

The influence of transducer operating point on distortion generation in the cochlea

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(Received 18 September 2003; revised 19 November 2003; accepted 15 December 2003)

Distortion generated by the cochlea can provide a valuable indicator of its functional state. In the present study, the dependence of distortion on the operating point of the cochlear transducer and its relevance to endolymph volume disturbances has been investigated. Calculations have suggested that as the operating point moves away from zero, second harmonic distortion would increase. Cochlear microphonic waveforms were analyzed to derive the cochlear transducer operating point and to quantify harmonic distortions. Changes in operating point and distortion were measured during endolymph manipulations that included 200-Hz tone exposures at 115-dB SPL, injections of artificial endolymph into scala media at 80, 200, or 400 nl/min, and treatment with furosemide given intravenously or locally into the cochlea. Results were compared with other functional changes that included action potential thresholds at 2.8 or 8 kHz, summing potential, endocochlear potential, and the $2f_1-f_2$ and f_2-f_1 acoustic emissions. The results demonstrated that volume disturbances caused changes in the operating point that resulted in predictable changes in distortion. Understanding the factors influencing operating point is important in the interpretation of distortion measurements and may lead to tests that can detect abnormal endolymph volume states. © 2004 Acoustical Society of America. [DOI: 10.1121/1.1647479]

PACS numbers: 43.64.Jb, 43.64.Nf [BLM]

Pages: 1219–1229

I. INTRODUCTION

These studies investigate the possibility that physiological response changes associated with changes in cochlear transducer operating point may be of value in the characterization of endolymph volume disturbances, such as endolymphatic hydrops. If the relationship between mechanical input and electrical output of the cochlear transducer can be described by a response curve, then the operating point represents the location on this curve in the absence of stimulation. It has been suggested that endolymph volume disturbances can mechanically disturb transduction in the ear through changes in static position and thus operating point of the organ of Corti (Kirk *et al.*, 1997). Changes in emitted distortion products consistent with operating point shifts have been demonstrated during low-frequency biasing experiments (Frank and Kössl, 1996; 1997) with both the f_2-f_1 and $2f_1-f_2$ emissions being modulated by a bias tone. Low-frequency biasing of the $2f_1-f_2$ emission has also been used to derive the cochlear transducer function (Bian *et al.*, 2002). Detailed studies of the relationships between operating point and acoustic emissions were reported by Kirk and Patuzzi (1997) and Kirk *et al.* (1997). In these studies it was found that exposure to a high-level, low-frequency tone produced marked changes of both the operating point and the f_2-f_1 emission. Subsequent studies have demonstrated that low-frequency tone exposures can produce a transient endolymphatic hydrops (Flock and Flock, 2000; Salt *et al.*, 2002; 2003). The study by Salt (2003) utilized chemical volume markers that were monitored in the endolymphatic space by ion-selective microelectrodes. Exposure to 200 Hz

tones at 115 dB produced marker concentration changes indicating an endolymph volume increase of approximately 30% in the second turn of guinea pigs. A similar degree of hydrops was reported during stimulation with a 140-Hz tone at 109 dB SPL by Flock and Flock (2000). They used confocal microscopy to view Reissner's membrane in the apical turn of the guinea pig cochlea and found substantial bowing of Reissner's membrane towards scala vestibuli.

The purpose of the present study was to quantify changes in operating point, distortion, and other functional measures during manipulations intended to disturb endolymph volume or endolymph homeostasis. We used three types of interventions: (1) Tone exposures at 200 Hz, 115 dB SPL that have been shown to cause transient endolymphatic hydrops (Salt, 2003; Flock and Flock, 2000); (2) Direct injections of artificial endolymph into scala media of the second cochlear turn (Salt and DeMott, 1997); (3) Treatment with furosemide, a blocker of ion transport in the lateral wall of the cochlea that results in substantial reduction of the endolymphatic potential and ionic changes of endolymph (Brusilow, 1976; Rybak and Morizono, 1982; Rybak and Whitworth, 1986).

In order to determine the cochlear transducer operating point, we have adopted an approach similar to that used by Kirk *et al.* (1997), in which operating point was derived from an analysis of the cochlear microphonic waveform. Operating point changes were compared with a number of measures of cochlear distortion, including harmonic distortions of the microphonic and acoustic two-tone distortion products $2f_1-f_2$ and f_2-f_1 . Operating point was also compared with a number of other functional measures including cochlear sensitivity as assessed by action potential thresholds and summing potentials.

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II. METHODS

A. Animal preparation

Pigmented NIH-strain guinea pigs weighing 350–500 g were used in this study. Animals were anesthetized with intraperitoneal sodium thiobarbital (Inactin, Sigma, St. Louis, 100–125 mg/kg) and placed on a thermistor-controlled heating pad to maintain core temperature at 38 °C. A central line was placed in the left external jugular vein for supplementary anesthetic and other drugs. The trachea was cannulated and the animal was ventilated. End tidal CO₂ was monitored and the tidal volume of the respirator was adjusted to maintain an end tidal CO₂ level of 38 mm Hg (5%). Heart rate was monitored. Prior to recordings, pancuronium bromide (Pavulon, Baxter, Irvine, approximately 0.05 mg, to effect) was given intravenously to minimize myogenic artifacts and to aid mechanical ventilation.

The caudal portion of the right jaw was removed and the auditory bulla was exposed by a ventral approach. The bulla was opened, leaving middle-ear structures intact. A longitudinal incision was made along the external auditory canal and a hollow earbar was inserted until it sealed to the canal wall.

The instrumentation for stimulus generation and data acquisition was Tucker-Davis System 3 hardware controlled by a custom-written VISUAL BASIC (Microsoft) program. An acoustic emissions system (Etymotic ER-10C, microphone and 2 speakers) and a high-intensity speaker (Sennheiser HD580) were incorporated into the hollow earbar so that each sound channel terminated near the tip of the earbar. Sound stimuli in the ear canal were calibrated at the beginning of each experiment using the ER10C microphone with an automated procedure that tracked a criterion sound level of 70 dB SPL across a range of frequencies (1 to 16 kHz for Etymotic speakers, 125 Hz to 16 kHz for the Sennheiser speaker) in 1/4-octave steps.

B. Cochlear evoked potentials

Cochlear responses were recorded from an Ag/AgCl ball electrode positioned at the junction between the round-window membrane and the bony otic capsule. The signal was amplified 1000× and high-pass filtered at 5 Hz. Signals were digitized at 48.8 kHz and averaged using Tucker-Davis RP-2 modules.

C. Responses to biphasic tone bursts

Cochlear potentials [cochlear microphonic (CM), summing potential (SP), and action potential (AP)] were recorded in response to phase-locked tone bursts of two opposing polarities. Typically ten responses to positive onset stimuli and ten responses to negative onset stimuli were averaged. Tone bursts were 12 ms in duration with 0.5-ms cosine-shaped onset. Waveforms containing the AP and SP were obtained by summing the responses to both polarities, and the CM was obtained by subtracting the responses to opposite polarities. Cochlear responses were typically recorded in response to 4-kHz stimulus at 90 dB SPL. In addition, the same collection paradigm was incorporated into

an automated routine by which the AP threshold was established using a 10- μ V criterion. The criterion magnitude was approximately 4 times greater than the background noise level in the averaged response. Stimuli were increased in 5-dB steps until an AP greater than 10 μ V was detected and then stimuli were reduced in 5-dB steps until the AP was below the 10- μ V criterion. The threshold was determined by interpolation between the above- and below-threshold amplitude values. Thresholds were monitored during experimental manipulations with stimuli at 2.8 and 8 kHz. In addition, prior to manipulations a frequency/threshold curve (AP audiogram) was measured in 1/4-octave steps from 1 to 16 kHz.

D. Single-phase CM analysis

The CM obtained as detailed above, by subtracting responses to opposite polarity stimuli, is not appropriate for operating point analysis because polarity-sensitive components are canceled in the waveform subtraction procedure. Instead, CM was averaged in response to a sustained 500-Hz tone, presented at high level to saturate the cochlear transducer. Data sampling was initiated 5 s after the tone commenced, phase-locked to positive-going zero crossings of the tone. Twenty epochs of a 2048-point waveform (42-ms window) were averaged. The waveform was Hamming windowed and the power spectrum was calculated. The peaks representing the primary, second harmonic, and third harmonic were measured. Harmonics were expressed in dB relative to the amplitude of the primary. Half of the waveform (1024 points) was then subjected to an analysis to determine operating point, as detailed below.

E. Acoustic emissions

Acoustic emissions were recorded in response to 4- and 4.8-kHz primaries, presented at 75 or 80 dB SPL. Frequencies of the primaries were optimized to ensure an exact number of cycles in the sample buffer. An 8192-point (168-ms) buffer was averaged, with phase based on synchronous positive zero crossings of both primaries. Stimuli were turned on for 5 s before data collection started. The spectrum of the buffer was then calculated and a number of spectra were averaged to produce the final spectrum. Typically, three spectra were averaged with each spectrum derived from an average of five time buffers. Spectral peaks were derived as the local maximum within an 11-point cursor centered at the expected frequency. The noise floor was established by averaging 6 points of the cursor distant from the peak, i.e., 3 points from each end of the 11-point cursor. At the stimulus levels used, $2f_1 - f_2$ amplitude was an average of 34.1 dB above the noise floor and $f_2 - f_2$ was an average of 24.8 dB above the noise floor.

F. Endolymphatic manipulations

1. Exposure to 200 Hz, 115 dB SPL

A 200-Hz tone was delivered to the ear from the Sennheiser speaker at a level of 115 dB for 3 min. This exposure has previously been shown to induce transient endolymph

phatic hydrops (Flock and Flock, 2000; Salt, 2003). During exposure to the tone, no sound-evoked potentials or acoustic emissions were collected.

2. Injection of artificial endolymph

The bone overlying stria vascularis in the second turn was thinned with a flap knife (Mueller AU13400) and a small, approximately 30- μm , fenestra was made with an angled pick (Storz N1705-80). Injections were performed from double-barreled glass pipettes, with their tips beveled to a diameter of 15–20 μm . The injection barrel was filled with an artificial endolymph consisting of 140-mM KCl and 25-mM KHCO_3 and was mounted on a Nanoliter 2000 microinjection pump [World Precision Instruments (WPI), Sarasota]. The second barrel of the pipette was filled with 500-mM KCl and was used to record endocochlear potential. The pipettes were inserted into endolymph through the access fenestra using registration of a stable positive voltage as verification the pipette tip was located in the endolymphatic space.

3. Treatment with furosemide

Animals were given furosemide (Vetus Furoject, 50 mg/ml, Burns, New York) intravenously at a dose of 100 or 50 mg/kg. Drug delivery was followed by 0.3 ml of lactated Ringer's to ensure the entire dose was expelled from the cannula.

In experiments where furosemide was injected locally in the cochlea, an injection pipette containing 10 mg/ml of furosemide was sealed into the basal turn of scala tympani. The stock 50-mg/ml furosemide was diluted 1:4 in artificial perilymph containing (in mM) NaCl (125), KCl (3.5), CaCl_2 (1.3), NaHCO_3 (25), MgCl_2 (1.2), NaH_2PO_4 (0.75), and glucose (5). The injection pipette was mounted on a WPI Ultra-pump. The otic capsule was thinned and the dry bone coated with thin cyanoacrylate adhesive. The thinned area was surrounded by a ring of two-part silicone adhesive (Kwik-Cast, WPI, Sarasota) and a small fenestra made in the middle with an angled pick. The injection pipette was inserted and droplet of cyanoacrylate was applied while wicking fluid away from the site. When a seal had been achieved the entire field was covered with more two-part silicone adhesive. Injections into scala tympani were performed at a rate of 100 nl/min for a period of 10 min.

G. Experimental design

Immediately after the cochlea was exposed, animals were evaluated with an AP threshold audiogram and the measurement and analysis of CM to low-frequency tones. Animals showing hearing loss, specifically with an AP threshold more than 2 standard deviations above the laboratory mean, were excluded from the study, as were any animals in which the cochlear microphonic to a 500-Hz, 90-dB SPL tone was less than 200 μV . This latter exclusion criterion was set arbitrarily to ensure that the signal-to-noise ratio was adequate to resolve low-level spectral peaks in the CM analysis. We also excluded some animals in which we observed tissue in the external canal contacting the tympanic membrane. This

could be seen through the tympanic membrane from the middle-ear side and occurred when sectioning the ear canal compromised venous drainage from the structure. These animals typically showed elevated AP thresholds to low frequencies, low CM amplitude, and a notably distorted CM to low frequencies. Congestion of the tissue was subsequently avoided by making the incision for the earbar parallel to the long axis of the ear canal. The baseline data obtained from the above measurements were summarized as representing the premanipulation state of the animals. Experimental procedures on the cochlea, such as perforating the otic capsule and placing injection pipettes, were performed after baseline data were collected.

The experimental study consisted of measuring cochlear potentials to biphasic and single phase stimuli, AP thresholds to 8- and 2.8-kHz tones, and acoustic emissions repeatedly. In endolymph injection experiments the endocochlear potential was also measured. It required approximately 35 s to collect a complete set of measurements, so the sampling interval was set to 40 s. Data collection was performed as a fully automated procedure. Measured parameters were graphed and stored as they were collected. When repeated measurements made over at least a 5-min period were judged by the experimenter to be stable (i.e., not drifting in one direction), the animal was subjected to one of the interventions listed above, during which the collection of responses continued.

The Animal Studies Committee of Washington University approved the experimental procedures used in this study under protocol numbers 19 990 029 and 20 020 010.

H. Calculated dependence of distortion on cochlear transducer operating point

The output signal (V) generated by the cochlear transducer responding to a sine wave input (P) was calculated using a first-order Boltzmann function to describe the transducer [Eq. (1)], an approach that has previously been used by Kirk *et al.* (1997)

$$V = -P_{\text{sat}} + (2 P_{\text{sat}}) / (1 + \exp(z(P + P_o))), \quad (1)$$

where P_{sat} is the saturation voltage of the transducer, z is the sensitivity of the transducer, and P_o is the operating point.

Based on this equation, the slope of the calculated transducer curve varies when the saturation voltage (P_{sat}) is changed. To avoid this problem, we therefore defined z in terms of a slope parameter (S) and P_{sat} [Eq. (2)]. When z was defined by this relationship, changing P_{sat} did not change the slope of the transducer curve

$$z = -2 S / P_{\text{sat}}. \quad (2)$$

Output waveforms were calculated for a sinusoidal input as operating point was systematically varied. Waveforms were subjected to the same methods of spectral analysis and determination of operating point as were the recorded cochlear microphonic data. This analysis of calculated waveforms was used both to validate the operating point analysis method and to aid in the interpretation of experimental data.

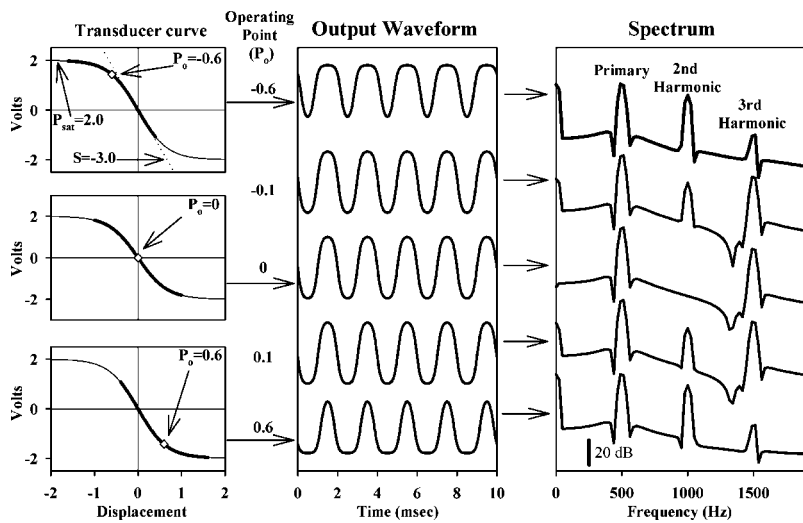


FIG. 1. Output waveforms (middle panel) calculated for transducers with varying operating points (P_o) as indicated. The three panels at the left show the transducer curves for operating point values of 0.6 (upper), 0 (middle), and -0.6 (lower). In each plot, the heavy line indicates the region of the transducer curve over which the stimulus is applied. Spectral analysis of each output waveform is shown in the right panel. For operating point set to zero, the output waveform is symmetric and shows low second harmonic distortion. Even small deviations in operating point (0.1) cause second harmonic distortion increases, even though distortion is not apparent in the time waveform. With operating point values further from zero (0.6) the time waveform is highly asymmetric and a prominent second harmonic distortion peak is apparent in the spectrum.

I. Operating point analysis of cochlear microphonic

A simulated microphonic waveform was calculated as described above and based on the Boltzmann parameters P_{sat} , S , and P_o together with parameters for frequency (f), phase (p), and dc offset (V_o). A curve-fitting procedure was then used in which the six parameters were simultaneously varied to best fit the calculated waveform to the experimentally recorded cochlear microphonic waveform. Fit was optimized by minimizing the sum of squares of differences between calculated and experimental curves. The fitting procedure was performed in real time during data collection, and the derived Boltzmann parameters were recorded. The amplitude of the cochlear microphonic determined the parameters S and P_{sat} . Under this analysis, the scale of operating point is such that a value of 1 is equal to the displacement of the transducer at the peak of the applied sinusoidal stimulus.

III. RESULTS

A. Simulations

The cochlear transducer has nonlinear, saturating characteristics and can be approximated by a Boltzmann function. With such a transducer, the output signal depends on where the input signal is “centered” with respect to the transducer, the so-called “operating point.” This concept and its consequences are shown in Fig. 1, where output waveforms were calculated for a sinusoidal input at 500 Hz with different values of operating point. The transducer curve, based on the Boltzmann function and its parameters, is shown in the left column. With the transducer operating point at zero (left center panel), a sinusoidal input gives a symmetric output voltage. Spectral analysis of this output shows an absence of second harmonic distortion. Positive or negative values of operating point produce an asymmetry of the time waveform which is detected in the spectrum as a second harmonic distortion. For small values of operating point (e.g., 0.1), little change in the time waveform is apparent, while the spectrum shows a prominent second harmonic. The polarity of operating point shown here is intended to represent displacement of structures in a similar manner to

an applied pressure in the ear canal. Positive displacement indicates positive pressure in the ear canal and displacement of the organ of Corti towards scala tympani. Since organ of Corti displacements towards scala tympani cause a negative voltage shift in that scala, the slope of the transducer (left column) is negative.

The calculated relationships between distortion and operating point are shown in Fig. 2. These curves show the magnitude of the second and third harmonics from spectra similar to those in Fig. 1, relative to the magnitude of the primary. This demonstrates graphically that second harmonic distortion is extremely sensitive to deviations of the operating point away from zero, with distortion increasing by almost 50 dB as operating point increases over the range shown. In contrast, the third harmonic distortion peak, which is dominated by the saturating characteristic of the transducer, is relatively unchanged for small operating point changes but decreases when operating point becomes large. These data suggest that measures of second harmonic distortion may be a potentially useful indicator of small changes of cochlear transducer operating point.

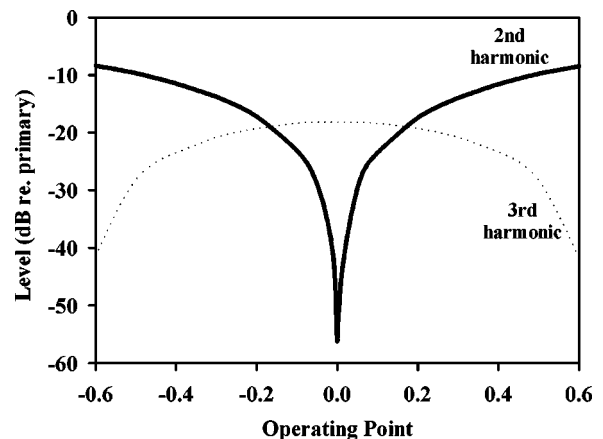


FIG. 2. Dependence of harmonic distortion amplitude on transducer operating point. Second harmonic distortion is extremely sensitive to small operating point changes and increases rapidly as operating point moves away from zero. Third harmonic distortion is less sensitive to small operating point changes but decreases for higher operating point values.

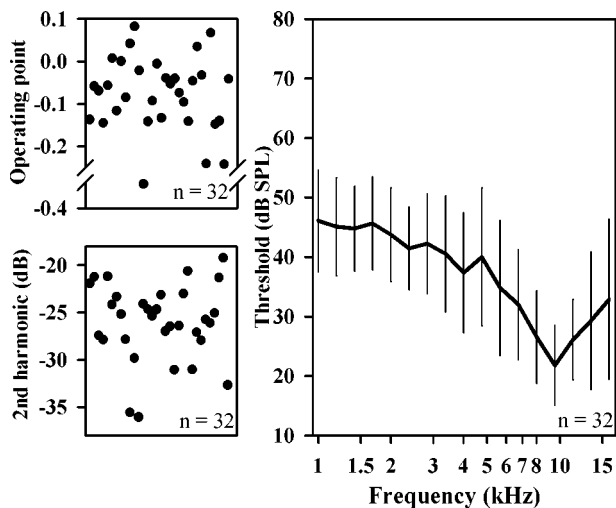


FIG. 3. Baseline measurements of operating point (upper left), second harmonic distortion (lower left), and of cochlear sensitivity as indicated by AP thresholds (right). Each data point in the plots at the left represent an individual animal. Bars on the AP threshold plot indicate standard deviation. Baseline measurements were made prior to any manipulations of the cochlea.

B. Baseline measurements

The variation of operating point, second harmonic distortion, and cochlear sensitivity prior to any experimental manipulations is given in Fig. 3. Both operating point and second harmonic distortion were highly variable across individual animals. Although the mean value of operating point was -0.08 (s.d. 0.09 , $n=32$), there were a number of animals with positive operating point values. Second harmonic distortion, measured in dB relative to the primary peak, varied from -36 dB to over -20 dB. All animals showed normal cochlear function as assessed by AP thresholds, even the animal with an outlying large negative operating point (-0.35).

C. 200-Hz tone exposure

Figure 4 shows the simultaneously measured operating point and second harmonic distortion changes resulting from

exposure to 200 Hz for 3 min at 115 dB SPL in two experiments. Both examples showed a movement of operating point in the positive direction following sound exposure, although the time courses differ slightly. In contrast, the behavior of the second harmonic differs markedly in the two experiments. In example 1, second harmonic distortion increased after the exposure, while in example 2 it showed a marked decrease. The difference between the two animals can be appreciated by plotting each pair of operating point and second harmonic distortion values against each other, as shown in the correlation plots in the right column of the figure. For example 1, the pre-exposure operating point was slightly positive. In this case, moving further positive, away from zero, would be expected to increase distortion, which is what was observed. In contrast, the animal in example 2 showed a negative operating point before exposure. In this case, a movement of operating point in the positive direction brings operating point closer to zero, with an expected reduction of second harmonic distortion. In this example, the relationship between measured operating point and second harmonic distortion closely follows the calculated curve.

Interpretation of harmonic distortion changes during experimental manipulations is therefore extremely difficult unless the baseline operating point of the individual animal is considered. Since distortion may differ in both magnitude and direction in different animals, calculating the deviation of responses across animals has little value. In the figures that follow, we have therefore shown individual response curves together with the mean value to interpret distortion changes.

The response changes resulting from 200-Hz, 115-dB SPL exposure are summarized in Fig. 5. This exposure condition has been shown to induce a transient endolymphatic hydrops that recovers back to normal over a 10–20-min period with a recovery half-time of 3.2 min (Salt, 2003). In the present study, the treatment produced a transient positive shift in operating point by approximately 0.1, consistent with a hydrops-induced displacement of the organ of Corti towards scala tympani. The operating point shift was transient, however, and showed a recovery back to baseline within 3–4

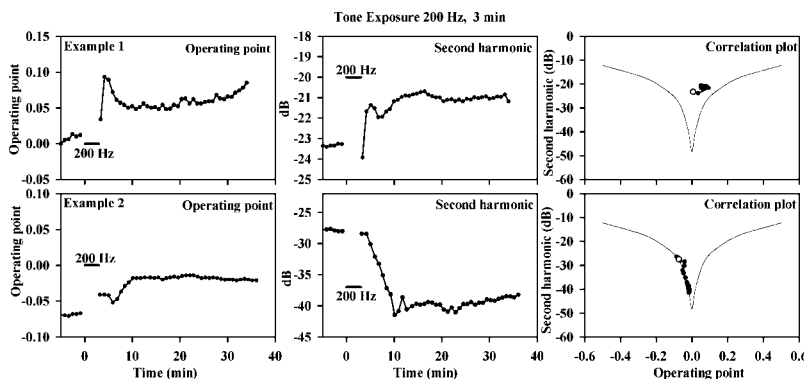


FIG. 4. Two examples in which changes of operating point and second harmonic distortion were recorded simultaneously before and after exposure to a 200-Hz tone for 3 min at 115 dB SPL. The exposure increases operating point in both examples. Second harmonic is increased in example 1 (upper row) and is decreased in example 2 (lower row). This difference is accounted for by consideration of the pre-exposure operating points for the two animals. The positive deflection moves operating point further from zero in example 1 (causing distortion to increase) and closer to zero in example 2 (causing distortion to decrease). The correlation plots (right column) show second harmonic distortion plotted as a function of operating point. The open symbol shows the pretreatment value and the thin line shows the calculated dependence from Fig. 2.

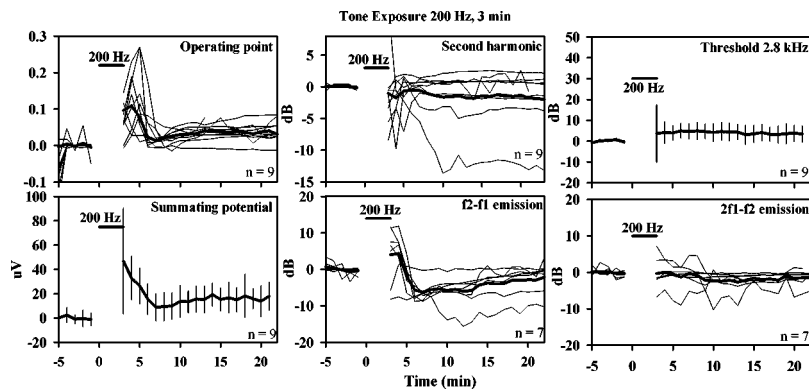


FIG. 5. Response changes elicited by exposure to a 200-Hz tone for 3 min at 115 dB SPL. In each panel, the mean curve is shown as a thicker line. Thin lines represent individual experiments. Bars indicate standard deviation. Operating point and SP are transiently increased due to the 200-Hz-induced endolymph volume increase. Second harmonic distortion and the f_2-f_1 emission show substantial changes that vary in direction, magnitude, and time course due to the complex dependence on operating point. Cochlear sensitivity, as assessed by thresholds at 8 and 2.8 kHz and by the $2f_1-f_2$ emission, is not markedly changed by the exposure.

min of the tone exposure ending. The SP similarly increased transiently and recovered back to baseline within a few minutes. The cochlear microphonic measured with single-phase 500-Hz stimuli or 4-kHz biphasic stimuli showed only small amplitude fluctuations relative to the mean baseline amplitudes of 1478 μV (s.d.=243, $n=9$) and 538 μV (s.d.=137, $n=9$), respectively (data not shown). Second harmonic distortion showed substantial changes in all animals, but the timing, magnitude, and polarity of the change were highly variable due to the reasons discussed above. The f_2-f_1 emission similarly showed substantial changes in all animals, typically larger than 10 dB. Both increases and decreases of f_2-f_1 were apparent, comparable to changes in the second harmonic. Measures of cochlear sensitivity [AP thresholds at 8 kHz (not shown), 2.8 kHz, and the $2f_1-f_2$ emission] indicated only minor changes during the tone-induced endolymph volume disturbance.

D. Artificial endolymph injection at 80 nl/min

Injection of artificial endolymph has previously been used to induce endolymph volume disturbances. In a prior study, 15-min injections at rates higher than 25 nl/min caused endolymphatic hydrops and a basally directed endolymph flow in the cochlea (Salt and DeMott, 1997). Figure 6 summarizes the effect of injections at 80 nl/min for 15 min (total 1.2 μL injected) on the measured responses. Rather

than operating point increasing due to the endolymph volume increase, in most cases we found that large oscillations of operating point occurred. The initial deflection, though large and positive in one animal, was negative in others. The mean curve showed a decrease during the injection, followed by a slow increase some time after the injections finished. The second harmonic also showed oscillations in amplitude with occasional larger deflections. In each experiment, the second harmonic distortion changes were accounted for by the operating point changes, as shown in the example correlation plot at the upper right. In this case, distortion decreased and passed through a minimum as operating point moved through zero, then increased as operating point became more positive. The observation of a minimum close to the measured zero operating point validates the accuracy of the operating point estimate. Two of the experiments showed distortion going through a minimum as the operating point of the animal passed through zero. The SP and f_2-f_1 emission similarly showed increased variation throughout the injection with stabilization afterwards. The injection produced only minor changes in EP, $2f_1-f_2$ emission, and thresholds at 8 kHz. In contrast, thresholds at 2.8 kHz showed a systematic increase during injection (average 15 dB) and a slow recovery afterwards, consistent with the injection-induced endolymph volume disturbance.

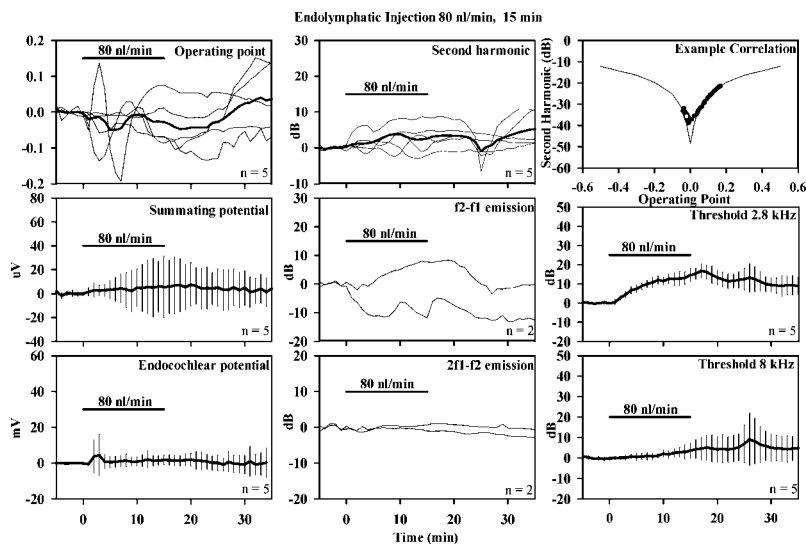


FIG. 6. Response changes elicited by artificial endolymph injection into the second turn of scala media at 80 nl/min for 15 min. Mean curves are shown as thicker lines. Thin lines represent individual experiments. Bars indicate standard deviation. Operating point shows injection-induced fluctuations but no overall increase indicating endolymphatic hydrops. Summating potential similarly shows changes that are not consistent. Second harmonic distortion and the f_2-f_1 emission show variations that depend on operating point. An example of the correlation between second harmonic distortion and operating point is shown at the upper right.

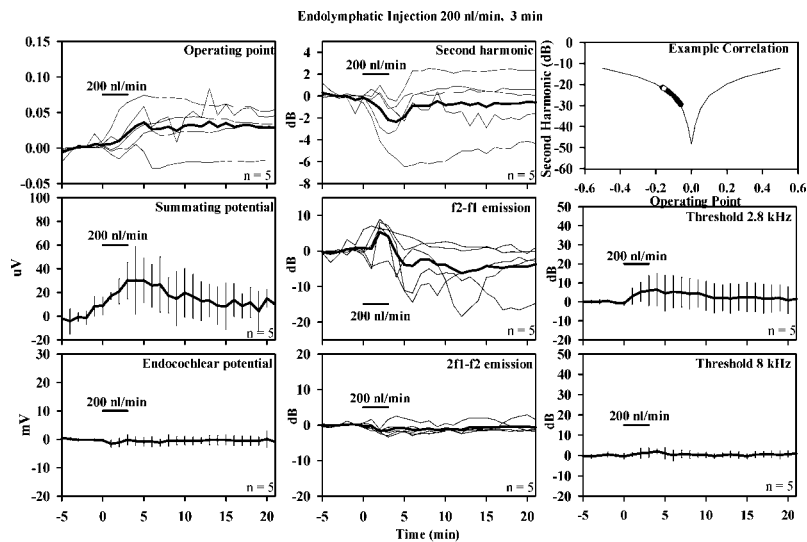


FIG. 7. Response changes elicited by artificial endolymph injection into the second turn of scala media at 200 nl/min for 3 min. Mean curves are shown as thicker lines. Thin lines represent individual experiments. Bars indicate standard deviation. In the majority of animals, operating point is increased by the injection, although cyclical fluctuations are also apparent and are reflected in second harmonic distortion and f_2-f_1 emission changes. An example of the correlation between second harmonic distortion and operating point is shown at the upper right.

E. Artificial endolymph injection at 200 nl/min

In view of the rapid recovery of operating point following hydrops induced by 200 Hz, and the absence of a major operating point increase with 80-nl/min injection, it appeared likely that operating point could adapt quickly to abnormal endolymph volume states. We therefore performed injections at higher rates to induce larger endolymph volume changes more rapidly. Figure 7 summarizes the response changes resulting from 200-nl/min injection of artificial endolymph into the second turn of the cochlea for 3 min, for a total of $0.6 \mu\text{L}$ injected. In four of five animals, operating point showed an increase consistent with endolymphatic hydrops. The fifth showed operating point oscillations, similar to those observed with lower injection rates, but no overall increase. The second harmonic showed transient changes during injection, as did the f_2-f_1 emission. Second harmonic distortion changes were more consistent in this group as all five animals had negative baseline operating points. In the example correlation plot, second harmonic distortion showed a sizable decrease, consistent with the movement of operating point towards zero and in a manner close to that predicted by the simulations. The SP showed an injection-induced increase.

Injection at this rate again had little influence on EP, thresholds at 8 kHz, and the $2f_1-f_2$ emission. Thresholds at 2.8 kHz were slightly elevated, with a mean increase of 6 dB. The smaller threshold increase at 2.8 kHz compared with the 80-nl/min injection group is accounted for by the smaller total volume injected.

F. Artificial endolymph injection at rate of 400 nl/min

The physiologic changes induced by artificial endolymph injection at the higher rate of 400 nl/min for 3 min are summarized in Fig. 8. The total volume delivered in this procedure was $1.2 \mu\text{L}$, the same as the injections shown in subsection D at 80 nl/min. Although four out of five animals showed a transient operating point increase during injection, almost all showed a subsequent operating point decrease to below the preinjection value. The decline in operating point appears to be associated with the larger total volume injected. The second harmonic typically showed transient decreases, followed by an increase as expected from the operating point changes, as shown by the example correlation plot. The SP also showed a prominent increase during injection but did not fall below baseline afterwards. The f_2-f_1

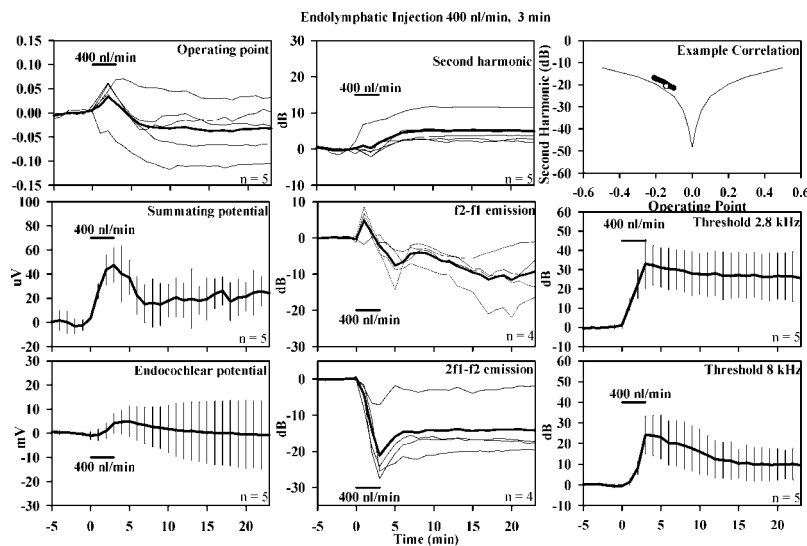


FIG. 8. Response changes elicited by artificial endolymph injection into the second turn at 400 nl/min for 3 min. Mean curves are shown as thicker lines. Thin lines represent individual experiments. Bars indicate standard deviation. In most animals, operating point was transiently increased by the injection and subsequently declined. The summating potential showed a similar time course of change. Second harmonic distortion increased, consistent with the operating point change as indicated in the correlation example. The f_2-f_1 emission decreased. Endocochlear potential and cochlear microphonic amplitude increased, while all measures of cochlear sensitivity (thresholds at 8 kHz, 2.8 kHz, and $2f_1-f_2$ emission) suggest sensitivity declines.

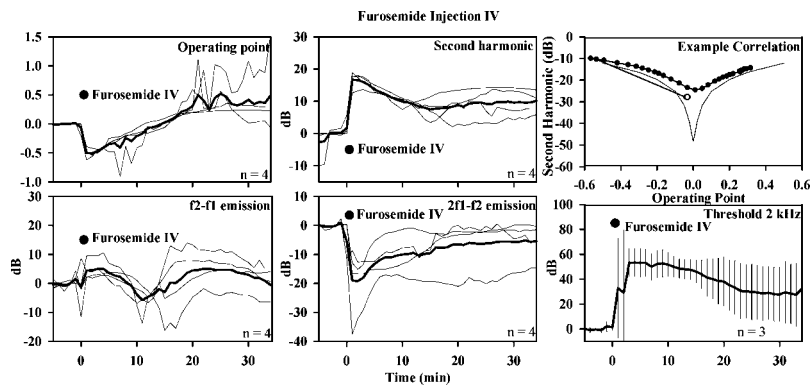


FIG. 9. Response changes elicited by intravenous administration of furosemide. Mean curves are shown as thicker lines. Thin lines represent individual experiments. Bars indicate standard deviation. Operating point transiently decreases, before increasing above the baseline value. Both second harmonic and the f_2-f_1 emission show an increase, followed by a decrease to a minimum caused by the transducer operating point passing through zero. An example of the relationship between second harmonic distortion and operating point is given. Cochlear sensitivity declines, as indicated by the reduction in $2f_1-f_2$ emission, elevation of threshold at 2 kHz, and decline in cochlear microphonic.

emission showed a transient increase during injection and a subsequent decline. Although the endocochlear potential and CM were both increased by injection, measures of cochlear sensitivity (thresholds at 8 kHz, 2.8 kHz, and the $2f_1-f_2$ emission) all indicated a substantial decrease in cochlear sensitivity from injection at this rate with little indication of recovery afterwards.

G. Intravenous furosemide injection

Administration of furosemide was found to produce substantial changes of operating point that allowed the dependence of cochlear distortion on operating point to be demonstrated. The changes induced by intravenous furosemide are summarized in Fig. 9. In all animals, furosemide caused an operating point decrease followed by a slow increase. This resulted in a rapid increase in second harmonic distortion, followed by a decrease reaching a minimum after approximately 10 min, before increasing again. The example correlation plot shows the dependence of distortion on the operating point movements. Of great interest is the observation that with this treatment, the f_2-f_1 emission showed changes comparable to those of the second harmonic. Specifically, f_2-f_1 showed an increase followed by a decline passing through a minimum before increasing again. This complex time course would not be interpretable without some understanding of the effects of operating point on distortion in the cochlea. The reduction in the f_2-f_1 emission to a minimum varies in time from animal to animal but undoubtedly represents the time when the cochlear transducer operating point passed through zero as it moves positive in the recovery phase. Intravenous furosemide caused a large reduction in cochlear microphonic amplitude (not shown)

and decreased cochlear sensitivity, as indicated by reduction of $2f_1-f_2$ emission and the elevation of thresholds at 2 kHz.

H. Furosemide injected locally into the cochlea

Locally applied furosemide had markedly different effects on operating point than when given intravenously, as summarized in Fig. 10. The operating point shows a substantial increase followed by a decline. Second harmonic distortion showed a substantial decline, in some cases becoming 40 dB lower than the primary as zero operating point was passed. The example correlation shows the clear dependence of distortion level on the cochlear transducer operating point. The magnitude of reduction of second harmonic distortion varied in different experiments, depending on the baseline operating point of each animal. With this treatment, the f_2-f_1 emission did not show changes similar to the second harmonic, as was noted previously with intravenous furosemide. This is accounted for by the fact that local furosemide may rapidly affect the basal cochlear turn but will only slowly influence higher cochlear turns. The slow reduction in f_2-f_1 emission, $2f_1-f_2$ emission, and slow elevation of threshold at 2 kHz are consistent with all of these responses originating from higher cochlear turns that are less influenced by furosemide. This supports the view that the operating point associated with the 500-Hz cochlear microphonic differs from the operating point associated with the f_2-f_1 emission, presumably because operating point can vary between different cochlear locations.

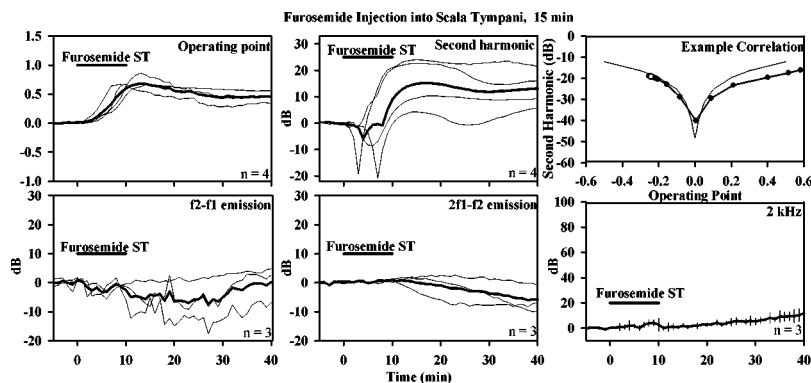


FIG. 10. Response changes elicited by local administration of furosemide into the basal turn of scala tympani. Mean curves are shown as thicker lines. Thin lines represent individual experiments. Bars indicate standard deviation. Operating point increases, causing a complex change in the second harmonic as expected, based on the correlation shown at the upper right. The f_2-f_1 emission, $2f_1-f_2$ emission, and 2-kHz threshold all show slow, minor changes, consistent with their generation at more apical sites that are less influenced by the applied furosemide.

IV. DISCUSSION

Our findings support the views of Frank and Kössl (1996) and Kirk and Patuzzi (1997) that the operating point of the cochlear transducer plays a critical role governing the levels of distortion in the ear. While the prior studies focused on f_2-f_1 , the present study also shows a strong dependence of second harmonic distortion of the cochlear microphonic on operating point. In addition, we found that baseline operating points measured prior to experimental manipulations varied over a considerable range, with animals showing positive or negative values. This distribution accounted for the variable levels of second harmonic distortion in the cochlear microphonic and explained sometimes oppositely directed distortion changes with endolymph manipulations. The baseline variation of operating points appears to have only minor influence on cochlear sensitivity measured by AP thresholds, with all animals in the study showing normal threshold curves across the frequency range.

The calculated relationship between second harmonic distortion and operating point shown in Fig. 2 provides a conceptual framework for the interpretation of distortion changes during experimental manipulations. The fact that an experimental treatment may cause distortion increases in some animals and distortion decreases in others, or may show complex patterns with minima, as in Figs. 9 and 10, would be difficult to interpret without taking into account their underlying dependence on transducer operating point. The observation that in some experiments the second harmonic distortion goes through a minimum as the measured operating point passes through zero (e.g., Figs. 6, 9, and 10) also validates the methodology used to derive operating point. It is notable that the pretreatment level of second harmonic distortion is not always low and in many cases the distortion level seen in the initial normal state was reduced by the experimental treatment (e.g., Figs. 4, 5, 6, 7, and 10). The average operating point measured with a 500-Hz, 90-dB stimulus was -0.08 , suggesting a bias of the cochlear transducer equivalent to a movement of the organ of Corti towards scala media. This observation agrees with the findings of Kirk *et al.* (1997) in which the examples they presented showed negative pretreatment operating points. However, the range of operating points and the possibility that some animals may have positive values have not previously been documented. The variation of operating point also explains some of the variations in prior studies. In the study of Frank and Kössl (1996), low-frequency bias tones were found to modulate the f_2-f_1 emission either at the frequency of the bias tone or in some animals at twice the frequency of the bias tone. We interpret this as showing that if the operating point is nonzero, and the bias amplitude is not sufficient to drive the transducer to reach zero, then the modulation by the bias would occur at the frequency of the bias tone. On the other hand, if operating point is near zero and the bias tone is large enough to drive the transducer through zero, then the distortion will show increases twice per bias cycle and the modulation will occur at twice the frequency of the applied stimulus. Operating point thus plays a key role in generating distortion and is central to our understanding and interpretation of second harmonic and f_2-f_1 distortion.

Disturbances of the endolymphatic system are shown to cause transient changes of operating point in the cochlea. Two hundred-Hz tones and injections into endolymph at high rates (200 nl/min and higher) cause a positive movement of operating point, consistent with an endolymph volume enlargement and displacement of the organ of Corti towards scala tympani. Comparison of the time courses of endolymph volume change induced by 200-Hz tone exposure (Salt, 2003) or by artificial endolymph injections (Salt and DeMott, 1997) with operating point data from the present study shows that operating point recovers substantially faster than does the endolymph volume disturbance. This is consistent with the observation that slowly occurring endolymph volume increases (80-nl/min injection rate) appear to produce instability or slow oscillations of operating point, rather than a sustained operating point change. These observations suggest that some form of compensation for the induced endolymph volume disturbance occurs at the level of the organ of Corti. Thus, operating point, while sensitive to some volume disturbances, is not a direct measure of endolymph enlargement but is influenced by compensation processes. Kirk *et al.* (1997) calculated that the recovery kinetics of operating point could be explained by two exponential processes with time constants of 30 and 220 s. Our data are consistent with this analysis.

An important finding of the study is that acute endolymph volume disturbances can be detected by noninvasive means and are seen as substantial changes of the f_2-f_1 emission. Although the f_2-f_1 changes temporally corresponded with the endolymph volume change, the magnitude, direction, and time course of change were not indicative of the magnitude or duration of endolymph volume change. It is apparent that the observation that f_2-f_1 changes can be large (greater than 10 dB) while $2f_1-f_2$ emission and AP threshold changes are minor (a few dB). Kirk *et al.* (1997) and Frank and Kössl (1996) interpreted this difference as suggesting that a fundamental change in distortion generation was occurring, rather than an alteration to the mechanical drive to a constant distortion generator. It is our interpretation that the large magnitude of the f_2-f_1 and second harmonic distortion changes is a reflection of the high sensitivity of distortion to small operating point changes, as shown in Fig. 3, especially when operating point approaches zero. The mechanism of generation is not fundamentally different but the dependence on operating point is highly nonlinear. At present, a quantitative interpretation of f_2-f_1 changes is not possible. Correlations of f_2-f_1 magnitude with operating point derived from CM recordings are poor, since both responses are collected with different stimulus conditions and involve different cochlear regions. It is likely that the operating points of different cochlear regions will vary according to the properties of the local hair-cell population. At present, we cannot determine the operating point at the specific sites involved in f_2-f_1 generation, at the frequencies and levels of stimulation used to elicit the emission. The theoretical foundation provided by our study may permit a more quantitative approach to the analysis of f_2-f_1 changes in the future.

Our findings with 200-Hz exposure replicate to a con-

siderable degree the findings of Kirk and Patuzzi (1997), although slightly different conditions were used in our study. We delivered the 200-Hz tone at a level of 115 dB SPL, which is higher than that used by Kirk and Patuzzi. It is a level that we have shown consistently produces endolymphatic hydrops in the second turn of the cochlea (Salt, 2003). In addition, our probe tone was delivered at 500 Hz and at 90 dB SPL, compared to Kirk and Patuzzi (1997), who used a 200-Hz tone at 95 dB SPL to obtain CM from the basal turn. We similarly found an increase in operating point with the exposure, consistent with an induced endolymphatic hydrops. Due to the higher level of tone exposure, we find the bounce in AP thresholds after the exposure to be less apparent, which has been reported previously (Salt, 2003).

We initially expected that endolymphatic injections would increase endolymph volume with minimal secondary effects and could possibly provide a well-controlled manipulation of operating point. Instead, we found that injections at rates that are known to produce hydrops caused fluctuations and oscillation of the operating point. Only with the higher injection rates was an increase in operating point apparent. However, after the injection of 1200 nl of artificial endolymph, either in 3 min (400 nl/min) or in 15 min (80 nl/min) the operating point in many animals decreased to a level below the starting value. The cause of this overcompensation is not known. In comparison, for those animals that received a total of 600 nl of artificial endolymph at 200 nl/min, operating point change remained positive in all except one. Operating point changes with injection thus seem to be affected by both the rate and the total volume of artificial endolymph injected.

Furosemide treatment produced large changes of operating point. Given intravenously, operating point showed a consistent decrease, followed by an increase and overshoot. With a local injection of a dose that caused a similar cochlear microphonic decrease, a consistent, but opposite, result was obtained. Operating point increased markedly, then recovered but remained elevated. Furosemide inhibits the Na/K/2Cl cotransporter (NKCC1) found in the marginal cells of stria vascularis and other cell types. This action would be expected to suppress K^+ currents circulating through the endolymph. Given intravenously, furosemide has been shown to reduce the endocochlear potential and to reduce endolymph K^+ and Cl^- (Brusilow, 1976; Rybak and Morizono, 1982; Rybak and Whitworth, 1986). It is likely the ion reductions would be accompanied by water movement out of endolymph and possibly an endolymph volume reduction, which would be consistent with the operating point decrease. However, we cannot conclude that the operating point change reflects an endolymph volume change as other mechanisms may predominate. As there are likely both current and voltage changes at the level of the hair cells, operating point changes may arise by a number of other mechanisms.

It is interesting that locally applied furosemide gave an oppositely directed operating point change. This is likely explained by different cochlear locations being affected by the drug. The first possibility is that different regions along the length of the cochlea may be involved, with intravenous fu-

rosemide affecting the entire length and local injection affecting primarily the base (Schmiedt *et al.*, 2002). It has also been shown that the cell types involved in ion transport processes vary from base to apex (Spicer *et al.*, 2003), so a variation of sensitivity to furosemide with location is possible. Alternatively, it is possible that within any segment of the cochlea furosemide may have access to different cell types when given intravenously compared to when it is given locally. Intravenous drug will have ready access to the vascularized intrastrial space and from there influence the marginal cells. In contrast, when given into scala tympani furosemide must first cross the basal and intermediate cell layer, with associated extremely tight junctions (Jahnke, 1975), in order to reach the marginal cells. Other cell types that contain the NKCC1 cotransporter would likely be exposed to relatively higher doses and may disturb radial ionic movements in a manner different from the suppression of ion transport in the marginal cells. The specific other cell types expressing the NKCC1 cotransporter are spiral ligament fibrocytes of type II, IV, and V, especially in the suprabasal region and in the vicinity of the outer sulcus, and spiral limbus fibrocytes (Crouch *et al.*, 1997; Sakaguchi *et al.*, 1998). If these cell types regulate ion currents in a manner different from the marginal cells, it may account for the different direction of operating point movement.

The acoustic emissions changes we observed with furosemide ($2f_1-f_2$ and f_2-f_1) were comparable to those observed by Mills *et al.* (1993). They found the f_2-f_1 to exhibit changes completely different from $2f_1-f_2$, characterized by sharp minima as we also observed. Based on phase measurements they interpreted the minima as representing zero crossings, an interpretation that our present study supports.

Under some conditions operating point, second harmonic, f_2-f_1 , and SP changes occurred with minimal changes of AP thresholds or of the $2f_1-f_2$ emission. Prior studies have noted that the f_2-f_1 emission and operating point are variable and influenced by a variety of factors that are not normally associated with cochlear sensitivity change. Psychophysical experiments in humans initially showed that the magnitude of the difference tone (f_2-f_1) was highly variable across subjects (Plomp, 1965; Humes, 1979). Modeling studies by Hall (1974) concluded that f_2-f_1 was dependent on the inclusion of asymmetric nonlinearities. With the advent of acoustic emissions, Brown (1988) characterized the f_2-f_1 response, showing that the magnitude decreased as stimulation was prolonged, during which time $2f_1-f_2$ remained stable. Brown also found the f_2-f_1 emission to be susceptible to anesthesia and suggested that the sensitivity change could be an efferent effect. Mountain (1980) found f_2-f_1 emission magnitude to be sensitive to electrical stimulation of the efferent system. Pharmacological manipulation studies by Kirk and Johnstone (1993) also gave support for efferent involvement. In contrast, similar studies of f_2-f_1 variation in rabbits were found to be inconsistent with an efferent involvement (Whitehead *et al.*, 1991). A subsequent study by Kujawa *et al.* (1995) documented changes in f_2-f_1 with the duration of stimulation, but pharmacologic treatments of the cochlea failed to support the

concept that f_2-f_1 was controlled by the efferent system. Based on an analysis of cochlear microphonic during electrical efferent stimulation, Patuzzi and Rajan (1990) also concluded that the stimulation-induced increase in microphonic amplitude occurred with little or no change in operating point. The possibility that distortion was linked to operating point was implied by Mills *et al.* (1993), in their interpretation of f_2-f_1 changes that “went through another apparent zero” during furosemide treatment. This concept was further developed by Frank and Kössl (1996) and Kirk and Patuzzi (1997), in which operating point changes induced distortion changes in a predictable manner based on the known characteristics of cochlear transduction, as discussed above. Combined with our present study, there is good evidence that second harmonic and f_2-f_1 distortion are highly dependent on operating point, but operating point appears to be insensitive to efferent effects.

V. CONCLUSIONS

A dependence of second harmonic distortion and f_2-f_1 emissions on transducer operating point has been demonstrated. The operating points shown by different animals varied prior to treatments, a difference which markedly influences the observed responses to experimental manipulations. Second harmonic and f_2-f_1 distortion are sensitive to manipulations of the endolymphatic system and may provide a means to monitor endolymph volume disturbances.

ACKNOWLEDGMENTS

This work was supported by research grants RO1 DC01368 (A.N.S.) and T32 DC00022 (D.S.) from the National Institutes on Deafness and Other Communication Disorders (NIDCD), National Institutes of Health.

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