tucker-Davis (TD) system-3 hardware was used to generate and control acoustic stimuli under the control of a custom-written Visual Basic (Microsoft) program. Three channels of sinusoidal stimuli were generated by two TD-RP2 modules, with each channel passed through a TD-PA5 attenuator to control level, before being amplified by headphone amplifier (TD-HB7). Two channels of probe stimuli (f_1 , f_2) were delivered to the ear canal by a modified Etymotic ER10C system coupled to the hollow earbar. A third channel of very low frequency (4.8 Hz) sound, used for acoustic biasing, was delivered with a Sennheiser HD 580 or HD 265 driver coupled to the ear bar. All sound stimuli were delivered and acoustic emissions were recorded within a closed system. The microphone within the ER10C system was used both for recording acoustic emissions and for the calibration of stimuli. In each animal, calibration curves were determined for all 3 transducers by tracking the attenuation required to generate a stimulus level of 70 dB SPL in the external canal as frequency was varied in $\frac{1}{4}$ octave steps. Calibration tables were generated, taking into account the frequency response of the microphone. Based on the calibration data, all acoustic stimuli were delivered on a dB SPL basis.

2.5 Cochlear Potentials Recording

Cochlear potentials were recorded from an Ag/AgCl ball electrode placed on the bone at the margin of the RW membrane or from the KCl-filled glass pipettes inserted into scala media and used to record EP. Signals from the RW electrode were recorded differentially with respect

to a needle electrode at the vertex using a TD HB4, optically-coupled amplifier with a gain of 1000 $\times$ and high-pass filtered at 5 Hz. Cochlear potentials and otoacoustic emissions (OAE) were collected simultaneously using up to 4 input channels of the TD-RP2 modules, sampling at 24 or 48 kHz. In addition, two channels of data were also sampled at 44 kHz and streamed to disk using a PC equipped with an internal sound card. Time-averaged waveforms were collected and analyzed as follows:

2.5.1 AP thresholds—Cochlear compound action potential (AP) waveforms were averaged in response to 10 positive-onset and 10 negative-onset, 12 ms duration tone-burst stimuli with 0.5 ms rise fall. AP detection threshold was an amplitude criterion of 10 μ V representing a positive response. At each frequency tested, stimulus levels were increased in 5 dB steps until a positive response was obtained, and then decreased in 5 dB steps until the response was below the criterion. Threshold was established by interpolation between above- and below-threshold responses. AP thresholds were either established in $\frac{1}{4}$ octave steps from 1 kHz to 22 kHz or were repeatedly measured as a function of time at specific frequencies.

2.5.2 CM/SP/AP to high frequency tones—Two averaged waveforms were recorded separately to equal numbers of positive- and negative-onset, 8 kHz tone-bursts, summing the waveforms to cancel CM and produce the AP/SP waveforms, and subtracting the waveforms to cancel CM/SP and produce a CM waveform. Changes in amplitude of the response components with time were followed by making repeated measurements.

2.5.3 CM and OAE responses to low-frequency tones—CM responses and microphone recordings to prolonged tones were averaged with data collection commencing 5 s after tone onset to avoid onset phenomena (Lukashkin and Russell, 2002). Prior to each epoch collection, the tone was ramped down, the phase reset, and then ramped up over 8 ms with data collection starting 30 ms afterwards. This allowed exact alignment of time epochs in the average, while minimizing acoustic transients caused by resetting phase. Typically, 10 phase-locked, 4096-point, 168 ms responses were averaged.

2.6 Spectra

Spectra of averaged CM waveforms or OAE were calculated using National Instruments routines. In each case, three individually-obtained spectra were averaged for each measurement. Probe frequencies were set to include an integer number of cycles in the time window, allowing rectangular windowing to be used. Although OAE measurements were routinely collected simultaneously with CM recordings, no OAE data are presented in this study.

2.7 Boltzmann Analysis

A Boltzmann analysis of the CM waveform was performed using a 21 ms segment of the averaged time waveform. The waveform was transferred online to an Excel spreadsheet and the “Solver” capability used to fit the CM with a waveform synthesized from a Boltzmann transducer curve relating voltage output to applied pressure input (Kirk et al., 1997; Sirjani et al, 2004). The variables fitted in the procedure included: P_{sat} : the saturation voltage of the curve; Slope: the steepness of the transducer function at zero input pressure; OP: the operating point, which represents the position on the transducer curve at the zero crossings of the probe stimulus.

2.8 Low-Frequency Biased CM

The biasing protocol utilized a 500 Hz probe tone in conjunction with a 4.8 Hz bias tone. The 208 ms period of the bias tone allowed 8 independent, equally-spaced, 21 ms collections of

the 500 Hz response to be performed during each bias cycle, as detailed elsewhere (Brown et al., 2009). Probe tone frequency was adjusted to ensure an integer number of cycles in each 512 point collection buffer. As the buffers were too short to obtain sharp spectra, four independent data collections were made (32 buffers total) the four waveforms for each of the eight points on the bias were concatenated, producing eight 2048 point buffers. Signals were high-pass filtered with the cutoff set at 5 Hz for RW measurements and 100 Hz for SM or ST measurements. Each of these eight independent buffers were subjected to spectral and Boltzmann analysis.

3. Results

3.1 Physiologic Changes Measured During Gel Injections

The morphology of the CM waveform recorded from the basal turn of the cochlea was markedly changed by gel injection into the apex. Figure 2 (left column) shows CM waveforms measured repeatedly in response to a 500 Hz, 90 dB SPL probe tone during a 10 min injection of 1 μ L Healon into the apex at 100 nL/min. During the injection (waveforms D, E, F) CM waveforms became highly asymmetric with a more pointed, positive-going component and a flattened, negative-going component. The CM waveform returned to near pre-injection morphology within minutes of the injection stopping. The waveform shape changes can be interpreted as the output of a non-linear transducer with Boltzmann characteristics, in response to a sinusoidal stimulus with a variable displacement added during the injection (Figure 2, right column). In each Lissajous figure in the right panel, the same CM waveform data (gray) are plotted against an input sinusoid added to an offset pressure. The magnitude of the offset is equivalent to the OP measure described previously. The thin black lines represent Boltzmann transducer curves with fixed P_{sat} and slope parameters. In each case OP, indicated by the open circles, was established by fitting the CM to the transducer curve with a least-squares procedure. The shape and amplitude changes of CM are largely explained by a displacement being added to the input stimulus, forcing the output to be generated by the sinusoidal stimulus operating over a different region of the transducer curve. The displacement caused by injection was equivalent to a positive pressure in the ear canal of approximately 1 Pa, which is comparable to the peak pressure of the 90 dB SPL probe stimulus.

A number of physiological changes quantified during a 10 min Healon gel injection at 100 nL/min are summarized in Figure 3. In this experiment, OP was derived from CM recorded simultaneously at 3 locations; from glass electrodes inserted into SM and ST of the mid basal turn and from the RW wire electrode. In each case, after a brief delay of around 3.5 minutes at the onset of Healon injection, OP was displaced in a positive direction, equivalent to positive pressure in the ear canal and indicating a displacement the organ of Corti towards ST. OP quickly recovered to the pre-injection state when injection stopped. Second harmonic distortion ($2f_1$) in the CM also showed a substantial increase, corresponding to OP moving further away from zero (Frank and Kössl, 1996; Sirjani et al., 2004; Brown et al., 2009), and then quickly returned to pre-injection levels when the injection stopped. EP was also changed with a similar time course, increasing by over 10 mV during the injection. Although an increase of EP is qualitatively consistent with a sustained displacement of the organ of Corti towards ST, reducing the conductance of transducer channels, the magnitude of this change was surprisingly large. It is quite unusual for EP to reach a value close to 100 mV in the mid-basal turn of the guinea pig. The SP, measured from the RW in response to an 8 kHz, 80 dB SPL probe tone, also showed a marked increase, becoming more positive during the injection while the amplitudes of CM and CAP responses to the fixed 8 kHz tone decreased slightly.

The effects of two Healon injections at a lower rate of 50 nL/min are summarized in Figure 4. Again, the injections caused sustained increases of OP, $2f_1$, EP and SP, consistent with sustained displacements of the organ of Corti towards ST. In this experiment, CAP thresholds

were also monitored during the injections and were elevated by 20 – 30 dB for both high- (16 kHz) and low-frequency (2 kHz) stimuli during the injection and recovered immediately afterwards. A gel withdrawal was also performed in this experiment, which produced transient, negatively-directed changes of OP, EP, and SP, and an increase in $2f_1$, as OP again moved away from zero. The transient nature of the response to withdrawal could have been due either to pipette blockage or possibly due to the apical region of ST becoming “unplugged” as gel was withdrawn. Only low-frequency thresholds were elevated during the withdrawal procedure.

In the above experiments, the common practice of high-pass filtering the sound evoked potentials removed the DC components from the CM waveforms. In order to look more closely at the relationship between gel-induced transducer displacement and the EP, we made DC-coupled CM recordings from the scala media electrode (containing both EP and CM components), an example of which is shown in Figure 5. These CM data only differ from those presented previously by presence of components below 5 Hz, that includes the DC level. The DC-recorded CM data are shown as Lissajous plots with the x-axis representing the sinusoidal input pressure added to a fitted OP (pressure offset). Before injection, OP in this animal was -0.01 Pa and the voltage at this point was 90.4 mV, which was close to the EP measured in quiet of 89.7 mV. The CM had a pk/pk amplitude of 7.95 mV with a maximum voltage during positive pressure excursions of 94.4 mV. The Boltzmann function fitted to these data had a P_{sat} of 4.3 mV so the theoretical maximum voltage for a large positive input pressure would be 94.7 mV. The CM data recorded during a 10 min gel injection into the apex at 100 nL/min indicate that OP was displaced by 0.38 Pa, which would account for only 2.2 mV of the EP increase. In fact, during injection the voltage at the OP was 99.8 mV and the EP in quiet was 100.9 mV, suggesting there was an additional 7.3 mV increase in the EP that was not due to the OP moving positively on the Boltzmann curve. As the CM data show, the entire transducer curve appears to be shifted positive by 7.3 mV during the injection. Prior to 13 gel injections into the apex, the mean EP measured in quiet was 88.6 mV (SD 4.6 mV). The mean EP increases caused by gel injections were 8.1 mV (SD 1.9 mV, $n=5$) at 50 nL/min, 12.4 mV (SD 2.0 mV, $n=4$) at 100 nL/min and 14.3 mV (SD 1.2 mV, $n=4$) at 200 nL/min. Both the OP shift and the DC shift in the Lissajous plots were sustained throughout the injection period and both recovered fully afterwards, confirming that the additional DC component does not arise from artifacts such as electrode drift. The EP increases during injection are therefore demonstrated to be considerably larger than can be explained by transducer-generated voltage changes.

The dependence of induced OP changes on the rate of Healon gel injection, and lack of OP changes during control injections of artificial perilymph are summarized in Figure 6. Different recording locations are indicated by different symbols. For injections, (positive injection rates) OP change was related to the injection rate, although a line fitted to the injection data had an R^2 of only 0.26. Control injections with artificial perilymph, rather than gel, produced little or no OP changes, as indicated by open symbols and were significantly different from gel injections at 50 ($p=0.03$) and 100 ($p=0.006$) nL/min injection rates (Mann Whitney Ranked Sum tests). Withdrawals performed after injection produced negatively-directed OP changes. The absence of withdrawal-induced OP changes in some animals could be due to the pipette tip plugging of the apex being insufficiently filled with gel.

3.2 Physiologic Changes Measured Following Gel Injections

Although most parameters (OP, $2f_1$, EP, SP, AP thresholds) quickly returned to near pre-injection values after gel injections, the enhanced modulation of OP by low-frequency biasing remained. Figure 7 shows an experiment in which non-biased CM measurements (upper panel) were made together with low-frequency biased CM measurements (lower 2 panels) during a 0.5 μ L Healon injection at 50 nL/min. The time interval between measurements in this

experiment is longer due to the additional time required to collect low-frequency biased data. For the biased data (middle panel) each time point shows 8 OP measurements taken at equally spaced intervals during a cycle of the 4.8 Hz, 110 dB SPL bias tone. The degree to which OP was modulated by the bias (maximum OP – minimum OP for the 8 points: OP_{mod}) is shown in the lower panel. OP derived from the non-biased CM waveform increased at the start of gel injection (Figure 7, upper panel), and at the same time the OP modulation induced by low-frequency biasing was increased almost 10-fold (Figure 7, lower 2 panels). Not only was OP_{mod} elevated during gel injection, but it remained elevated when the injection ceased, even as the non-biased OP returned to the pre-baseline state.

The sustained increase in sensitivity to the bias tone was also apparent in bias-modulated CM waveforms measured before and after a 0.5 μ L Healon injection, as shown in Figure 8. The eight waveforms were recorded at equally-spaced intervals throughout the bias cycle, as indicated by the stated bias time. Before injection, the CM waveform changes induced by the bias were subtle and could be accounted for by a small modulation of OP. Panel B of the figure shows the same CM data shown in panel A (Y-axis), plotted against a sinusoid (X-axis), displaced along the pressure axis (X-axis) to obtain the best fit to a Boltzmann transducer curve. The open circles show the derived OP value, which was modulated during the bias cycle, as indicated by the dotted lines joining the symbols. After Healon injection, the bias-induced changes of CM waveshape were more dramatic and were clearly visible in the waveform plotted on a time axis (Figure 8C). When plotted against the input sinusoid (Figure 8D), much larger OP shifts were required to account for the CM waveform changes. It is also apparent that after the Healon injection, the bias-induced modulation of the OP shifted in phase by approximately 90°.

Bias-induced changes measured before and after a 0.5 μ L Healon injection (from the same experiment as shown in Figure 8) are quantified in Figure 9. The sinusoidal modulation of OP by the bias (Figure 9A) increased in amplitude and changed in phase by approximately 90° after Healon injection. The bias-induced modulations of the second harmonic, $2f_1$ (Figure 9B) and the third harmonic, $3f_1$ (Figure 9C) were altered both in amplitude and in characteristics after Healon injection. These changes were explained by the known relationship between the OP and distortion generation by a Boltzmann transducer, as shown in Figure 9F. Prior to the injection the baseline OP in this preparation was slightly positive and the bias-induced displacements were not sufficient to cause OP to cross zero (Figure 9A and 9F, open symbols). As a result, distortions exhibited a sinusoidal modulation. Following Healon injections, the OP changes induced by the bias were much larger and pushed OP further to positive and negative values. Because $2f_1$ is increased and $3f_1$ is reduced as OP moves further from zero (Figure 9F), high-amplitude low-frequency biasing resulted in a bimodal modulation of the distortion product amplitudes. The EP change (Figure 9D) induced by the bias tone alone (in the absence of a probe stimulus) was essentially a low-frequency CM in response to the 4.5 Hz bias signal, and was consistent with an increase in bias-induced displacement of the organ of Corti after gel injection. Similarly, the changes of SP evoked by an 8 kHz, 80 dB SPL tone-burst (Figure 9E), in which SP changed polarity at some points in the bias cycle, were also consistent with a greater influence of the bias after Healon injection. The apparent phase differences between EP, OP and SP with respect to the bias were due to the different high-pass filtering requirements for each of the measures. Nevertheless, the 90° change in phase of the bias effect on each measurement *after* Healon injection was a systematic physiological effect of helicotrema blockage.

In a number of experiments, the dependency of OP_{mod} on the level of the bias tone was measured before and after gel injections. Figure 10 shows the average relationship between OP_{mod} and bias level measured before injection (thick black line, $n=10$, error bars=SD) and for six individual experiments after varying amounts of Healon were injected. Healon

injections into the apex increased OP_{mod} for all levels of the bias. Before Healon injection, bias levels of 80 dB and lower resulted in small OP_{mod} values, representing a noise floor at which the bias effects were below the typical variation of OP. Following Healon injections, bias levels as low as 60 dB SPL produced OP_{mod} levels that were greater than the noise floor of OP_{mod} measurements, indicating an elevated sensitivity to the 4.8 Hz bias stimulus. Control injections of artificial perilymph (open symbols) had no influence on the sensitivity of OP_{mod} to the bias. In all experiments, Healon injection increased the sensitivity of OP_{mod} to bias tones in a volume-dependent manner, with the sensitivity increasing by as much as 30 dB. The OP_{mod} data shown were derived from CM measurements made from SM. Almost identical results were obtained from recordings made simultaneously from the RW (data not shown).

The frequency-dependence of the sensitivity changes induced by Healon injections was assessed by determining the stimulus threshold level required to elicit a CM amplitude of 500 μ V. CM thresholds were only accepted if they were at least 10 dB above the noise floor, measured by an identical data collection without the stimulus. Figure 11 shows CM thresholds measured as stimulus frequency was varied from 4 Hz to 4 kHz and AP thresholds from 1 kHz to 22 kHz. Both CM and AP thresholds were measured before and after artificial perilymph injection as a control (upper row) or after one or two Healon injections (middle and bottom rows) totaling 1 or 2 μ L. Control injections of 1 μ L artificial perilymph into the apex did not alter CM or AP thresholds. In contrast, injections of Healon markedly increased the sensitivity to very low frequency tones. For 1 μ L, injections, the CM sensitivity changes occurred with minimal changes of AP sensitivity to high-frequency tones. After 2 μ L of Healon was injected, larger AP threshold elevations occurred.

3.4 Bias-induced Operating Point Changes in Animals with Endolymphatic Hydrops

As part of a prior study (Salt et al., 2005), low-frequency biased CM waveforms were collected in a group of control animals and in a limited number of animals with chronic endolymphatic hydrops induced by surgical ablation of the endolymphatic sac and duct. The OP_{mod} recordings were made from the RW membrane using exactly the same protocol as in the present study. The dependence of OP_{mod} on bias level in this data set is summarized in Figure 12. The control animals in this group ($n=22$) did not differ appreciably from those presented in Figure 9. OP_{mod} was found to be markedly elevated in the 3 animals with endolymphatic hydrops, an observation that was mentioned in the prior publication but the data were not included as its significance was not appreciated at the time. The enhanced effect of 4.8 Hz bias tones in hydropic animals may be accounted for by a restriction of communication through the perilymphatic spaces at the apex in the hydropic cochlea. Figure 13 shows mid modiolar sections through the apical turns of a normal cochlea (left column) and two hydropic cochleae (mid and right columns) with the fluids spaces highlighted in the lower row. These specimens are from different animals from those in which OP_{mod} was recorded, but they demonstrate that the perilymphatic spaces near the apex (shown orange) become occluded to a degree that the cross-sectional area of scala vestibuli may be smaller than that of scala tympani. In the specimen with more severe hydrops (right column), Reissner's membrane appears to occlude the helicotrema. A restriction of perilymph communication between scala vestibuli and scala tympani would be expected to increase mechanical sensitivity to low frequency sound in a manner comparable to that shown for gel injections into the apex.

4. Discussion

4.1 Sustained Displacements of the Organ of Corti

The sustained changes of OP, EP and SP measured in the basal cochlear turn during Healon gel injections into the apex are consistent with an induced local displacement of the organ of Corti in the basal turn. The displacement is presumed to result from a small pressure differential

across the organ. This represents the first *in vivo* demonstration that the OP can be displaced towards scala tympani for as long as 20 minutes, producing sustained changes in cochlear potentials, which then rapidly recover with minimal temporary or permanent changes in cochlear function, other than the hypersensitivity to infrasound caused by the helicotrema blockage. A Boltzmann analysis of the cochlear microphonics (Patuzzi and Moleirinho, 1998) demonstrates that many of the physiologic changes (including changes in CM waveform morphology and a portion of the EP increase) result from a displacement-induced partial closure of transducer channels, reducing current through the hair cells. The magnitude of the mechanical displacements, as quantified by OP changes, was partially dependent on the rate of gel injection, although animals differed in their sensitivity to injections. Induced changes were found to recover quickly when injections ceased with no indication of sustained trauma caused by the procedure. Oppositely-directed changes could be produced via gel withdrawals performed after an initial gel injection. Injection-induced changes of the CM waveform, specifically substantial increases of $2f_1$ and $3f_1$ distortion components, were accounted for by displacement of the transducer away from the mid-point state. This highlights the importance of the resting position of the organ or Corti in the generation of distortion products. AP thresholds to high-frequency stimuli were elevated by approximately 30 dB as OP was displaced by injection, demonstrating OP displacements as a potential contributing factor in the cause of transient or permanent hearing impairments caused by endolymphatic hydrops or other pathologies. It is presumed that the reduction in AP thresholds was due to the displacement of the OP towards scala tympani, away from the optimal sensitivity position at the middle of the transducer, and reducing the active amplification process of the OHCs (Kirk and Patuzzi, 1997; Kirk et al., 1997). This is supported by the fact that AP threshold changes fully recovered immediately following gel injections, when the OP had returned to its pre-injection position. These findings demonstrate that gel injections into the apex may provide a tool to help understand the relationships between the resting position of the organ of Corti and cochlear function *in vivo*. In prior studies, basilar membrane position shifts were induced in an *in vitro* cochlear preparation by varying perfusion pressures, causing changes of CM waveforms and harmonics (Fridberger et al., 1997). However, the EP in this preparation was unavoidably low, around 8 mV, making it difficult to compare with the normal cochlea. Our current preparation offers several advantages, allowing similar manipulations to be performed in a normal cochlea, with normal hearing sensitivity and in a fully reversible manner so that the findings are more readily interpreted. Sustained displacements of the OP and EP, with some slight initial adaptation, have also been achieved by applying a constant force to the outer wall of the cochlea *in vivo* (Zou et al., 2006). This manipulation typically resulted in movements of the OP towards scala vestibuli and a reduction of EP. Furthermore, previous measurements of basilar membrane displacement during the presentation of high-level (>90 dB SPL) tones have demonstrated a net displacement of the basilar membrane position during tonal stimulation (LePage, 1987; Cooper and Rhode, 1992), which can also be seen as a displacement of the OP derived from CM as a function of stimulus level (Patuzzi and Moleirinho, 1998). It remains controversial, however, whether or not high-levels of acoustical stimulation induce a net displacement of the basilar membrane. With high level, low frequency stimulation, position changes may be a mechanical consequence of the transient endolymphatic hydrops that such stimulation produces (Salt, 2004).

An important finding demonstrated by the study related to the quantitative relationship between transducer displacement and the EP, in which the EP increase caused by gel injection was substantially greater than could be accounted for by the OP change when the transducer properties were considered. The source of this substantial voltage increase remains uncertain. Other than a change in the mechano-electric transducer (MET) channel conductance as the gel injection displaced the organ of Corti, the EP might have been increased by either i) an increase in the stria current in response to the gel injection via some yet unexplained mechanism, ii) an increase in the electrical resistance at one of the other electrical resistance boundaries of

scala media, which suggests there is another pressure transducer in the cochlea not directly linked to the non-linear, acoustically stimulated MET channels, or iii) an increase in the electrical resistance at the basolateral wall of the outer hair cells, which might occur in response to the initial hyperpolarization of the cell due to the increased resistance of the MET channels during the gel injection (Patuzzi and Moleirinho, 1998; O'Beirne and Patuzzi, 2007). Whatever the mechanism underlying the relatively large EP increase during gel injections, it indicates that EP is not solely determined by MET channel conductance and suggests an additional process may be active in EP regulation.

The observation that OP, EP and SP changes were sustained throughout gel injections of 10 – 40 min in duration might indicate that stereociliary adaptation, comparable to that seen in vestibular hair cells (Eatock, 2000) and accounted for by a tensioning of tip links, is not active in the mammalian cochlea. However, such adaptation might still occur if it were to a partial extent that did not fully compensate the changes induced by the gel injections. If such adaptation exists it must have a time course shorter than our measurements could resolve, and most likely shorter than the development of the pressure difference across the organ of Corti induced at the onset of the gel injection. Further studies on this issue are ongoing, using step pressure pulses applied to the external canal after the helicotrema has been blocked by gel, to clarify the degree and rate of adaptation. Preliminary data show that OP changes monitored with high time resolution show sustained changes during acoustic pressure pulses after the helicotrema is occluded with gel, in contrast to the transient responses observed before the helicotrema is obstructed. It is also notable that the EP increase produced by gel injection showed no indication of adaptation back towards the normal level, suggesting that ion transport processes in the lateral wall are not modulated by the demands of the transducer system over the time scale studied here.

Gel injection induced EP changes in the present study that were larger than those found in prior studies in which EP was recorded during fluid injections into the endolymphatic spaces (Takeuchi et al, 1990; Salt and DeMott, 1997; Sirjani et al, 2004) or into the perilymphatic spaces (Salt and DeMott, 1998). This suggests that the organ of Corti was displaced relatively little towards ST by simple fluid injection methods. Previous studies of pressure and flow measurements during endolymphatic injections into the cochlea have demonstrated that the injected fluid rapidly flows basally toward the sacculus, which is more compliant than the cochlea, so that any endolymph-perilymph pressure differential in the cochlea is rapidly dissipated (Wit et al., 2000; Salt and DeMott, 1997). In prior studies, fluid injections into perilymph in the absence of an outlet resulted in relatively small EP changes (compared to the changes induced by Healon injections into the present study), as pressure increases were quickly balanced between the perilymphatic scalae (Salt and DeMott, 1998). The control injections in the present study confirm the general finding that perilymphatic injections do not typically displace the organ of Corti. It is therefore apparent that Healon injections causing helicotrema blockage provide a better tool to produce sustained displacements of the organ of Corti *in vivo* than do fluid injections into the endolymphatic or perilymphatic spaces.

The precise fluid mechanics of how gel injections into the apex of the cochlea manifests as a pressure differential across the cochlear partition in the base of the cochlea requires consideration of the fluid space dimensions, boundary compliances and the location of fluid outlets. Injections into the apex of fluid containing a marker that was detected by ion-selective electrodes have shown that the primary outlet for fluid efflux is the cochlear aqueduct (Salt et al., 2006). The narrow perilymphatic spaces at the apical end of scala tympani would impede the flow of gel towards the cochlear aqueduct, resulting in a pressure increase in the apical region of scala vestibuli. It is unlikely that endolymph is “squeezed” basally from the apex, producing a local endolymphatic hydrops at the base, because this would require an equal displacement of perilymph volume out from scala vestibuli, which is bound by the bony walls

and which would also be pressurized by the gel injection. Instead, the gel injections probably increase the hydrostatic pressure in scala vestibuli and scala media equally, with minimal fluid flow or pressure leak in either scala vestibuli or scala media because the primary pressure outlet in the cochlea is the cochlear aqueduct.

In the initial gel injection of each animal there was typically a 2-3 minute delay before OP and EP changes were induced. This was consistent with the time necessary to plug the apical region of scala tympani, after which the fluid pressure differential across the organ of Corti would be altered substantially more by further gel injection. Subsequent injections produced almost immediate changes in each of the recorded potentials. Physiological changes of OP and EP occurred at the start of injections when only nanoliter volumes of gel had been injected. The effects of such small volumes of gel applied to the apex on function in the basal turn are best explained by a mechanical disturbance of the organ of Corti, as opposed to a pharmacologic effect of gel on the basal turn, as insufficient volume was applied to reach basal locations. It was possible that a change in the OP during gel injection was due to the effects of fluid pressure changes on the supporting cells in the basal turn, on which the OHCs are situated, producing similar changes to the effects of traumatic tones observed by Flock et al., (1999) using *in vitro* preparations of guinea pig cochleae. However, we would expect mechanical distortion of supporting structures to produce temporary or permanent changes in cochlear sensitivity, as was the case in the study by Flock et al., (1999), yet our results demonstrated an almost immediate recovery of CAP thresholds, EP and SP. It therefore appears more likely that the primary effect of the gel was a displacement of the organ of Corti without damage to supporting cells, similar to that which would be expected from non-traumatic acoustic stimulation.

4.2 Helicotrema Plugging

Our measured changes of CM sensitivity as stimulus frequency was varied, after gel injections into the helicotrema, confirm the role played by this structure in attenuating very low-frequency stimuli, primarily those in the infrasonic range. In guinea pigs, CM sensitivity decreases with frequency at approximately 6 dB/octave, which has been accounted for in terms of fluid viscosity at the helicotrema (Dallos, 1970). In animals with shorter cochleae and larger helicotremas (e.g. humans, chinchillas, cats) a steeper cutoff of 12 dB/octave is explained in terms of fluid inertia impeding perilymph movements (Dallos, 1970, Marquardt et al, 2007). In either case, increasing the viscosity of fluid at the helicotrema would increase the sensitivity to infrasonic tones. This accounts for the enhanced modulations of OP, SP and EP measurements during 4.8 Hz bias tones found in this study. The 90° phase shift in the effects of the 4.5 Hz bias on each of OP, EP and SP after gel injection is presumably due to an increase in the acoustic impedance at the helicotrema, which would alter the phase of cochlear responses to acoustic stimuli below 150 Hz (Dallos, 1970; Puria and Allen, 1990).

4.3 Apical Gel Injections Compared to Endolymphatic Hydrops Models

An enhancement of sensitivity to infrasonic bias tones, comparable to that found after plugging the helicotrema with gel was also found in guinea pigs with surgically-induced endolymphatic hydrops. In the hydropic animals, it is likely that the enhancement results from an occlusion of the perilymphatic pathway between scala vestibuli and scala tympani by endolymphatic hydrops, as shown in Figure 13. An enhanced sensitivity to pressure changes was previously found in studies where sustained pressures were applied to the middle ear of hydropic animals (Sakikawa et al., 1999). Nystagmus was induced by this manipulation occurred at lower applied pressures in hydropic animals compared to normal controls. However, a combined pressure waveform with both sustained and infrasonic (9 Hz) components is routinely applied to the ears of patients with Ménière's disease, as a form of therapy with the Meniett device (Odkvist et al., 2000). There has been no indication that ears of Ménière's patients are any more sensitive to this procedure than are non-Ménière's subjects. There are anecdotal reports that Meniere's

patients are sensitive to atmospheric pressure fronts or infrasonic sounds from other sources, but no rigorous studies have been performed on this subject.

Many studies have investigated the possibility of using low-frequency biasing to detect endolymphatic hydrops either with AP (Tono and Morizono, 1995), SP (Klis and Smoorenburg, 1988), acoustic emissions (Hirschfelder et al., 2005; Rotter et al., 2008) or psychophysical measures (Hof-Duin and Wit, 2007). The majority of these studies have used bias frequencies of 20 Hz or higher. Our findings suggest that greater differences between hydropic and normal ears will occur as bias frequency is reduced. Alternatively, there are now multiple studies in which the cutoff slope of low-frequency hearing has been quantified in humans using low-frequency biasing of otoacoustic emissions (Marquardt et al, 2007; Bian and Scherrer, 2007). If endolymphatic hydrops occludes the apical perilymphatic spaces in the human, we would anticipate that the cutoff slope measured by these methods would be reduced.

The mechanical displacement of the organ of Corti in the basal turn during apical gel injections may be mechanically comparable to that produced by endolymphatic hydrops, since both would displace the organ of Corti towards scala tympani. However, basilar membrane displacements might also occur due to changes induced by hydrops unrelated to fluid pressure changes, such as outer hair cell contractions (Zenner, 1986) or changes in stereocilia stiffness (Fettiplace and Ricci, 2003). Basilar membrane displacements have been demonstrated in the temporal bones from Ménière's patients (Xenellis et al., 2004). Characterizing both displacement-induced and EP-induced sensitivity changes could help understand some aspects of Ménière's disease, such as the fluctuating hearing sensitivity and tinnitus. Our measurements show that organ of Corti displacements suppress hearing sensitivity, increase distortion, and increase both the SP and EP. However, as discussed above, it is unlikely that the apical gel injections will produce endolymphatic hydrops in the basal turn so there may be substantial differences between the two models with regard to endolymph volume and Reissner's membrane displacement. One major difference between the two models is the substantial rise in EP observed during apical gel injections while EP was systematically lowered by 10 mV or more in animals with surgically-induced hydrops (Salt and DeMott, 1994). A high EP is necessary for sensitive hearing (Hibino and Kurachi, 2006), and reduced EP causes a decrease in hearing sensitivity by approximately 1 dB/mV (Sewell 1984). On the other hand, an abnormally high EP might contribute to tinnitus (Patuzzi et al., 2004) due to increased receptor current, depolarization of the inner hair cells and increased spontaneous neurotransmitter release. The primary afferent firing rate is known to vary proportionally with EP (Sewell, 1984) so, if elevated spontaneous neural firing was perceived as a sound, EP changes could be related to tinnitus.

Acknowledgements

This study was supported by research grant RO1 DC01368 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health and by a grant to Daniel Brown from the Ménière's Research Fund Group of New South Wales, Australia. We would like to acknowledge contribution of Ruth Gill, for assisting in the preparation of the manuscript and of Dr. Barbara Bohne for taking the photographs of histological specimens.

References

- Assad JA, Corey DP. An active motor model for adaptation by vertebrate hair cells. *J Neurosci* 1992;12:3291–3309. [PubMed: 1527581]
- Bian L, Chertoff ME, Miller E. Deriving a cochlear transducer function from low-frequency modulation of distortion product otoacoustic emissions. *J Acoust Soc Am* 2002;112:198–210. [PubMed: 12141345]

- Bian L, Linhardt EE, Chertoff ME. Cochlear hysteresis: Observation with low frequency modulated distortion product otoacoustic emissions. *J Acoust Soc Am* 2004;115:2159–2172. [PubMed: 15139627]
- Bian L, Scherrer NM. Low-frequency modulation of distortion product otoacoustic emissions in humans. *J Acoust Soc Am* 2007;122:1681–1692. [PubMed: 17927428]
- Brown DJ, Hartsock JJ, Gill RM, Fitzgerald HE, Salt AN. Estimating the Operating Point of the Cochlear Transducer Using Low-Frequency Biased Distortion Products. *J Acoust Soc Am*. 2009in review
- Böhmer A. Hydrostatic pressure in the inner ear fluid compartments and its effects on inner ear function. *Acta Otolaryngol Suppl* 1993;507:3–24. [PubMed: 8273452]
- Choi CH, Chertoff ME, Bian L, Lerner D. Constructing a cochlear transducer function from the summing potential using a low frequency bias tone. *J Acoust Soc Am* 2004;116:2996–3007. [PubMed: 15603145]
- Cohen J, Morizono T. Changes in EP and inner ear ionic concentrations in experimental endolymphatic hydrops. *Acta Otolaryngol* 1984;98:398–402. [PubMed: 6524337]
- Cooper NP, Rhode WS. Basilar membrane mechanics in the hook region of cat and guinea-pig cochleae: sharp tuning and nonlinearity in the absence of baseline position shifts. *Hear Res* 1992;63:163–190. [PubMed: 1464568]
- Dallos, P. *The Auditory Periphery*. Academic Press; NY: 1970. p. 180-187.
- Dallos P. The active cochlea. *J Neurosci* 1992;12:4575–85. [PubMed: 1464757]
- Dunnebie EA, Segenhout JM, Dijk F, Albers FW. Sensory cell damage in two-phase endolymphatic hydrops: a morphologic evaluation of a new experimental model by low-voltage scanning techniques. *Otol Neurotol* 2001;22:655–661. [PubMed: 11568675]
- Eatock RA. Adaptation in hair cells. *Annu Rev Neurosci* 2000;23:285–314. [PubMed: 10845066]
- Fettiplace R, Ricci AJ. Adaptation in auditory hair cells. *Curr Opin Neurobiol* 2003;13:446–451. [PubMed: 12965292]
- Frank G, Kössl M. The acoustic two-tone distortions 2f₁-f₂ and f₂-f₁ and their possible relation to changes in the operating point of the cochlear amplifier. *Hear Res* 1996;98:104–115. [PubMed: 8880185]
- Flock A, Flock B, Fridberger A, Scarfone E, Ulfendahl M. Supporting cells contribute to control of hearing sensitivity. *J Neurosci* 1999;19:4498–4507. [PubMed: 10341250]
- Fridberger A, van Maarseveen JT, Scarfone E, Ulfendahl M, Flock B, Flock A. Pressure-induced basilar membrane position shifts and the stimulus-evoked potentials in the low-frequency region of the guinea pig cochlea. *Acta Physiol Scand* 1997;161:239–252. [PubMed: 9366967]
- Hibino H, Kurachi Y. Molecular and physiological bases of the K⁺ circulation in the mammalian inner ear. *Physiology (Bethesda)* 2006;21:336–345. [PubMed: 16990454]
- Hirschfelder A, Gossow-Müller-Hohenstein E, Hensel J, Scholz G, Mrowinski D. Diagnosis of endolymphatic hydrops using low frequency modulated distortion product otoacoustic emissions. *HNO* 2005;53:612–617. [PubMed: 15565422]
- Hof-Duin NJ, Wit HP. Evaluation of low-frequency biasing as a diagnostic tool in Menière patients. *Hear Res* 2007;231:84–89. [PubMed: 17658231]
- Horner KC. Cochlear and vestibular epithelia from a patient with Menière's disease: a case study. *Scanning Microsc* 1992;6:1115–1127. [PubMed: 1295082]
- Klis JF, Smoorenburg GF. Cochlear potentials and their modulation by low-frequency sound in early endolymphatic hydrops. *Hear Res* 1988;32:175–184. [PubMed: 3360677]
- Kirk DL, Moleirinho A, Patuzzi RB. Microphonic and DPOAE measurements suggest a micromechanical mechanism for the 'bounce' phenomenon following low-frequency tones. *Hear Res* 1997;112:69–86. [PubMed: 9367230]
- Kirk DL, Patuzzi RB. Transient changes in cochlear potentials and DPOAEs after low-frequency tones: the 'two-minute bounce' revisited. *Hear Res* 1997;112:49–68. [PubMed: 9367229]
- Konishi T, Nielsen DW. The temporal relationship between basilar membrane motion and nerve impulse initiation in auditory nerve fibers of guinea pigs. *Jpn J Physiol* 1978;28:291–307. [PubMed: 713181]
- Kros CJ, Rusch A, Richardson GP. Mechano-electrical transducer currents in hair cells of the cultured neonatal mouse cochlea. *Proc R Soc Lond B Biol Sci* 1992;249:185–193.

- LePage EL. Frequency-dependent self-induced bias of the basilar membrane and its potential for controlling sensitivity and tuning in the mammalian cochlea. *J Acoust Soc Am* 1987;82:139–154. [PubMed: 3624635]
- Long CH 3rd, Morizono T. Hydrostatic pressure measurements of endolymph and perilymph in a guinea pig model of endolymphatic hydrops. *Otolaryngol Head Neck Surg* 1987;96:83–95. [PubMed: 3118301]
- Lukashkin AN, Russell IJ. Modifications of a single saturating non-linearity account for post-onset changes in 2f1-f2 distortion product otoacoustic emission. *J Acoust Soc Am* 2002;112:1561–1568. [PubMed: 12398462]
- Marquardt T, Hensel J, Mrowinski D, Scholz G. Low-frequency characteristics of human and guinea pig cochleae. *J Acoust Soc Am* 2007;121:3628–3638. [PubMed: 17552714]
- Ninoyu O, Meyer zum Gottesberge AM. Changes in Ca^{++} activity and DC potential in experimentally induced endolymphatic hydrops. *Arch Otorhinolaryngol* 1986;243:106–107. [PubMed: 2424416]
- O'Beirne GA, Patuzzi RB. Mathematical model of outer hair cell regulation including ion transport and cell motility. *Hear Res* 2007;234:29–51. [PubMed: 17981412]
- Odkvist LM, Arlinger S, Billermark E, Densert B, Lindholm S, Wallqvist J. Effects of middle ear pressure changes on clinical symptoms in patients with Ménière's disease--a clinical multicentre placebo-controlled study. *Acta Otolaryngol Suppl* 2000;543:99–101. [PubMed: 10908991]
- Patuzzi RB, Brown DJ, McMahon CM, Halliday AF. Determinants of the spectrum of the neural electrical activity at the round window: transmitter release and neural depolarisation. *Hear Res* 2004;190:87–108. [PubMed: 15051132]
- Patuzzi RB, Moleirinho A. Automatic monitoring of mechano-electrical transduction in the guinea pig cochlea. *Hear Res* 1998;125:1–16. [PubMed: 9833960]
- Patuzzi R, Sellick PM, Johnstone BM. The modulation of the sensitivity of the mammalian cochlea by low frequency tones. I. Primary afferent activity. *Hear Res* 1984a;13:1–8. [PubMed: 6706858]
- Patuzzi R, Sellick PM, Johnstone BM. The modulation of the sensitivity of the mammalian cochlea by low frequency tones. III. Basilar membrane motion. *Hear Res* 1984b;13:19–27. [PubMed: 6706859]
- Puria S, Allen JB. A parametric study of cochlear input impedance. *J Acoust Soc Am* 1991;89:287–309. [PubMed: 2002170]
- Rotter A, Weikert S, Hensel J, Scholz G, Scherer H, Hölzl M. Low-frequency distortion product otoacoustic emission test compared to ECoG in diagnosing endolymphatic hydrops. *Eur Arch Otorhinolaryngol* 2008;265:643–649. [PubMed: 18026744]
- Russell IJ, Cody AR, Richardson GP. The responses of inner and outer hair cells in the basal turn of the guinea-pig cochlea and in the mouse cochlea grown in vitro. *Hear Res* 1986;22:199–216. [PubMed: 3733540]
- Rydmarker S, Horner KC. Atrophy of outer hair cell stereocilia and hearing loss in hydropic cochleae. *Hear Res* 1991;53:113–122. [PubMed: 2066279]
- Sakikawa Y, Wall C, Kimura RS. Vestibular responses of normal and hydropic ears of the guinea pig to middle ear pressure application. *Ann Otol Rhinol Laryngol* 1999;108:271–276. [PubMed: 10086621]
- Salt AN, DeMott JD. Endolymph calcium increases with time after surgical induction of hydrops in guinea-pigs. *Hear Res* 1994;74:115–121. [PubMed: 8040082]
- Salt AN, Henson MM, Gewalt SL, Keating AW, DeMott JE, Henson OW Jr. Detection and quantification of endolymphatic hydrops in the guinea pig cochlea by magnetic resonance microscopy. *Hear Res* 1995;88:79–86. [PubMed: 8576007]
- Salt AN, DeMott JE. Longitudinal endolymph flow associated with acute volume increase in the cochlea. *Hear Res* 1997;107:29–40. [PubMed: 9165344]
- Salt AN, DeMott JE. Longitudinal endolymph movements induced by perilymphatic injections. *Hear Res* 1998;123:137–147. [PubMed: 9745962]
- Salt AN. Acute endolymphatic hydrops generated by exposure of the ear to non-traumatic low frequency tone. *JARO* 2004;5:203–214. [PubMed: 15357421]
- Salt AN, Rask-Andersen H. Responses of the endolymphatic sac to perilymphatic manipulations: Evidence for the presence of a one-way valve. *Hear Res* 2004;191:90–100. [PubMed: 15109708]

- Salt, AN.; Hensen, HE.; Gill, RM.; Sirjani, DB. Even-order distortions as indicators of endolymphatic hydrops. In: Lim, David J., editor. Proceedings of the 5th International Symposium on Meniere's Disease and Inner Ear Homeostasis Disorders; Los Angeles, CA: House Ear Institute; 2005. p. 115-116.publishers
- Salt AN, Hale SA, Plonke SK. Perilymph sampling from the cochlear apex: a reliable method to obtain higher purity perilymph samples from scala tympani. *J Neurosci Methods* 2006;153:121–129. [PubMed: 16310856]
- Sewell WF. The effects of furosemide on the endocochlear potential and auditory-nerve fiber tuning curves in cats. *Hear Res* 1984;14:305–314. [PubMed: 6480516]
- Shinomori Y, Spack DS, Jones DD, Kimura RS. Volumetric and dimensional analysis of the guinea pig inner ear. *Ann Otol Rhinol Laryngol* 2001;110:91–98. [PubMed: 11201817]
- Sirjani DB, Salt AN, Gill RM, Hale SA. The influence of transducer operating point on distortion generation in the cochlea. *J Acoust Soc Am* 2004;115:1219–1229. [PubMed: 15058343]
- Takeuchi S, Takeda T, Saito H. Pressure relationship between perilymph and endolymph in guinea pigs. *Acta Otolaryngol* 1990;109:93–100. [PubMed: 2309564]
- Thorne M, Salt AN, DeMott JE, Henson MM, Henson OW jr, Gewalt SL. Cochlear fluid space dimensions for six species derived from reconstructions of 3-D magnetic resonance images. *Laryngoscope* 1999;109:1661–1668. [PubMed: 10522939]
- Tono T, Morizono T. Low-Frequency biasing of round window responses in the guinea pig and chinchilla. *Audiol* 1995;34:47–56.
- Valk WL, Wit HP, Albers FW. Changes in distortion of two-tone cochlear microphonic and otoacoustic emission signals during an acute endolymphatic hydrops in the guinea pig. *Eur Arch Otorhinolaryngol* 2006;263:430–434. [PubMed: 16380806]
- Wit HP, Warmerdam TJ, Albers FW. Measurement of the mechanical compliance of the endolymphatic compartments in the guinea pig. *Hear Res* 2000;145:82–90. [PubMed: 10867280]
- Xenellis JE, Linthicum FH Jr, Webster P, Lopez R. Basilar membrane displacement related to endolymphatic sac volume. *Laryngoscope* 2004;114:1953–9. [PubMed: 15510021]
- Zenner HP. Motile responses in outer hair cells. *Hear Res* 1986;22:83–90. [PubMed: 3733547]
- Zou Y, Zheng J, Ren T, Nuttall A. Cochlear transducer operating point adaptation. *J Acoust Soc Am* 2006;119:2232–2241. [PubMed: 16642838]

Abbreviations

AP	Action potential
CM	cochlear microphonic
EP	endocochlear potential
MET	mechano-electric transducer
OP	operating point
OAE	otoacoustic emissions
OHC	outer hair cell
RW	round window

ST

scala tympani

SP

summating potential

Figure 1.

The label abbreviations are ST: scala tympani; SV: scala vestibuli; OC: organ of Corti; RM: Reissner's membrane; CA: cochlear aqueduct. **Left:** Reconstruction of the 3D anatomy at the apical region of the guinea pig cochlea, shown with the outer wall cut away. The fluid space at the apex is dominated by SV, with ST entering near the midpoint of the spiral. This opening, termed the helicotrema, is not a narrowing but rather is just the termination of ST. **Right:** Schematic showing fluid movements associated with Healon gel injections into the cochlear apex. When fluid is injected into the sealed cochlea, flow is directed towards the cochlear aqueduct at the base of ST. Healon will enter ST until the scala is occluded, after which pressure will be applied to SV and to the entire vestibule, and to the endolymphatic space by pushing on Reissner's membrane (see image at left). As pressure is primarily released by the cochlear aqueduct acting as an outlet, this will cause a pressure differential between endolymph and ST, resulting in displacement of the OC.

Figure 2.

Left: Cochlear microphonic (CM) waveforms recorded from the round window in response to a 500 Hz, 90 dB SPL probe tone before, during and after injection of 1 μ L of Healon gel into the cochlear apex at 100 nL/min. The time that the measurement was made is indicated at the left. **Right:** The same CM waveforms (Y) plotted against a sinusoid (X), forming a Lissajous figure, and fitted to a saturating Boltzmann function by varying only the operating point, as indicated by the open circle on each plot. The amplitude and shape changes of CM can be largely accounted for by operating point changing the region of the transducer over which the sinusoidal stimulus operates.

Figure 3.

Response changes during the injection of 1 μL of Healon into the cochlear apex at 100 nL/min. After a few minutes delay, operating point (OP) and second harmonic distortion ($2f_1$) measured simultaneously from 3 sites with 500 Hz probe tones was increased, falling rapidly back to near pre-injection values when the injection stopped. Endocochlear potential (EP) was reversibly elevated by over 10 mV. In response to 8 kHz probe tones, summing potential (SP) was elevated while cochlear microphonics (CM) and cochlear action potential (AP) were reduced.

Figure 4.

Response changes during 2 injections and 1 withdrawal of Healon at 50 nL/min. OP measured simultaneously from the round window (RW) and the basal turn of scala media (SM) increased during injections and transiently decreased during the withdrawal. $2f_1$ increased during injections and withdrawals, with increases corresponding to OP moving away from zero. EP and SP changes were similar to those of OP, with EP elevated by almost 10 mV during injections. AP thresholds to high and low frequency stimuli were elevated during injections whereas only low frequency thresholds were affected by the withdrawal.

Figure 5.

Lissajous plots of DC-coupled scala media CM waveforms (black lines) plotted against the summed sinusoidal input and fitted operating point (OP: white circles). The plots show data measured before (lower plot) and during (upper plot) 1 μ L gel injection into the apex at a rate of 100 nL/min. During the injection OP was displaced by 0.38 Pa, an amount which would increase output voltage by 2.2 mV. In contrast, EP was increased by 9.5 mV, leaving 7.3 mV that could not be accounted for by mechanical displacement of the transducer.

Figure 6.

Dependence of operating point changes on the rate of injection (positive rates) or withdrawal (negative rates). Solid symbols show operating point measured at the round window (RW: inverted triangles) or from scala media (SM: triangles). The fitted line has an equation: OP Change = 0.0044 * Injection Rate + 0.42, with an R^2 of 0.26. Open symbols show the response to control injections of artificial perilymph (AP). Withdrawals after injections caused oppositely-directed operating point changes in most animals. The average OP changes for different injection rates were as follows:

	20-25 nl/min	50 nl/min	100 nl/min	200-250 nl/min
Healon	0.47 SD 0.38; n=3	0.63 SD 0.56; n=15 * p=0.03	0.86 SD 0.33; n=11 * p=0.006	1.31 SD 0.54; n=9
Artificial Perilymph		0.02 n=2	0.01 SD 0.01; n=4

Figure 7.

Comparison of low-frequency biased and unbiased operating point changes during a 10 min Healon injection into the apex at 50 nL/min (0.5 μ L total volume injected). **Upper panel:** Operating point derived from non-biased responses, showing an increase during injection and a decline afterwards. **Middle Panel:** At each time point, operating point determined at 8 equally-spaced intervals during one cycle of the 4.8 Hz bias stimulus are plotted. **Lower Panel:** Amount of operating point modulation (maximum OP - minimum OP for the 8 points measured during a cycle of the bias tone). At the start of injection, sensitivity to the low-frequency bias was increased markedly and remained elevated after injection.

Figure 8.

Low-frequency (4.8 Hz) biased cochlear microphonic (CM) waveforms recorded from scala media of the basal turn in response to a 500 Hz, 90 dB SPL probe stimulus before (A,B) and after (C,D) injection of 0.5 μ L Healon into the cochlear apex. In each block, 8 waveforms measured at equally-spaced intervals on the bias cycle are shown, with times indicated. Where CM is plotted against time (A,C) the heavy curves show the waveforms at the extreme points of the bias cycle. The Lissajous figures (B,D) show the same CM waveforms (heavy lines) plotted against a sinusoidal input, displaced to best fit the transducer curve (thinner line). The open circle indicates the displacement, which corresponds to the operating point at that point on the bias cycle. The larger bias induced changes of the CM waveform (C) produced by gel injection are accounted for by larger displacements of the 500 Hz probe stimulus along the transducer curve.

Figure 9.

A to E) Parameters measured at 8 points on the bias cycle before (open circles) and after (filled circles) injection of 0.5 μ L Healon into the cochlear apex. Operating point (OP) changes were larger after injection and changed in phase by approximately 90° (A). Modulation of $2f_1$ (B) and $3f_1$ (C) was markedly increased and changed from a sinusoidal to a bimodal pattern. This was anticipated based the known relationships between the relative amplitude of $2f_1$ or $3f_1$ distortion products and operating point (F), where before and after Healon injection $2f_1$ (open circles: before, closed circles: after) was followed a V-shaped relationship to OP, and $3f_1$ (open squares: before, closed circles: after) followed an inverse parabola relationship. Bias induced EP changes were markedly increased (D) as was the effect of bias on the SP evoked by an 8 kHz 80 dB SPL tone-burst (E).

Figure 10.

Bias-induced modulation of operating point as a function of level. **Heavy line with SD error bars:** Mean modulation of operating point as a function of bias tone level in 10 animals prior to any manipulation. **Lines with filled symbols:** Modulation of OP with bias level in individual animals after varying amounts of Healon were injected into the apex (Squares 0.5 μ L; Circles 1 μ L; Triangles 1.5 – 2.5 μ L injected). **Lines with open symbols:** Responses measured after control injections of artificial perilymph. The approximate noise floor for modulation measurements is indicated. Healon gel injections into the cochlear apex were systematically found to increase the sensitivity of the cochlea to low frequency bias stimuli.

Figure 11.

CM thresholds (left column) and AP thresholds (right column) measured before (open symbols) and after (solid symbols) injections into the cochlear apex. The upper row shows a control injection of artificial perilymph, while the lower two rows show two examples of the effects of Healon injections. Healon made the ear less sensitive at some frequencies, but markedly increased the sensitivity to sounds in the infrasonic range (<20 Hz). AP thresholds were little affected by injections of 1 μ L, but were suppressed as the injected volume became larger.

Figure 12.

Bias-induced modulation of operating point as a function of level in normal animals (different data set compared to Figure 9, heavy line with SD error bars). Lines with symbols show biased-induced modulation of operating point measured in 3 animals with surgically-induced endolymphatic hydrops.

Figure 13.

Partial occlusion of the perilymphatic spaces at the apex by endolymphatic hydrops. In the lower row, the endolymphatic spaces of the specimens are shown blue and the perilymphatic spaces are shown orange. In the normal cochlea (left column), scala vestibuli (SV) and scala tympani (ST) communicate through the helicotrema, with the predominant resistance to fluid flow provided by the ST that has a smaller cross-sectional area than SV. In hydropic cochleae (middle and right columns), Reissner's membrane distends into SV, in some turns reducing SV cross-sectional area below that of ST. In the specimen at the right, Reissner's membrane is seen to occlude the helicotrema in the apical turn. The capacity to shunt pressure differences between SV and ST of the basal turn by fluid flow through the helicotrema is therefore likely to be impaired in hydropic cochleae.