Effects of Furosemide Applied Chronically to the Round Window: A Model of Metabolic Presbyacusis

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Hearing thresholds in elderly humans without a history of noise exposure commonly show a profile of a flat loss at low frequencies coupled with a loss that increases with frequency above ~2 kHz. This profile and the relatively robust distortion product otoacoustic emissions that are found in elderly subjects challenge the common belief that age-related hearing loss (presbyacusis) is based primarily on sensory-cell disorders. Here, we examine a model of presbyacusis wherein the endocochlear potential (EP) is reduced by means of furosemide applied chronically to one cochlea of a young gerbil. The model results in an EP that is reduced from 90 to ~60 mV, a value often seen in quiet-aged gerbils, with no concomitant loss of hair cells. Resulting measures of cochlear and neural function are quantitatively similar to those seen in aging gerbils and humans, e.g., a flat threshold loss at low frequencies with a high-frequency roll-off of approximately −8.4 dB/octave. The effect of the EP on neural thresholds can be parsimoniously explained by the known gain characteristics of the cochlear amplifier as a function of cochlear location: in the apex, amplification is limited to ~20 dB, whereas in the base, the gain can be as high as 60 dB. At high frequencies, amplification is directly proportional to the EP on an ~1 dB/mV basis. This model suggests that the primary factor in true age-related hearing loss is an energy-starved cochlear amplifier that results in a specific audiogram profile.

Key words: hearing; aging; gerbil; endocochlear potential; otoacoustic emissions; compound action potentials; presbyacusis

Schuknecht (1974) has described four types of human presbyacusis: (1) sensory, mainly affecting cochlear hair cells and supporting cells; (2) neural, typified by the loss of afferent neurons in the cochlea; (3) metabolic, in which the stria vascularis and lateral wall of the cochlea atrophy; and (4) mechanical, in which there is a stiffening of the basilar membrane and organ of Corti. In a later report, Schuknecht and Gacek (1993) described atrophy of the stria as the predominant lesion in temporal bones of elderly humans and sensory cell loss as being the least important cause of hearing loss in the elderly, especially if confounding factors, such as noise and drug exposures and genetic defects, are eliminated.

The recent findings of Gates et al. (2001) using distortion product otoacoustic emission (DPOAE) and audiogram data support the conclusion that sensory loss is not as prevalent in the aging population as once thought. Indeed, Gates et al. conclude that metabolic presbyacusis is the predominant cause of hearing loss with age. Other animal models that exclude noise history or genetic problems lend support to that conclusion. These models include rabbit (Bhattacharyya and Dayal, 1985), and CBA mice (Spongr et al., 1997). Even old C57BL/6 mice develop strial pathologies (Ichimiya et al., 2000).

Three of the four types of presbyacusis are known to occur in the gerbil model, the one type not yet demonstrated being mechanical. Scattered outer hair cell (OHC) losses are seen in the base and apex of the cochlea in the quiet-aged gerbil (Tarnowski et al., 1991; Schmiedt and Schulte, 1992). Neuronal loss is evident in spiral ganglion cell counts in Rosenthal's canal (Keithley et al., 1989; Slepecky et al., 2000; Suryadevara et al., 2001). Finally, lateral-wall degeneration, most specifically that of the stria vascularis, is almost always seen in quiet-aged gerbils (Gratton and Schulte, 1995; Gratton et al., 1996, 1997). A functional consequence of metabolic presbyacusis is to decrease the quiescent value of the endocochlear potential (EP) in scala media, even while leaving the potassium concentration in endolymph relatively normal (Rybak and Morizono, 1982; Schulte and Schmiedt, 1992; Schmiedt, 1996).

Furosemide is a selective and reversible inhibitor of the EP generator and has often been used to examine the effects of reduced EP on cochlear function (Evans and Klinke, 1982; Sewell, 1984a,b,c; Ruggero and Rich, 1991; Rybak et al., 1992; Mills et al., 1993; Rybak, 1993; Mills and Rubel, 1994; Rubsamen et al., 1995; Mills, 1997a,b). Here we describe the effects of chronic infusion of low levels of furosemide into the cochlea with regard to changes in the EP, DPOAEs, and the population response of auditory-nerve fibers. The population response measure used is the compound action potential (CAP). The furosemide model of metabolic presbyacusis yields results quantitatively similar to those obtained from aged gerbils. Moreover, the model supports the hypothesis that age-related hearing loss in the absence of external insults, such as noise or drug exposure, is primarily the result of a reduced EP rather than loss of sensory cells.

MATERIALS AND METHODS

Animals. This study represents data from 145 Mongolian gerbils (*Meriones unguiculatus*) collected over a period of 7 years. Sixty young animals of between 4 and 8 months of age were implanted with pumps. Data from the young furosemide-treated gerbils were compared with two groups of

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quiet-aged gerbils: the first cohort of gerbils aged 36 months comprised 26 animals, and a second group of 38 gerbils were aged 36 months (n = 27) and between 38 and 45 months (n = 11). The two groups of aged animals were tested ~3 years apart. Control groups were run in parallel with the aged animals and comprised a total of 21 animals aged between 4 and 8 months. Control data for the young gerbils implanted with pumps were obtained from the untreated left ears. Both sexes with healthy external ears were used in these experiments. Animals were born and reared in an acoustically controlled colony in which median sound pressure levels (SPL) were typically <40 dBA. The animal facilities have full accreditation from Association for Assessment and Accreditation of Laboratory Animal Care, and all experimental procedures were approved by the local Institutional Animal Care and Use Committee and met National Institutes of Health guidelines for animal care.

Surgical procedures. The survival surgery for pump implantation was done under sterile conditions. Antibiotics were not used, and none of the animals showed signs of any past or ongoing infections. Animals were anesthetized with sodium pentobarbital (50 mg/kg) and were given atropine to reduce secretions. Alzet (Durect, Cupertino, CA) miniosmotic pumps (model 2004) were used in all of the experiments. These pumps have a fill volume of ~200 μl and a mean pumping rate of 0.25 μl/hr. Under these conditions, they will last ~28 d. Cannulas were surgical-grade silicon tubing. Pumps and cannulas were sterilized, filled, and allowed to equilibrate at 37°C for 2 d before implantation. Pumps were filled with 1, 2, 3, 5, and 10 mg/ml (corresponding to a range of ~3–30 mM) furosemide. The main focus in this report is on animals treated with 5 mg/ml for 7 d, a dosage schedule that yielded physiological data closest to the mean data obtained from our aged gerbils. The pump was placed subdermally behind the scapulae, and the cannula was threaded through holes in the bulla to the round window (RW) niche (Chamberlain, 1977) and fixed in place at the surface of the bulla with dental cement. The bulla was then fully closed with the cement. Incisions were closed with suture, and the animal was allowed to recover in its cage on a heating pad. Any postsurgical discomfort was treated with buprenorphine, but most animals showed no signs of discomfort.

Pumps were allowed to remain in the animal for between 2 and 28 d. The bioactivity of furosemide kept in pumps at 37°C for 28 d was checked with HPLC and found to be stable. Moreover, in a few animals, the cannulas became disconnected from the pumps, allowing controls for cannula placement on hearing thresholds and otoacoustic emissions. In all cases, with chronic implantation of just the cannula, hearing thresholds, EP values, and DPOAE levels were similar to the control ear. During the terminal experiment, placement of the cannula within the RW niche was checked. Data from those few animals wherein the cannula was displaced from the niche were discarded. Finally, the pump delivery of 0.25 μl/hr was easily absorbed through the RW and into the surrounding bone, resulting in a dry middle-ear cavity with no adverse effects on middle-ear acoustics or on the recording of the CAP response.

Surgical procedures for the recording of the CAP response and the EP have been described in detail in previous articles (Schmiedt and Zwislocki, 1977; Schmiedt, 1993, 1996; Hellstrom and Schmiedt, 1996; Schmiedt et al., 1996). Briefly, the animal was anesthetized with sodium pentobarbital (50 mg/kg) and placed in a sound- and vibration-isolated booth that was heated to maintain the cochlea at or near body temperature. Supplemental doses of anesthesia were given as needed. The core temperature of the animal was controlled by a closed-loop DC heating pad. The pinna and surrounding glands were removed, and the bulla was opened widely. The CAP electrode, an Ag–AgCl wire, was placed on the bony rim of the RW niche. CAP potentials were voltage amplified by 10,000 and then lead to an oscilloscope and computer. The acoustic assembly consisting of a probe tube microphone (B&K 4134; Brüel & Kjær, Norcross, GA) and driver (model DT-48; Beyer Dynamic, Farmingdale, NY) was sealed to the bony ear canal with closed-cell foam. In implanted ears, the cannula was left in place during the CAP and EP recordings.

Physiological procedures. CAP thresholds were obtained audiovisually at half octave frequencies from 0.5 to 16 kHz and at 20 kHz. The tone pips were generated in the frequency domain by Tucker-Davis Technologies (Gainesville, FL) equipment and software, and the spectrum was normalized to the average ear canal SPL found in 30 gerbils. The pips had an overall duration of 1.8 msec with a $\cos^2$ rise–fall time of 0.55 msec. CAP input/output (I/O) functions were obtained by averaging 24 epochs using the same tone pips. CAP tuning and suppression boundaries were obtained using the forward masking and unmasking methods described by Dallos and Cheatham (1976, 1977) and Hellstrom and

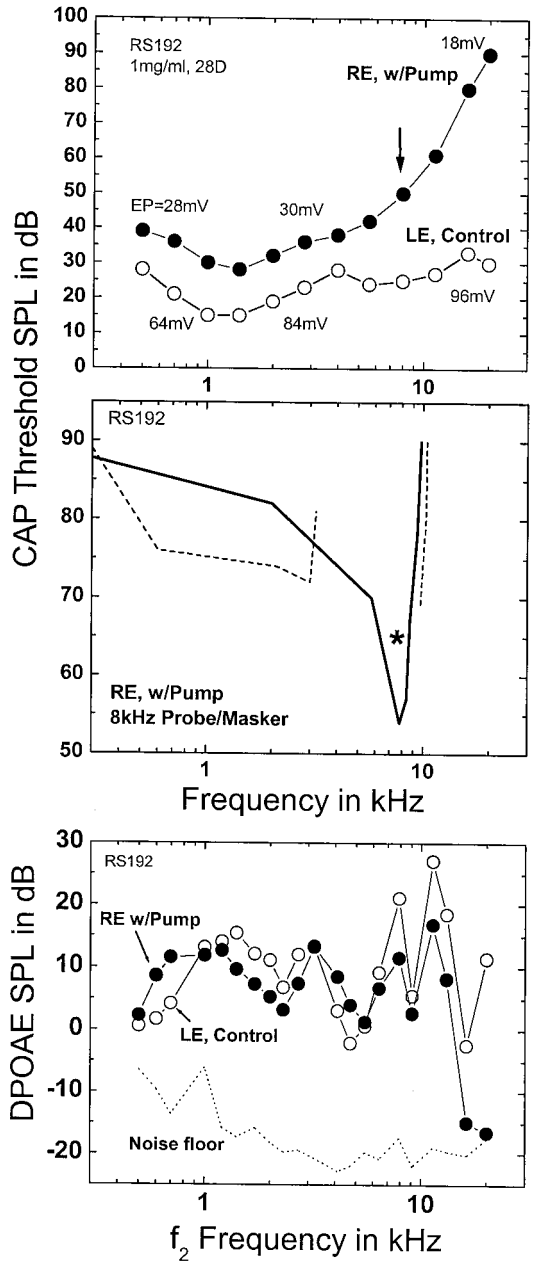

Figure 1. Neural (CAP) responses and DPOAEs in a gerbil infused with furosemide for 28 d. *Top*, CAP thresholds to tone pips as a function of frequency for the right ear (*RE*) treated with furosemide and left, untreated ear (*LE*). The *curves* plot the audibility curves of each ear. Control thresholds are within the normal range for the gerbil. EP values measured in the basal (*T1*; 16 kHz), middle (*T2*; 2 kHz), and upper (*T3*; 1 kHz) turns are shown for each cochlea. Note that the EP was shifted 40–50 mV in the apical turns, yet the corresponding neural shifts were only ~15 dB. In the base, the EP shift was ~70–80 mV and corresponded to a 60 dB shift of the neural response. *Arrow* marks the probe frequency used to obtain the tuning curve data. *Middle*, CAP tuning (*solid line*) and suppression (*dashed lines*) boundaries obtained with masking procedures from the furosemide-treated ear. The probe frequency was 8 kHz, at which neural thresholds were elevated by ~25 dB. The tuning is still sharp, and the suppression boundaries indicate that two-tone suppression is still present, despite an EP reduced to 30 mV. *Bottom*, DPOAE amplitudes obtained in the same ears with low-level primaries fixed at 50 dB SPL ($L_1 = L_2$) and swept across frequency with a ratio of $f_1/f_2 = 1.2$. The resulting DPOAE amplitudes from the treated ear show only minor changes from control values, except at the highest frequencies. *Dotted curve* is acoustic noise floor.

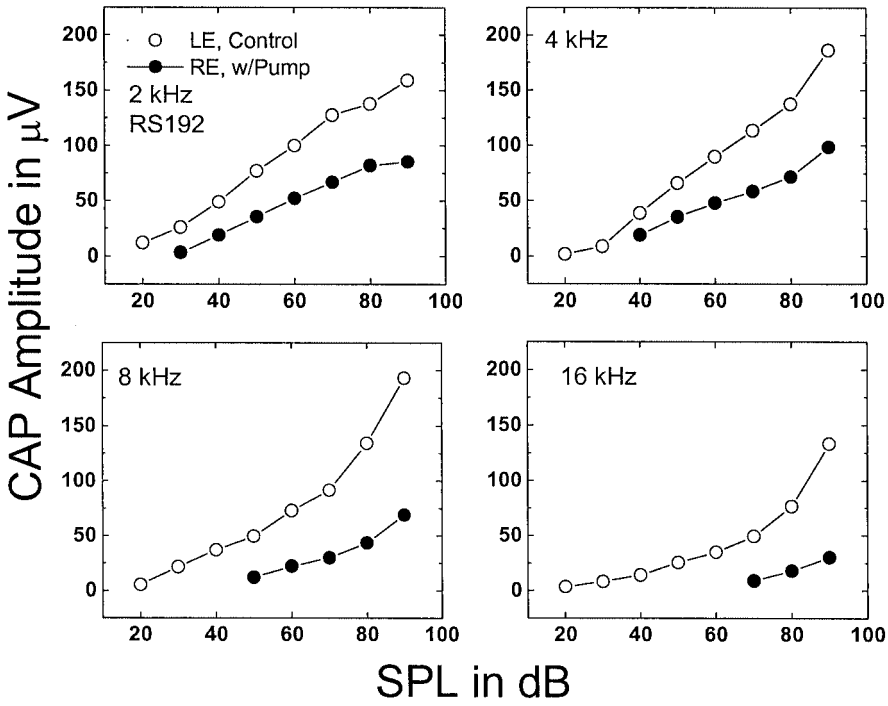

Figure 2. Neural I/O functions plotting the CAP peak amplitude as a function of the intensity of acoustic probe tones at 2, 4, 8, and 16 kHz. Data are from the animal of Figure 1 and represent an average of 24 epochs at each intensity level. All furosemide-treated ears showed similar trends compared with control ears, i.e., reduced slopes with much-reduced maximum amplitudes.

Schmiedt (1996) in the gerbil. The forward masker (and unmasker) were 60 msec tones with 5 msec rise–fall times terminating 10 msec before the standard 1.8 msec exciter tone pip.

Endocochlear potentials were recorded at the RW and in cochlear turns 1, 2, and 3 corresponding to best frequencies of ~20, 16, 2.2, and 1 kHz according to the single-fiber map of Müller (1996). The electrode approach in the cochlear turns was through the bony lateral wall of the otic capsule via a small hole made with a slowly rotating drill bit. EP microelectrodes were filled with 0.2 M KCl and averaged 30 MΩ impedance. EP was always measured as the voltage difference between scala media and a pool of isotonic saline on the neck muscles for each turn.

DPOAEs were measured with an ARIEL (Ariel, Cranbury, NJ) board and CUBeDISP (ETYMOTIC Research, Elk Grove Village, IL) software using the B&K 4134 microphone, probe tube, and frequency equalizer. The intensity levels of both primaries were fixed at 50 dB SPL. Other primary levels were used, mostly $L_1 = 50$, $L_2 = 40$ dB SPL, but the results did not differ substantially from those obtained with $L_1 = L_2 = 50$ dB SPL and are not shown in this report. Primary levels below ~65 dB SPL are known to be quite vulnerable in the gerbil, whereas those above 70 dB are not (Mills et al., 1993). Primary tones were swept from f_2=20.0 to 0.5 kHz with an f_2/f_1 ratio of 1.2 and a resolution of 10 points per octave. For clarity in the figures presented here, every other point was dropped for a resolution of 5 points per octave. Additional methodology for obtaining the DPOAEs can be found by Boettcher and Schmiedt (1995). Complete physiological evaluations, including CAP thresholds and I/O functions, DPOAEs, and EPs were obtained in all of the ears examined in this study, and identical procedures were done in both the left (control) and right ears of each animal.

Morphological procedures. Surface preparations followed the procedures described by Tarnowski et al. (1991) with minor modifications. Briefly, anesthetized animals were exsanguinated by transcardial perfusion with 10 ml of 0.9% saline solution containing 0.1% sodium nitrite, followed by 50 ml of a freshly prepared mixture of 4% paraformaldehyde and 2% glutaraldehyde. The inner ears were then immersed in fixative overnight at 4°C, decalcified with EDTA, and postfixed with a 1% OsO_4–1.5% K_4 $Fe(CN)_6$ solution for 2 hr in darkness. Specimens were dehydrated and embedded in Epon LX112 resin. After partial polymerization, the cochleas were bisected and dissected into half turns, which were reembedded in Epon as a flat surface preparation.

Hair cells were evaluated with a long working distance 40× oil immersion objective (numerical aperture 0.85; Epiplan; Zeiss, Oberkochen, Germany). A calibrated ocular reticule was used to measure cochlear distance. Distances along the cochlear duct were converted to frequencies using the single fiber map of Müller (1996), which is similar to our previous map (Tarnowski et al., 1991). Hair cells were recorded as absent based on spaces created by missing cells. A computer program divided the cochlea into 50 bins of equal length, and an averaging algorithm was applied to calculate hair cell densities. Percentage of loss was calculated from mean data of known hair cell densities obtained from young control cochleas (Tarnowski et al., 1991).

RESULTS

A complete data set obtained from one furosemide-treated cochlea and its contralateral control ear are illustrated in Figures 1–3. In this animal, the furosemide treatment consisted of 1 mg/ml for 28 d. The results from this animal typify data obtained from all of the furosemide-treated gerbils. CAP thresholds and EP values recorded in all three turns of both the treated and control ears are shown in the *top panel* of Figure 1. The control ear showed a normal range of EP values and neural thresholds. In contrast, the EP measured in the treated ear was reduced between 40 and 70 mV from normal, with CAP thresholds greatly elevated at high frequencies but only ~15 dB at low frequencies.

Masked and unmasked CAP tuning and suppression boundaries obtained with an 8 kHz exciter tone are plotted in the *middle panel* of Figure 1. These curves have been shown to be analogs of single-fiber tuning and suppression boundaries (Dallos and Cheatham, 1976, 1977; Harris and Dallos, 1979). Tuning from the furosemide-treated cochleas was sharply defined despite significant threshold shifts at the probe frequency arising from the EP loss. In the example shown here, the threshold shift at 8 kHz is 25 dB compared with the control ear. Coupled with the sharp tuning, two-tone rate suppression was present in the furosemide-treated cochleas until threshold shifts exceeded 40–50 dB. Similar results from single-fiber and CAP responses have been found in quiet-aged gerbils (Schmiedt et al., 1990; Hellstrom and Schmiedt, 1996). Thus, chronic EP-deprived cochleas usually maintain nonlinearities that are derived from OHC function. In contrast, threshold shifts caused by OHC loss are commonly associated with decreased sharp tuning and an absence of suppression (Schmiedt et al., 1980; Dallos, 1992; Robles and Ruggero, 2001).

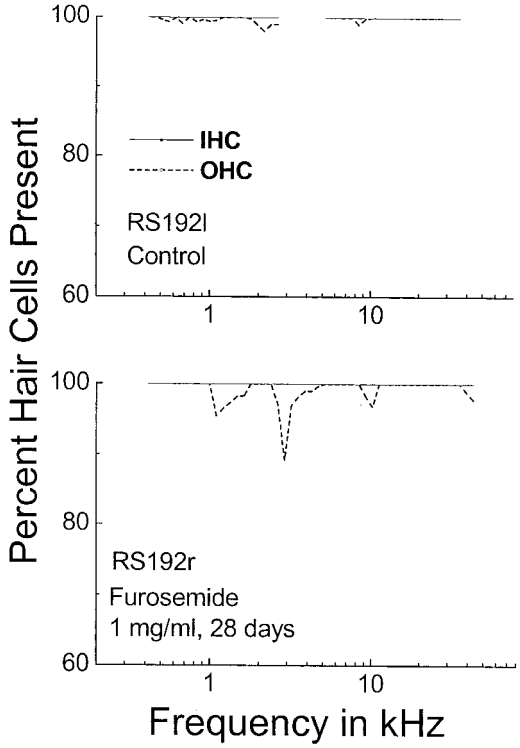

Figure 3. Hair cell counts along the cochlear spiral. Frequency–distance map of the cochlear length is taken from Müller (1996). *Top*, Counts obtained from the control cochlea show almost no loss. Blank region was caused by missed section during processing. *Bottom*, Hair cell loss in the treated ear was very minor and was within normal control bounds. These profiles are similar to the three other furosemide-treated cochleas processed for hair cell counts. There was no evidence that the furosemide at pump concentrations of between 1 and 10 mg/ml affected hair cell survival.

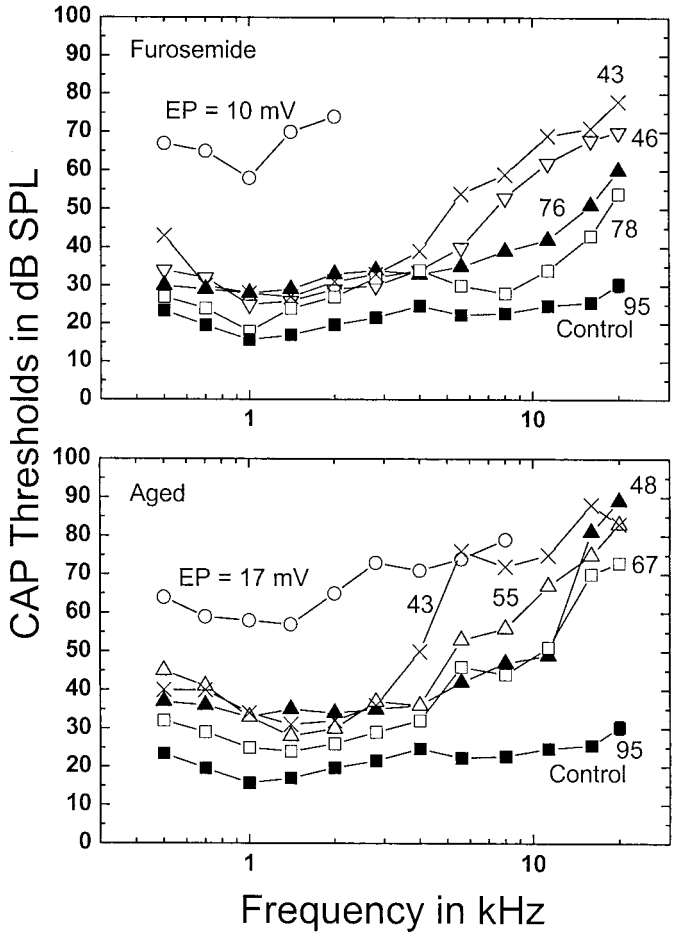

Figure 4. Neural threshold data from furosemide-treated and aged gerbils compared with their respective control groups. EP measured at the round window is shown for each *curve*. *Top*, CAP thresholds obtained from right ears of five gerbils after 7 d of 5 mg/ml furosemide treatment. Control means are from seven control ears; error bars are SEM. *Bottom*, Same as above but for a group of five aged ears. Controls are the same as the *top panel*. Note parallel shift of treated and aged ears at low frequencies coupled with an increasing loss at high frequencies.

The *bottom panel* of Figure 1 plots DPOAE amplitudes from the control and treated ears in this animal as a function of f_2 in the standard "DPgram" format (Schmiedt and Addy, 1982; Schmiedt, 1986; Probst, 1990). As judged by the DPOAE amplitudes (Probst, 1990), OHC function was nearly unaffected by the 50 mV drop in the EP, except at the very basal locations above ~16 kHz at which the DPOAE amplitudes fall near the noise floor of the measuring system. Relatively robust DPOAEs (relative to the threshold shifts) were recorded in all animals of the furosemide group, except in a few cases in which thresholds were raised over 40 dB.

The CAP I/O functions plotted in Figure 2 were obtained from the same animal as shown in Figure 1. CAP I/O functions from treated ears with a lowered EP were always significantly shallower in slope than those from untreated ears, with markedly reduced maximum amplitudes. Like the CAP and DPOAE data shown in Figure 1, these I/O results are very similar to those found in quiet-aged gerbils, in which the EP is reduced to an average of ~60 mV in 36-month-old gerbils (Hellstrom and Schmiedt, 1990; Schmiedt, 1996).

Hair cell counts in the form of cochleograms were obtained in four animals treated with doses of furosemide ranging between 1 and 10 mg/ml for 10–28 d. In all cases, hair cell loss was not significantly different from the control ear, i.e., the hair cell counts were normal in the furosemide-treated ears. Cochleograms from the treated and untreated ears of the animal associated with Figures 1 and 2 are shown in Figure 3. The blank region in the control ear was caused by an unusable plastic-embedded section.

Similarities among profiles of CAP thresholds obtained from five furosemide-treated and five aged ears are shown in Figure 4. Control data for both *panels* are from seven untreated ears from the furosemide set of animals. The *top panel* illustrates the range of thresholds found with 5 mg/ml furosemide applied for 7 d. Individual variability ranged from almost-normal thresholds with a 78 mV EP measured at the RW, to a cochlea with an EP of 10 mV that had only responses to low-frequency tone pips. The overall pattern of the CAP threshold shifts was a constant shift at low frequencies coupled with an increasing shift above ~4 kHz. Data from aged animals plotted in the *bottom panel* of Figure 4 showed similar trends, both in terms of individual variability and the overall profile of the thresholds.

DPOAE amplitudes in the furosemide-treated and aged ears were also similar as shown in Figure 5. The *top* and *middle panels* illustrate the DPOAE data from control ears and from either furosemide-treated (5 mg/ml for 7 d) or aged ears, respectively. The controls for the furosemide group were the untreated ears. Control data for the aged group were obtained from a young cohort in a similar time frame as the aged data. The noise floor of the measuring system is indicated by the *dashed lines* in the *top*

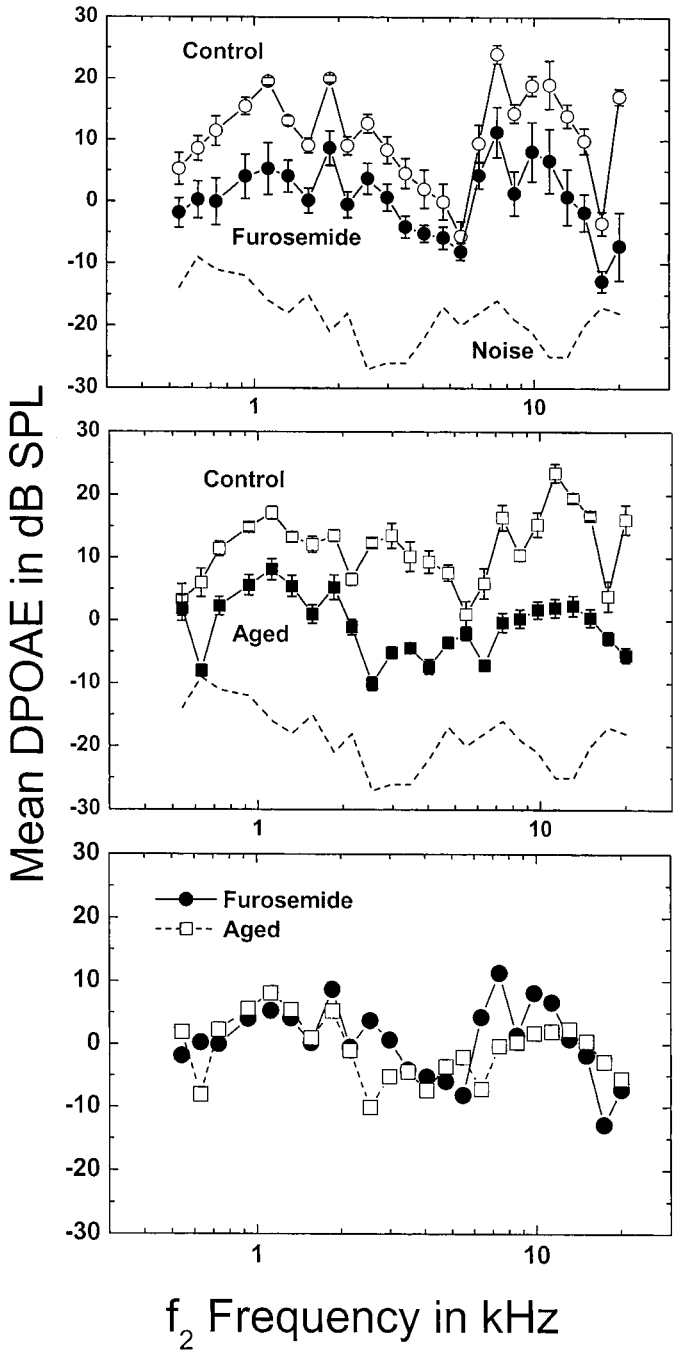

Figure 5. Mean ± SEM DPOAE amplitudes from furosemide-treated and quiet-aged gerbils obtained in a similar manner to those in the *bottom panel* of Figure 1. *Top*, Mean DPOAE amplitudes from six control and 11 furosemide ears from gerbils. *Middle*, Mean DPOAEs from 10 young control ears and 38 gerbils aged between 36 and 45 months. *Bottom*, Comparison of mean DPOAE amplitudes from the *top* and *middle panels*. Note the quantitative similarity of the amplitudes from the two groups. The DPOAE amplitudes are approximately flat across frequency and do not reflect typical neural threshold shifts shown in this figure and in Figures 1 and 7.

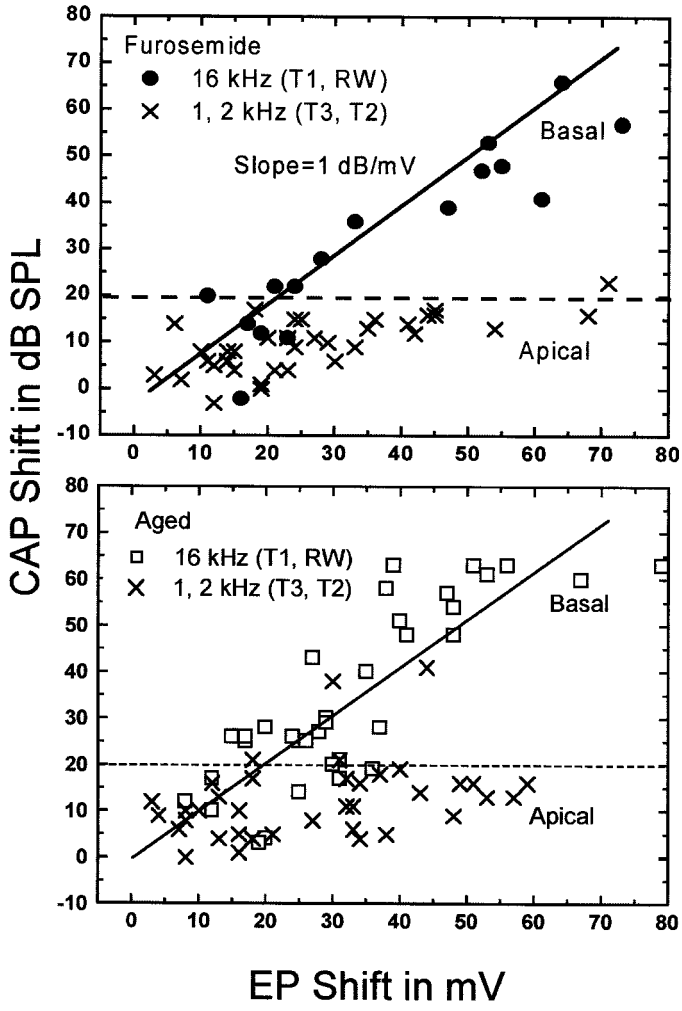

Figure 6. Scatter plots of CAP threshold shifts and EP shifts compared in the same cochlea at similar locations. CAP threshold shifts at 16 kHz are thus plotted with EP measures taken at turn 1 (*T1*) or the RW, whereas CAP shifts at 2 and 1 kHz are plotted with EP measures taken at turns 2 (*T2*) and 3 (*T3*), respectively. Thus, each *point* represents a neural shift plotted against a corresponding EP shift in a given cochlea. *Lines* are not best-fits to data but are drawn either as an upper bound at 20 dB (*dashed*) or with a slope of 1 dB/mV (*solid*). *Top*, Data from 16 furosemide-treated animals; the contralateral, untreated cochlea served as individual control in each animal. *Bottom*, Data from 35 aged gerbils; control data were taken from a group of 10 young cochleas. The 16 kHz data in both groups correlate well with EP loss; however, the 1 and 2 kHz data shift asymptotically to 20 dB for EP reductions >20 mV. The similarity between the data strongly support the EP loss as being the defining factor in the presbyacusis of the quiet-aged gerbil.

and *middle panels*. Note that the DPOAEs, although reduced from the control values, are still robust in both groups. The *bottom panel* directly compares the data from the furosemide and aged groups. Both groups show an approximately flat loss across frequency, despite the increasing neural threshold shifts seen at the higher frequencies in both groups (Fig. 4). DPOAE amplitudes from elderly humans show quantitatively similar trends (Castor et al., 1994).

CAP threshold shifts are plotted against corresponding EP shifts from furosemide-treated gerbils (*top*) or 36-month-old gerbils (*bottom*) in Figure 6. Controls are the untreated ear for the furosemide group and mean data from a young parallel cohort for the aged ears. Each *point* corresponds to a single measure of EP in one cochlea. Data in the furosemide group are shown from all dosage schedules and not just 5 mg/ml. Apical threshold shifts at 1 and 2 kHz are plotted as *X symbols* and are compared with EP shifts obtained in turns 3 (*T3*) and 2 (*T2*), respectively. The best frequencies of these locations according to frequency–distance

maps of the gerbil cochlea are ~1 and 2 kHz, respectively (Tarnowski et al., 1991; Müller, 1996). Similarly, 16 kHz CAP threshold shifts are plotted against EP measures taken in the first turn (*T1*) or through the RW. The *dashed lines* are drawn at a 20 dB shift, which seems to be an upper bound for the low-frequency data. The *solid lines* are not best fits to the basal (high-frequency) data but signify a slope of 1 dB/mV. The basal data primarily follow the 1 dB/mV line for both groups of animals. Interestingly, the CAP threshold shifts at high frequencies of the aged group tend to be on the high side of the *solid line*, whereas the threshold shifts of the furosemide group tend to be on the low side. The greater CAP threshold shifts for the aged animals at high frequencies may be caused by the scattered OHC and ganglion cell loss often seen in the cochlear base of the aged gerbil (Tarnowski et al., 1991). Conversely, it is clear that large decreases in the EP were associated with an asymptotic maximum of ~20 dB of CAP threshold shift in the apical turns. Thus, neural thresholds were highly correlated with EP at high frequencies but shifted only 20 dB, regardless of EP shift at low frequencies.

An overall comparison of mean CAP threshold shifts obtained from the furosemide model to similar mean data from three groups of aged gerbils is shown in the *top panel* of Figure 7. Mean EP values taken at the RW are also shown for each group. Error bars are ±SEM. The 36-month-old animals are split into two groups, with 3 years separating the two groups. The 38-month-old group was a subset of the second 36-month-old group. The furosemide data models very well the aged data with a constant low-frequency loss coupled with an increasing loss at high frequencies. A line is fitted to the furosemide data above 4 kHz with a slope of −8.4 dB/octave. The break point between the *horizontal* (*dashed*) *line* and the *sloped line* is 4.2 kHz in the gerbil data. The increased loss at low frequencies evident in the 38-month-old group is probably the result of scattered OHC loss in the apex of the cochlea, which is often greater than that in the base (Bhattacharyya and Dayal, 1985; Tarnowski et al., 1991).

Human audiograms of elderly males and females with no significant noise history are plotted in the *bottom panel* of Figure 7. The data are from Jerger et al. (1993). The human data follow the same trends as the gerbil models, both aged and furosemide-treated. The low-frequency loss is flat with a break point at 1.3 kHz, at which a steeper loss is apparent. The *solid line* indicates the same slope as that shown in the *top panel* for the gerbils. It has a slope of −8.4 dB/octave and is fitted to the female data at 2 kHz and above. These results suggest that it is possible to distinguish between metabolic and sensory presbyacusis from the shape of the audiogram, i.e., a flat loss at low frequencies with a loss rolling off at approximately −10 dB/octave above ~2 kHz. A steeper roll-off at either high or low frequencies would suggest an additional sensorineural loss.

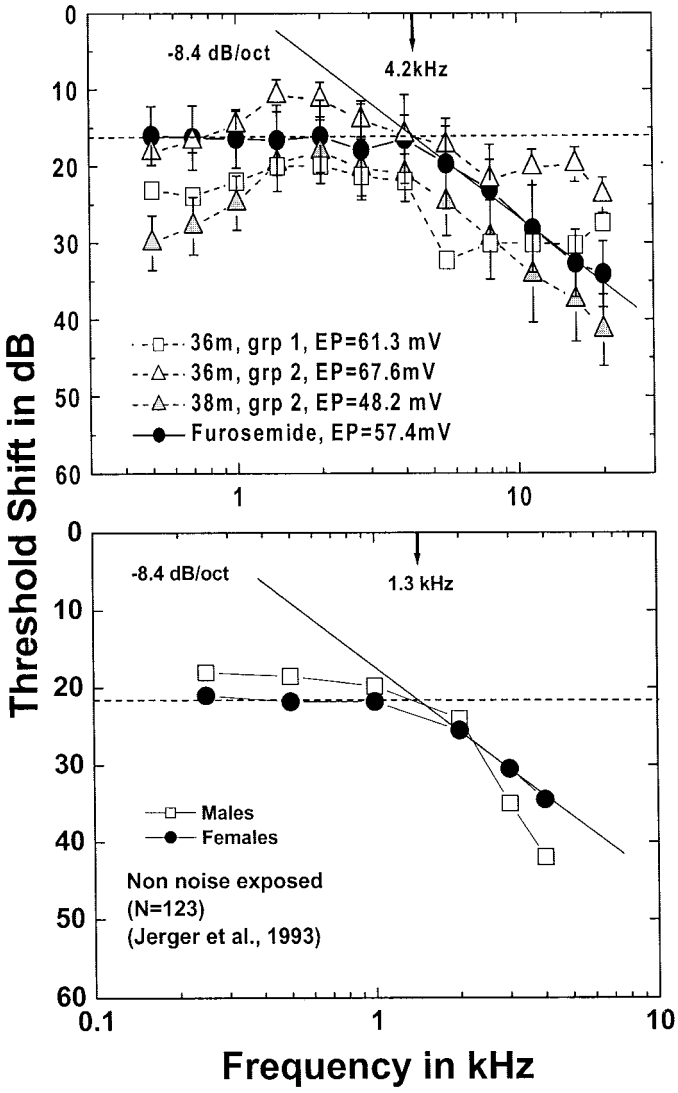

Figure 7. Mean ± SEM CAP threshold shifts in aged and furosemide-treated gerbils compared with human audiograms. Hearing loss is relative to the respective control data for the gerbils. *Top*, Mean data obtained from three groups of aged gerbils are shown. An early cohort of 36-month-old gerbils (*open squares*), a second cohort tested 3 years after the first (*open triangles*), and a third cohort of very old gerbils tested at between ages 38 and 45 months (*gray triangles*). Mean data from 10 gerbils treated with 5 mg/ml furosemide for 7 d are also plotted (*filled circles*). Mean EP values obtained at the RW are shown for each group. Note the relatively flat loss at low frequencies coupled with an increasing loss at high frequencies. *Dashed line* is a best fit through the furosemide data at 4 kHz and below. *Solid line* is best fit through the furosemide data above 4 kHz and has a slope of −8.4 dB/octave. Break point between the *dashed* and *solid lines* is at 4.2 kHz. The increased loss at low frequencies for the 38-month-old gerbils most likely has origins in apical hair cell loss that is seen in these very old gerbils (see Results). *Bottom*, Audiograms from 123 elderly humans without a significant history of noise exposure (from Jerger et al., 1993). *Dashed line* is a best fit for female data at low frequencies. *Solid line* is drawn through the female data at high frequencies with the same slope as found from the furosemide model in *top panel*. *Arrow* indicates the break point in humans at 1.3 kHz.

DISCUSSION

The data presented strongly support the hypothesis that EP reduction is a primary factor in true age-related hearing loss. Our model using furosemide applied chronically to the RW to reduce the EP in a young cochlea quantitatively replicates cochlear function in quiet-aged gerbils, including neural (CAP) threshold shifts, I/O functions, DPOAE amplitudes, and the values of EP measured along the cochlear spiral. The threshold profile of a flat loss at low frequencies coupled with an sloping loss at high frequencies is similar to that found in elderly humans (Gates et al., 1990), especially women and those screened against a previous history of noise and drug exposures (Jerger et al., 1993). Thus, OHC loss, although certainly present in all aging cochleas, is not necessarily the primary cause of the high-frequency hearing loss prevalent in the elderly.

The model assumes that furosemide acts solely on the generator of the EP. However, a caveat in this respect is that Santos-

Sacchi et al. (2001) have shown recently using an isolated OHC preparation that furosemide can have direct effects on OHC function. We believe that the *in vivo* perilymph concentrations of furosemide in our model are much smaller than those used by Santos-Sacchi et al. It is certainly true that furosemide concentrations will be highest nearest the RW, decreasing with cochlear distance toward the apex (Salt, 2001; Salt and Ma, 2001). Using the cochlear diffusion model of Salt (2001) with blood and middle-ear clearance half-lives set to 30 min with direct (as opposed to RW) perfusion into the perilymph at 0.25 μl/hr, steady-state concentrations of furosemide reach ~1 mM on average throughout the perilymph in scala tympani. As expected, the highest concentrations are nearest the RW and can reach ~6 mM, which is approximately where furosemide begins to have direct effects on OHC function (Santos-Sacchi et al., 2001). The cochlear frequencies associated with the highest concentrations are between 20 and 40 kHz, the upper end of the audibility curve for the gerbil and above the frequencies tested here.

On the other hand, arguments against furosemide acting directly on OHC function rather than through decreasing the EP in our model are as follows: (1) the results correspond quantitatively to those obtained with aged cochleas in which the EP is reduced without the use of furosemide; (2) the EP in our model is often reduced to a more or less constant value throughout the cochlea, similar to aged ears; (3) there is no hair cell loss found in the cochleas treated chronically with furosemide for as long as 28 d; and (4) the neural thresholds shifts seen at high frequencies are similar to those reported by Sewell (1984a,b,c) with intravenous injections of furosemide, which minimize its direct effects on OHCs (Santos-Sacchi et al., 2001). The high-frequency CAP–EP shifts found by Sewell essentially followed a 1 dB/mV slope, similar to the data presented here in which furosemide was applied to the RW.

Assuming that furosemide acts mainly on the EP, how can the threshold shift profile found in this model be explained? A parsimonious explanation lies in how the cochlea amplifier (Davis, 1983; Russell, 1983) responds to decreases in the EP along the cochlear spiral. These data can be found in studies of basilar-membrane vibration patterns measured in the base and apex of the cochlea (Cooper and Rhode, 1997; Robles and Ruggero, 2001). The gain of the cochlear amplifier in the base of the cochlea is closely related with and extremely dependent on OHC function and can approach 60 dB in a healthy cochlea. [Gain can be defined in terms of vibration response amplitudes measured in a healthy preparation versus the those found postmortem (for review, see Robles and Ruggero, 2001).] On the other hand, the gain of the cochlear amplifier in the apex is limited to only ~20 dB.

To complete the picture, the relationship between the EP and cochlear amplifier must be examined. This has been done in some detail by many investigators using furosemide as the modulating agent for the EP (Evans and Klinke, 1982; Sewell, 1984a,b,c; Ruggero and Rich, 1991; Mills et al., 1993; Mills and Rubel, 1994; Mills, 1997a,b, 2000) or extrinsic currents (Nuttall, 1985; Xue et al., 1993, 1995; Ren and Nuttall, 1998). These studies have shown a direct relationship of the cochlear amplifier response to the value of the EP. Indeed, Sewell (1984a) showed in cat that CAP thresholds to clicks were almost exactly proportional to the EP in a ratio of 1 dB/mV. Low-CF fibers were least affected by the furosemide, and high-CF fibers were most affected.

Together, these results invoke the following hypothesis: that threshold shifts seen in aged ears are the result of the decreased EP lowering the gain of the cochlear amplifier. In other words, threshold elevations in metabolic presbyacusis are the result of an energy-starved cochlear amplifier. Threshold loss at low frequencies asymptotes at ~20 dB because that is the limit of the cochlear amplifier gain in the apex. Similarly, basal thresholds are much more dependent on the EP because the gain of the amplifier is so much greater in the base, operating on a 1 dB/mV slope over a large dynamic range (Fig. 6). If true, then thresholds may regain normal values if the EP could be renormalized in aged cochleas with hair cell and neural systems that are primarily intact.

Whereas high-frequency neural thresholds are tightly coupled to cochlear amplifier gain, DPOAE levels are not. Indeed, there is a constant shift in the DPOAE levels across frequency in chronic furosemide-treated and aged gerbils (Fig. 5) and in many aged humans (Castor et al., 1994; He and Schmiedt, 1996). Thus, in cases in which EP is chronically decreased, equating DPOAE level shifts to cochlear amplifier gain changes or neural threshold shifts is problematic. On the other hand, the constant decrease of DPOAEs across frequency does match the constant loss of the EP along the cochlear spiral seen with age and chronic furosemide treatment.

Obviously, the inner hair cell (IHC) system is also dependent on the EP for depolarizing currents. With large EP decrements, it is clear the IHC depolarization will be adversely affected, and threshold shifts at low frequencies could become greater than the 20 dB loss associated with the cochlear amplifier (Nuttall, 1985; Rubsamen et al., 1995). This is born out by acute applications of furosemide in which threshold shifts across all frequencies can be much greater than 20 dB (Rybak, 1982, 1993; Sewell, 1984a,b). Perhaps the chronic application of low levels of furosemide allows the hair cell system, especially the IHCs, to adapt to the drop in the EP (Mills et al., 1993; Mills and Rubel, 1994; Mills, 1997a,b), thereby yielding a threshold profile similar to that in quiet-aged gerbils.

The gain profile of the cochlear amplifier as a function of frequency in the gerbil is similar to that found in elderly humans, especially females, resulting in a high-frequency neural loss with a slope of −8.4 dB/octave (Fig. 7). In gerbils, the break point is ~4.2 kHz, whereas in humans it is ~1.3 kHz. Thus, the cochlear amplifier seems much more active at lower frequencies in humans than in gerbil, suggesting that the human cochlea has evolved sharper tuning at lower frequencies than that found in rodents. It is also interesting to note that the ratio of these break point frequencies is approximately equal to the ratio of the cochlear lengths of the gerbil and human, ~12 versus ~33 mm, respectively (Schuknecht, 1974; Tarnowski et al., 1991).

If the slope of the high-frequency roll-off found in gerbils and humans is indeed similar for a given decrement in the EP, then one prediction of our model is that metabolic presbyacusis can be differentiated from sensory presbyacusis by the audiogram profile. Metabolic presbyacusis should yield audiograms like those in the *bottom panel* of Figure 7, in which the slope of the roll-off is approximately −8.4 dB/octave. On the other hand, sensory cell loss (OHC loss) will result in a slope much steeper than that associated with metabolic presbyacusis (Gates et al., 1990; Jerger et al., 1993). At low frequencies, a similar argument can be made. Metabolic presbyacusis should result in an essentially flat loss, whereas apical sensorineural loss will be additive, yielding a profile that slopes toward low frequencies. This sloping loss at low frequencies is seen in our oldest gerbils (Fig. 7), which have substantial hair cell loss in the apex despite being raised in a low-noise environment. Apical hair cell loss is also found in other aging models (Bhattacharyya and Dayal, 1985). Thus, our furo-

semide model of presbyacusis suggests ways in which human audiograms can be interpreted with regard to specific cochlear pathologies. Moreover, it provides insight as to the operation of the cochlear amplifier along the cochlear spiral.

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