Chapter 2
The Physiology of Cochlear Presbycusis

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2.1 Introduction

The effects of pure aging on the physiology and morphology of the human peripheral auditory system are difficult to study given the variability inherent in genetics and the environment with which the system must cope. Environmental exposures accumulated over a lifetime often combine mild, continuous noise exposures occurring daily, with occasional punctate episodes of very high decibel trauma associated with loud music, power equipment, and small arms fire. Moreover, the human experience includes many drugs that often have unintended side effects on the auditory periphery. Some drugs have well-known ototoxic properties; others are more insidious, like the continuous high-level use of some narcotics. Noise and drug injuries tend to preferentially damage the hair cells in the cochlea.

Genetics must then respond to an individual's environment, resulting in the very large variability present in the hearing capabilities of elderly humans. It is clear that animal models of age-related hearing loss are required to tease out the effects of aging alone from the effects of environment and genetics. Yet up until ~25 years ago, much of the research in presbycusis was accomplished by using human temporal bones and clinical data (Bredberg 1968; Schuknecht 1974; Gates et al. 1990; Schuknecht and Gacek 1993). Only in the last 30 years or so have animal models been established where the environment, diet, and genetics are strictly controlled (Keithley and Feldman 1979, 1982; Henry 1982; Keithley et al. 1989; Mills et al. 1990; Hequembourg and Liberman 2001; Ohlemiller and Gagnon 2004; for reviews see Willott 1991; Frisina and Walton 2001, 2006; Gates and Mills 2005; Canlon, Illing, and Walton, Chapter 3). Animals raised under these controlled conditions nonetheless show age-related declines in auditory function, consistent with the notion that presbycusis includes effects unique to aging and is not just the result of the combined effects of noise and other ototoxic factors over a lifetime.

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The deleterious effects of aging are often seen first in highly metabolic tissues in the body, coincident with a degradation of mitochondrial function. Mitochondrial dysfunction with age has been attributed to the buildup of reactive oxygen species (ROS), although this hypothesis still elicits controversy (Gruber et al. 2008). In the cochlea, it is the lateral wall where aerobic metabolism is extremely high because it is needed for maintenance of the K^+gradient between the endolymph and perilymph and the generation of the endocochlear potential (EP). The high K^+ and EP are both present in the endolymph of the scala media. It is not surprising then that there is now substantial evidence that age-related hearing loss uncomplicated by environmental and genetic variables is largely the result of pathologies in the cochlear lateral wall rather than just a general loss of hair cells. This chapter reviews some of the current literature on peripheral presbycusis and how lateral wall dysfunction, leading to a lowered EP, can result in audiograms in animal models that mimic those obtained from elderly humans.

2.2 Overview of Normal Mammalian Auditory Physiology

A concise yet accurate way of understanding normal cochlear physiology and how it breaks down with age is to segregate its functional aspects into three interlocking systems: the cochlear amplifier, its power supply, and the transduction mechanism. The three systems and their relationships are schematized in Fig. 2.1. The discussion here is necessarily brief and relates only to those ideas important for understanding the pathologies relating to presbycusis. Further details can be found in the cited references.

2.2.1 Cochlear Amplifier

The cochlear amplifier relies on an active process located in the outer hair cells (OHCs) to physically amplify the traveling wave vibrations along the basilar membrane (Davis 1983; Russell 1983; Cooper and Rhode 1997; Robles and Ruggero 2001). The amount of amplification is highly dependent on a potential (voltage) between the scala media and scala tympani, thereby present across the OHCs. This voltage is the EP, which is ~90 mV within the scala media when referenced to a neck muscle ground. Indeed, the amplification dependency is logarithmic-linear such that about a 1-dB decrease in amplification (corresponding to a 1-dB increase in threshold) results from a 1-mV decrease in EP (Sewell 1984; Ruggero and Rich 1991; Schmiedt 1993). The basilar membrane amplification from the active OHCs also shows a strongly compressive nonlinearity: vibrations from low-level sounds are amplified most, whereas those from intense sounds are amplified least. This compression of dynamic range at the level of the basilar membrane results in a relatively constant vibratory stimulus exciting the inner hair cells (IHCs) over a wide range of acoustic

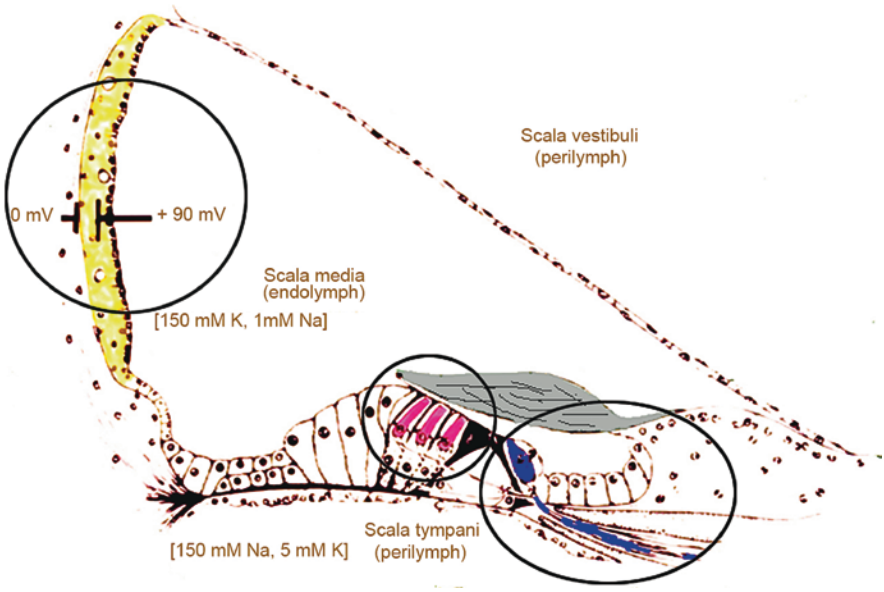

Fig. 2.1 Schematic cross section of a single turn of the cochlea. The three systems underlying basic cochlear function are outlined (circles). The left circle focuses on the lateral wall and stria vascularis and the production of the 90-mV endocochlear potential (EP) present in scala media. The middle circle centers on the outer hair cells (OHCs) and the micromechanics involved in the cochlear amplifier. The right circle is drawn around the inner hair cell (IHC) and the associated primary afferent nerve fibers that make up the transduction process where mechanical vibrations are transduced to neural impulses that are sent to the brain via the auditory nerve. (Adapted with permission from Mills et al. 2006b.)

intensities (Robles and Ruggero 2001) and is also the basis for two-tone suppression. Thus a healthy cochlea is strongly nonlinear in its response to signal intensity and multiple frequencies, resulting in various suppression phenomena and otoacoustic emissions (OAEs). (OAEs are acoustic distortion products that can be measured in the ear canal at frequencies that result when two tones are combined in a nonlinear fashion [Probst 1990]. The strongest in the ear are the cubic difference tones corresponding to frequencies of $2f_1 - f_2$.)

A final factor in understanding the normal cochlear amplifier is that its maximum gain varies along the basilar membrane. In the cochlear apex tuned to lower frequencies, the gain is only ~20 dB, yet in the base, the gain can be as high as 50-70 dB (Ruggero and Rich 1991; Mills and Rubel 1994; Cooper and Rhode 1997; Robles and Ruggero 2001; see Fig. 2.2a, b). Thus if cochlear amplification is totally lost, either from OHC loss or from a very low EP, one would expect to see the least effect at low frequencies and the most at high frequencies. This relationship is borne out in gerbil ears treated chronically with furosemide to artificially reduce the EP as well as in quiet-aged ears with a naturally reduced EP (Schmiedt et al. 2002b; Fig. 2.2c, d).

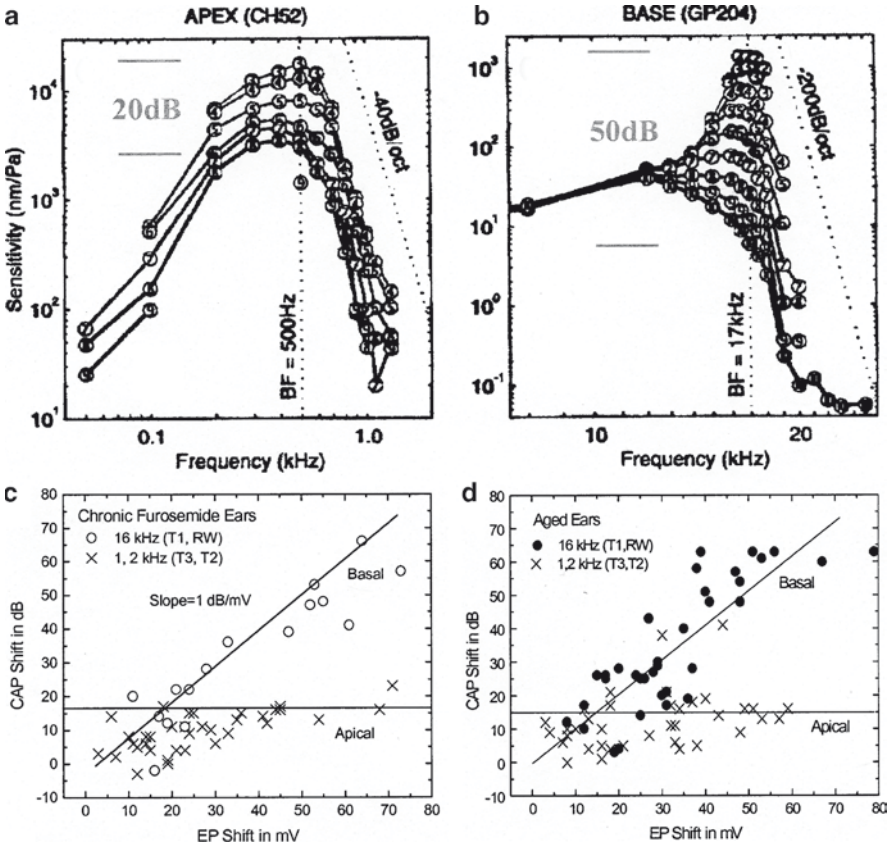

Fig. 2.2 Effects of cochlear location and condition on the vibration amplitudes of the basilar membrane in the apex of a chinchilla cochlea **(a)** and the base of the guinea pig cochlea **(b)**. Two species were used to allow the best recordings from the apex and base. The top lines were obtained first with the OHC amplifier in good condition. The bottom lines were obtained after cochlear death. Note that the apical gain from the OHC amplification is ~20 dB, whereas that from the base is 50 dB. BF, baseline frequency. (Adapted with permission from Cooper and Rhode 1997). Neural shifts in threshold derived from the compound action potential (CAP) response at 1 and 2 kHz (apical) and 16 kHz (basal) are plotted against shifts in the corresponding EP measured in gerbil ears chronically treated with furosemide **(c)** and ears aged 36 months **(d)**. Furosemide is a drug that reversibly decreases the EP and allows studying cochlear function under conditions of lowered EP but with normal hair cells and neurons in a young adult animal (see text; Schmiedt et al. 2002b). **c** and **d** emphasize the points made in a and b; i.e., the OHC amplifier gain at low frequencies is only ~20 dB, whereas at higher frequencies, it is around 50-60 dB. Additionally, **c** and **d** show that the gain in decibels is a linear function of the EP in millivolts at basal locations in the cochlea. The straight lines are not best fits but have slopes of one to show the asymptotic threshold shift for the low-frequency data and unity to show the linear relationship with EP for the high-frequency data. (Adapted with permission from Schmiedt et al. 2002b.)

2.2.2 Cochlear Power Supply

The second system is the cochlear power supply comprising the lateral wall tissues, including those of the stria vascularis where the EP is generated. This power supply is intimately related to the K^+-recycling pathway, which actively pulls K^+ back into the endolymph as it is effluxed from the hair cells into the perilymph. The pathway uses a network of supporting cells and fibrocytes (specialized cells that can turnover and often have stem cell precursors) along the basilar membrane and lateral wall, respectively, connected by gap junctions (Spicer and Schulte 1991, 1996; Marcus and Chiba 1999). A final step in K^+ recycling is the actual generation of the EP within the stria vascularis (Salt et al. 1987; Wangemann et al. 1995; Marcus et al. 2002; Wangemann 2002; Schulte 2007).

Note that K^+ recycling works against both concentration and electrical gradients: the K^+ concentration in the endolymph is ~150-170 mM compared with ~1 mM in the perilymph, and the potential present in the endolymph is ~90 mV (the EP) as compared with the 4-mV potential in the perilymph (Salt et al. 1987; Schmiedt 1996). Thus pushing K^+ along this route takes energy that is largely generated by Na^+-K^+-ATPase pumps in concert with the Na^+-K^+-$2Cl^-$ (NKCC) transporter (Wangemann 2002). The NKCC transporter is an important tool in our studies of the effects of EP changes on auditory function in that furosemide, a fairly specific, reversible antagonist against NKCC, provides a means to experimentally turn off and on the recycling pathway and subsequently the EP (Evans and Klinke 1982; Sewell 1984; Schmiedt et al. 2002b; Mills and Schmiedt 2004). Furosemide delivered either intravenously or via a round window application can reduce the EP to near 0 mV, with recovery from a single dose taking between tens of minutes to over a month if osmotic pumps are used for delivery (Sewell, 1984; Mills and Rubel 1994; Schmiedt et al. 2002b).

The EP serves as the cochlear battery. It is generated within the stria across the intrastrial space and is present in the endolymph along the entire cochlear duct (Wangemann 2002). (Note that EP generation in the stria is dependent on the ion flux provided by the fibrocytes in the lateral wall. In this context, strial and lateral wall pathologies can both result in a lowered EP.) The EP is produced largely by the stria in the basal turn where it is the highest and drops by ~10 mV in the more apical turns of the cochlea. Destruction of the stria or lateral wall in the basal turn results in a significantly lowered EP throughout the cochlear spiral, whereas destruction of the stria in the higher turns with an intact basal stria yields relatively minor reductions in the overall EP (Salt et al. 1987; Wu and Hoshino 1999). Thus apical strial pathology, as often seen with presbycusis, does not necessarily correlate with significantly lowered EP values, whereas basal atrophy is highly correlated with a reduced EP.

2.2.3 Cochlear Transduction

The third system in the transduction of cochlear vibration to neural impulses comprises the IHCs and the afferent fibers of the auditory nerve (see Fig. 2.1).

The IHCs function as passive detectors of basilar membrane vibration and excite afferent fibers via ribbon synapses around the base of the cell (Robles and Ruggero 2001). IHCs are more resistant to noise and chemical trauma than the OHCs and tend to survive with comparatively less pathology in aged ears. Even so, in animals raised their entire lives in quiet (quiet-aged ears), there is a significant loss and shrinkage of the afferent nerve fibers and their cell bodies, the spiral ganglion cells (SGCs) in Rosenthal's canal. The loss and shrinkage with age occur even with the IHCs present and seemingly normal both in animal models and in humans (Schuknecht 1974; Keithley and Feldman, 1979, 1982; Mills et al. 2006a).

In young healthy ears that have been raised in quiet, afferent fibers can be segregated into two or three groups corresponding to spontaneous rates (spont) and sensitivity (Liberman 1978; Schmiedt 1989). Typically, the most sensitive fibers have high rates of spontaneous activity (high-spont, 18 spikes/s and higher), with somewhat less sensitive fibers forming a middle group with spontaneous rates from 0.5 to 18 spikes/s (medium spont). The third group comprises the low-spont fibers with sensitivities that can be up to 50-60 dB lower than those of the high-spont group and have spontaneous rates below 0.5 spikes/s. Thus the sensitivity range of the three groups of afferents in young ears largely covers an intensity range between 0 and 90 dB SPL.

2.3 Schuknecht's Four Types of Presbycusis

Schuknecht (1974) has described four types of human presbycusis: (1) sensory, mainly affecting the cochlear hair cells and supporting cells; (2) neural, typified by the loss of afferent neurons in the cochlea; (3) metabolic, where the lateral wall and stria vascularis of the cochlea atrophy; and (4) mechanical, where there seemed to be a so-called "stiffening" of the basilar membrane and organ of Corti. To date, no real evidence has been found that the mechanical structure of the organ of Corti stiffens with age. The diagnoses of a mechanical presbycusis was derived from a flat loss of 30-40 dB in hearing threshold and was often coupled with degeneration in the spiral "ligament" along the cochlear lateral wall. The spiral ligament originally was thought to offer structural support to the basilar membrane (thus the descriptive term ligament); however, the spiral ligament is now known to consist largely of ion-transport fibrocytes involved in the recycling of K^+ efflux from the hair cells back to the endolymph. Thus it is very likely the mechanical presbycusis described by Schuknecht is simply a severe case of metabolic presbycusis. Indeed, animals with very low EP often show a flat audiometric loss of 40 dB and greater at low frequencies, similar to that ascribed to mechanical presbycusis.

In a later report, Schuknecht and Gacek (1993) described atrophy of the stria to be the predominant lesion in the temporal bones of elderly humans and sensory cell loss as being the least important cause of hearing loss in older humans, especially if the confounding factors of noise, drug exposure, and genetic defects are eliminated. The recent results of Gates et al. (2002) 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. (2002) and Gates and Mills (2005) conclude that metabolic presbycusis is the predominant cause of human hearing loss with age. Many animal models that exclude noise history or genetic mutations lend support to that conclusion. These models include chinchilla (Bhattacharyya and Dayal 1985), rabbit (Bhattacharyya and Dayal 1989), and CBA mice (Spongr et al. 1997). Even C57 and other mutant mice, if actually aged, develop strial pathologies (Ichimiya et al. 2000; Hequembourg and Liberman 2001; Ohlemiller and Gagnon 2004; Ohlemiller et al. 2008). CBA/J mice, however, seem to show only a hair cell loss with a fairly intact lateral wall with age as discussed in Section 2.4 below (Sha et al. 2008).

2.4 Sensory Presbycusis

Loss of sensory hair cells in the human aging ear is well documented (Bredberg 1968; Schuknecht 1974; Gates and Mills 2005). Indeed, morphologically, hair cell loss is one of the most apparent changes in temporal bones both in humans and in animals of advanced age (Dayal and Bhattacharyya 1989). Species studied include rabbit, guinea pig, cat, rats of various genetic backgrounds, chinchilla, mice of various genetic backgrounds, gerbil, and primate (see Willott 1991 for review). The other universally noticeable pathological change in aged temporal bones is the shrinkage and loss of SGCs in Rosenthal's canal, so it is understandable that presbycusis is commonly thought to be of "sensorineural" origin by many in the field of hearing.

When human audiograms were matched to temporal bone pathologies, it seemed clear that the high-frequency loss so often seen in presbycusis matched the OHC loss in the basal coil of the cochlea (Bredberg 1968; Johnson and Hawkins 1972; Schuknecht 1974). A caveat here is that excess noise exposure is commonplace in western society, and many people, especially men, have been exposed throughout life. Thus the underlying cause of the hair cell loss is problematic. Animals aged in quiet also lose hair cells but more at the apex than at the base of the cochlea. Thus the cochleograms often take on the shape of an inverted "U" (Dayal and Bhattacharyya 1989; Tarnowski et al. 1991). The OHC loss is typically scattered with most, if not all, the IHCs surviving. When neural or behavioral audiograms from the animal models are compared with the OHC loss, there is often a poor correlation.

Fig. 2.3 is an illustration of this last point. Shown in the four panels are the cochleograms and neural threshold shifts of four quiet-aged gerbils. The neural thresholds were obtained with the compound action potential (CAP) response. In all cases, there is a significant scattered OHC loss at the apex, with little or no IHC loss. All the threshold shifts have little relationship to the OHC loss.

Some mouse and rat models do show profound sensory losses with age. They are typically mice with a C57BL/6J background, which has a genetic mutation where hair cell loss begins a few months after birth (Spongr et al. 1997;

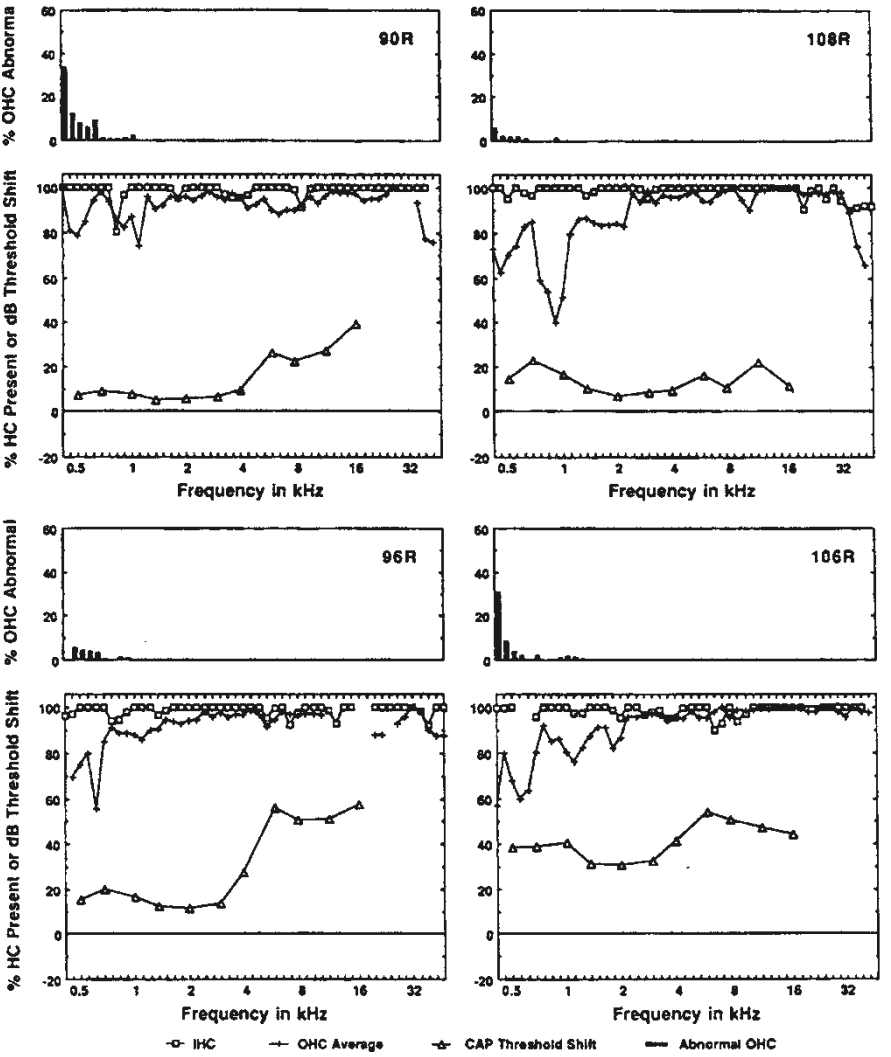

Fig. 2.3 Plots of hair cell condition, hair cell loss, and neural threshold shifts in 4 quiet-aged gerbils at 36 months of age. Percent of IHCs (open circles) and OHCs present (+) and the dB shift of the CAP (open triangles) are plotted against frequency using a gerbil frequency-distance map (Schmiedt and Zwislocki 1977). Note that OHC abnormalities and losses can be significant but are scattered and located mostly in the cochlear apex. IHCs are well preserved throughout the cochlear spiral and CAP shifts have little relationship to hair cell loss, especially with regard to frequencies above 3 kHz. (Adapted with permission from Tarnowski et al. 1991.)

Hequembourg and Liberman 2001). The OHC loss is nearly 100% and progresses from base to apex, with the IHC survival somewhat more robust than that of the OHC. The C57 mouse has been used extensively as a model for sensory presbycusis

and the genetic mutation responsible for the hair cell loss is often termed the "age-related hearing loss" mutation (Johnson et al. 1997). Although important as a model of sensory loss, the age-related hearing loss mutant is problematic with regard to being a true aging model. In the human situation, if a teenager is diagnosed with a progressive high-frequency loss caused by sensory cell degeneration, it is unlikely the condition would ever be called presbycusis.

The CBA/J mouse is a model with true sensory presbycusis (Sha et al. 2008). In this model, the sensory cells are progressively lost from the apex with some loss in the base, with little strial involvement (Lang et al. 2002). In both C57BL/6J and CBA/J mice, the EP remains normal throughout the life span of the animal, although subtle changes in the lateral wall of the C57BL/6 mice have been reported (Ichimiya et al. 2000; Hequemberg and Liberman 2001). Both these models with substantial IHC losses have neural losses with age similar to those found in other mutants without the increased IHC loss, i.e., the neural presbycusis seems not to depend directly on the survival of the IHCs. It is interesting to note that the hearing thresholds of the CBA/J model obtained from auditory brainstem recordings (ABRs) are often not well correlated with the hair cell loss, similar to findings obtained from the gerbil (Fig. 2.3). Finally, not all mice exhibit sensory presbycusis. There are some mutants, such as BALB/cj and NOD/ShiLtJ mice, that do show a decrease in EP with age (Ohlemiller et al. 2006, 2008). Given the mutant data, it is of great interest that wild-caught mice have similar patterns of hair cell loss with age as those of gerbils (Dazart et al. 1996).

2.5 Metabolic Presbycusis

2.5.1 Audiometric Data

For reference, audiograms from human subjects between the ages of 50 to more than 85 years of age are shown in Fig. 2.4. The profile of the hearing loss comprises a flat loss of between 10 and 40 dB at frequencies below ~1.5 kHz, coupled with a sloping loss at higher frequencies. In men, the high-frequency hearing loss is greater than in the women with a correspondingly steeper slope. If subjects are screened for noise history, this gender discrepancy is minimized (Jerger et al. 1993). Thus the audiograms from men are probably a mix of pure aging and cumulative noise exposure with concomitant excessive OHC loss. Note from the discussion on the cochlear amplifier that a complete loss of the OHCs in the base should lead to a flat hearing loss of between 50 and 70 dB above ~4 kHz, which is evident in the male audiograms, but not in those of the females.

The audiogram profile found in humans is also found in many animal models. Fig. 2.5 shows audiograms from an aged chinchilla, aged SJL/J mice, and three groups of gerbils raised in quiet. All show a flat loss at low frequencies coupled with a sloping loss at higher frequencies.

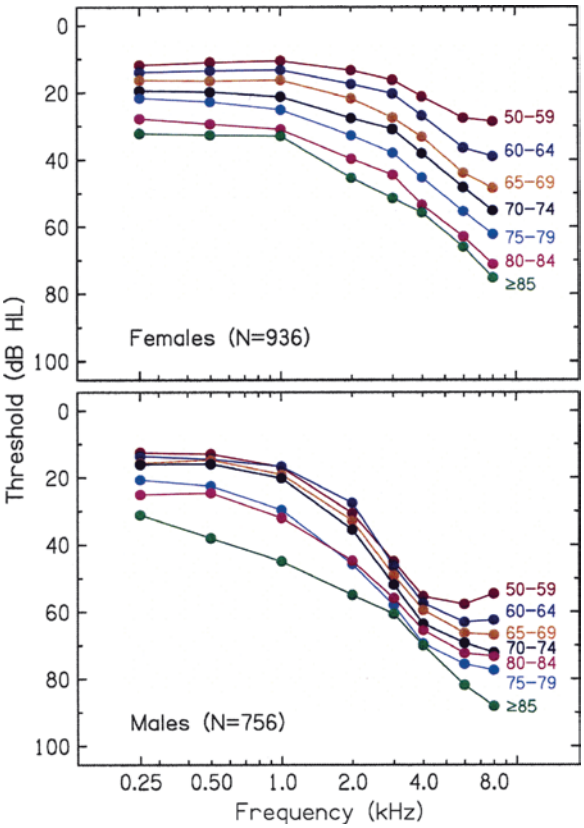

Fig. 2.4 Audiometric mean hearing losses (HL) in female (top) and male (bottom) participants in the ongoing study of age-related hearing loss at the Medical University of South Carolina (Lee et al. 2005; Dubno et al. 2008). The parameter is subject age at the time of enrollment. Note the characteristic profile of human age-related HL: a flat loss at low frequencies coupled with a sloping loss at frequencies above ~1 kHz. These subjects were not screened for noise history, and men typically show more threshold shifts at high frequencies than women, presumably from additional noise exposure (Jerger et al. 1993). Screening for noise history tends to minimize the gender difference. (Adapted with permission from Mills et al. 2006b.)

Individual hearing loss (HL) data are shown for five quiet-aged gerbils in Fig. 2.6 top. (Note that these data have been normalized to young-adult average thresholds that is represented by the 0-dB line and the 90-mV EP.) The EP decreases with age in the gerbil concomitant with a loss of strial volume and Na^+-K^+-ATPase activity along the lateral wall and stria (Schulte and Schmiedt 1992; Gratton et al. 1996, 1997; Spicer et al. 1997). Again, we see the standard presbycusic profile, which is also evident in some of the threshold shift curves plotted in Fig. 2.3. There is little or no correlation of these curves to the OHC loss in any of these animals. However, if we plot the curves with regard to the EP values found in the basal turns of the individual cochleas, a clear pattern emerges. The high-frequency loss is highly correlated with the amount of EP reduction. It is rare that the EP falls below

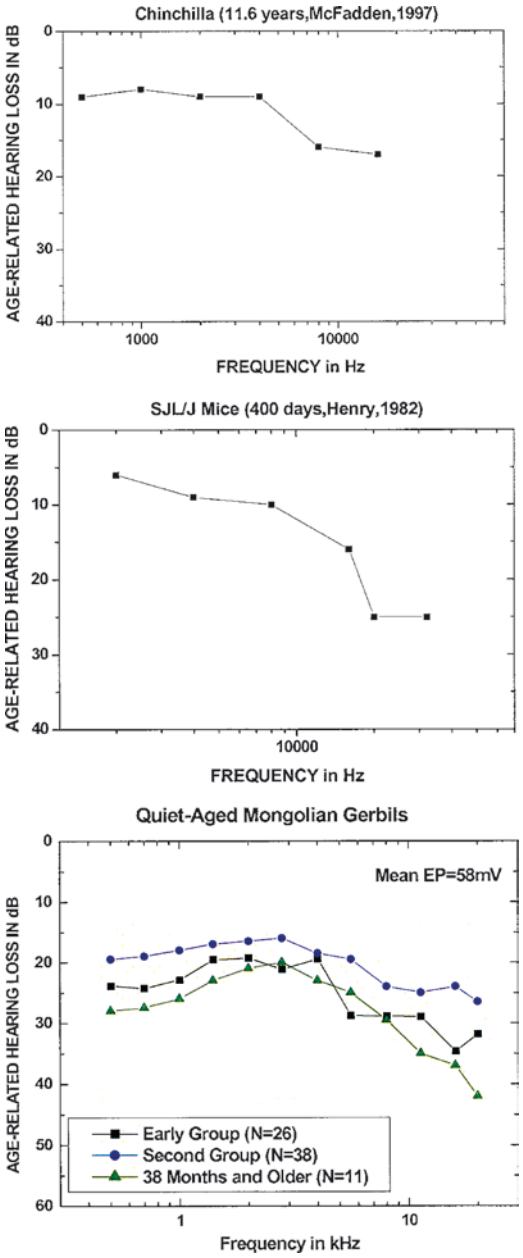

Fig. 2.5 Behavioral and neural hearing losses in three animal models. Top: neural evoked potentials in an 11.6-year-old chinchilla (data redrawn from McFadden 1997a). Middle: mean thresholds from the auditory brainstem response (ABR) in a group of SJL/J mice at 400 days of age (data redrawn from Henry 1982). Bottom: mean CAP thresholds of 3 groups of 36-month-old, quiet-aged gerbils (adapted from Schmiedt et al. 2002b). The early and late groups of gerbils were raised from different genetic stock, whereas the 38-month-old group comprised 11 animals from the late group that were aged an additional 2 months. These models show the classic profile of age-related hearing loss seen in humans: a small flat loss at low frequencies coupled to a sloping loss at higher frequencies. Some of the increased loss at low frequencies in the 38-month group may be the result of excess losses of apical OHCs seen with extreme age (see Fig. 2.3).

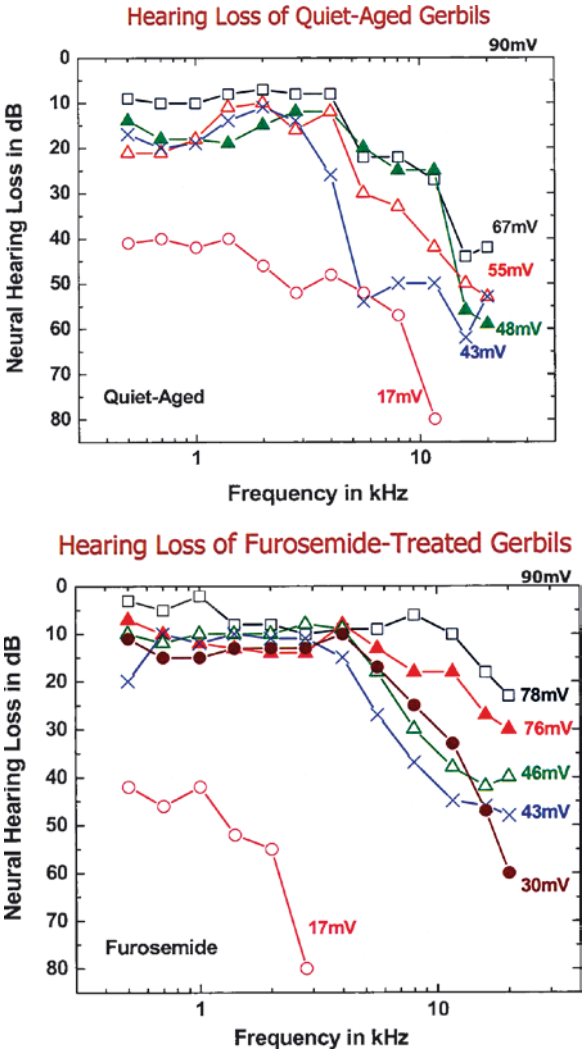

Fig. 2.6 HL profiles for individual ears from 36-month-old gerbils (top) and furosemide-treated ears from young gerbils (bottom). The aged and furosemide curves are normalized to the mean thresholds of young adult control animals. The EP recorded in the base of the cochlea is shown in mV for each animal. Note that young normal control animals have an EP of ~90 mV in the base of the cochlea as represented by the 0-dB HL abscissa. Furosemide was delivered to the round window of one ear via a cannula led from an osmotic pump. The opposite ear served as a control. The HL at high frequencies is well ordered by the amount of EP loss, whereas the loss at low frequencies is largely independent of EP as suggested by Fig. 2.2c, d. When EP drops below 25 mV, the thresholds even at low frequencies correspondingly increase, probably because the very low EP inhibits the IHC transduction process (Schulte and Schmiedt 1992). These 40-dB and greater flat losses may be analogs to the mechanical presbycusis described by Schuknecht (1974).

~40 mV, but when it does, the hearing loss even at low frequencies is greater than the 20-dB loss ascribed to the OHC amplifier. One hypothesis is that this excess loss is probably caused by an IHC transduction process that has been desensitized by the very low EP. The resulting flat 40-dB loss at low frequencies is similar to that seen in some cases of "mechanical" and neural presbycusis. One hypothesis is that these profiles represent an extreme form of metabolic presbycusis.

To test the hypothesis that the EP is the main variable in metabolic presbycusis and in the shaping of the audiogram in age-related hearing loss, furosemide was used to chronically lower the EP in one ear of a young gerbil (Schmiedt et al. 2002b). Furosemide is a potent but reversible inhibitor of the NKCC transporter and is well known to specifically block generation of the EP. Furosemide was applied to the intact round window via a cannula attached to an osmotic pump. The pump was placed between the scapulae, the cannula was led through the bulla, and the bulla was resealed with dental cement. The pumps could be sized to deliver the furosemide for up to one month. Cochleograms obtained from the pump animals showed almost no loss of hair cells and fairly normal strial morphology after several days of chronic exposure. To best mimic the EP loss seen in the 36-month-old gerbils, 5 mg/ml of furosemide were chronically delivered for seven days at a flow rate of 0.25 μl/h. The result is an animal model with essentially one young ear and one old ear. The similarity of the furosemide ear to an aged ear is remarkable in its breadth, from single-fiber responses and otoacoustic emissions to audiometric data (Schmiedt et al. 2002b).

Audiometric data obtained from the furosemide model are shown in the bottom panel of Fig 2.6. The audiometric profiles match those of the quiet-aged data in Fig. 2.6, top. Likewise, the profiles are ordered by the EP parameter. Another interesting point is that the furosemide dose was the same for all the young animals, yet the variation between the treated animals is similar to that seen with quiet-aged gerbils at 36 months of age. Perhaps the resistance or lack thereof to the furosemide threshold shift is somehow predictive of the amount of age-related hearing loss of an individual at a given age?

If the mean CAP threshold shifts obtained from the quiet-aged and furosemide-treated gerbils are compared, there is good quantitative agreement between the two groups (Fig. 2.7, top). The slope of the high-frequency roll-off for the furosemide data is −8.4 dB/octave, with a breakpoint of 4.2 kHz. If the hypothesis of the EP-controlled OHC amplifier gain is correct, this slope and breakpoint represent the distribution of the OHC amplifier gain along the cochlear spiral. This gain distribution may be expected to differ among species depending on the frequency-distance map of the particular cochlea.

Fitting the mean furosemide gerbil data to that of the human audiometric profile is shown in the bottom panel of Fig. 2.7. The human data are from two sources and have been screened for noise history to minimize the effects of OHC loss. The breakpoint for the human data appears to be around 0.9 kHz and the slope might be somewhat steeper than that of the gerbil data, suggesting that the OHC amplifier gain distribution along the cochlear spiral for the human is biased toward lower frequencies than in the gerbil. The main point of comparison, however, is that the overall audiometric profiles of the quiet-aged and furosemide-treated gerbils and

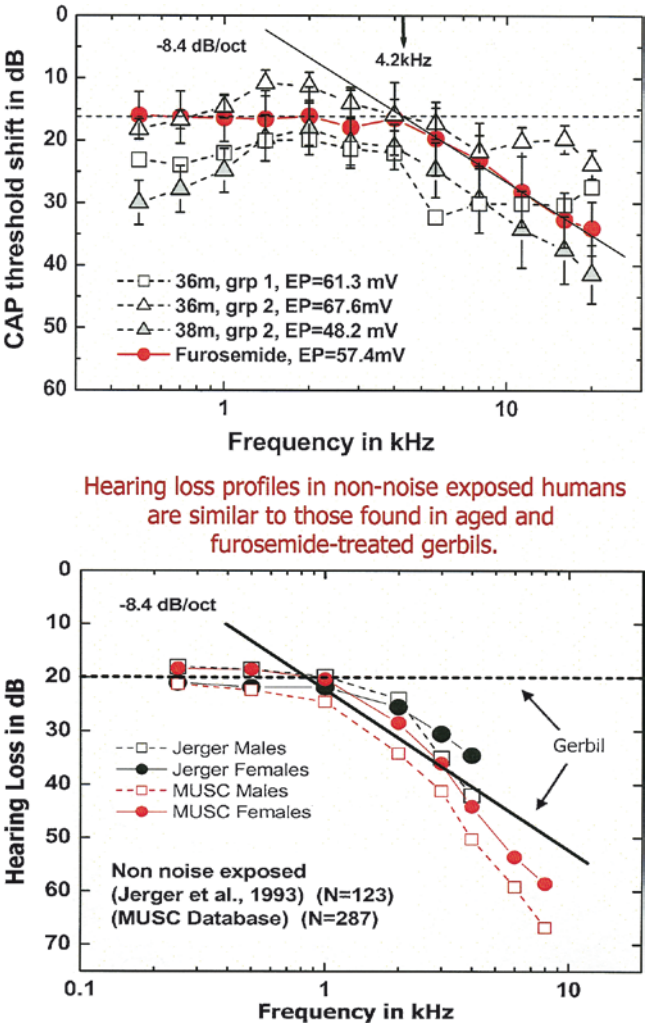

Fig. 2.7 Hearing loss profiles of three groups of quiet-aged and one group of furosemide-treated gerbils (top; adapted with permission from Schmiedt et al. 2002b.) and two groups of non-noise-exposed humans (bottom; adapted with permission from Mills et al. 2006a). The overall profile of the gerbil data arising from EP reduction from chronic furosemide treatment has been fitted to the human data (bottom panel). The flat loss at low frequencies is shifted to 20 dB, and the breakpoint for the shallow high-frequency roll-off has been shifted to ~0.9 kHz. It is clear that the metabolic model comprising EP reduction explains much of the HL profile of human presbycusis screened for noise and genetic histories.

the human data are remarkably similar. These data are strong evidence that the greatest factor underlying human presbycusis is EP loss arising from lateral wall degeneration with age. In other words, true age-related hearing loss in humans is largely of metabolic origin.

2.5.2 *Suprathreshold Data*

2.5.2.1 Single Tones

Suprathreshold measures obtained from animals are most often in the form of behavioral, neural, acoustic reflex, or otoacoustic data. A very clear suprathreshold result of aging in all animals is seen in the neural CAP response, an evoked electrical waveform recorded from the auditory nerve to either a click or tone pip. The CAP depends on a large number of auditory nerve afferents firing synchronously, resulting in a single negative wave as monitored by a gross electrode near the nerve. Fig. 2.8

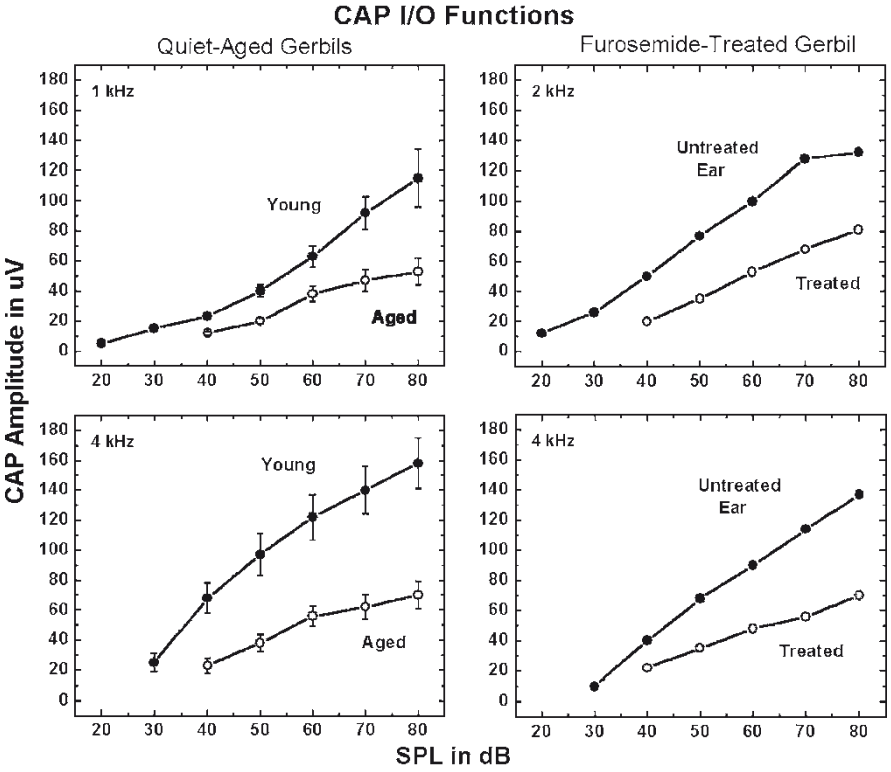

Fig. 2.8 Effects of metabolic presbycusis on suprathreshold measures of neural responses. CAP response amplitudes as a function of tone pip intensity (input/output [I/O] functions) at 1 and 4 kHz are shown for aged gerbils (left; values are means ± SE) and from a gerbil treated with chronic furosemide (right) at 2 and 4 kHz. Characteristics of the I/O function shared by both the aged and furosemide-treated ears are shallow slopes and diminished maximum amplitudes as compared with their control curves. Note that the furosemide-treated ears are in young animals with a full complement of hair cells and primary fibers. Thus the minimized CAP response with lowered EP is not simply from an anatomical reduction in the numbers of fibers available for excitation (see text).

show curves of CAP amplitude as a function of sound intensity in response to tone pips (an input/output function or I/O function) from control, quiet-aged, and furosemide-treated ears. There are the expected threshold shifts along the *x* axis; however, the most apparent changes with age and with EP reduction are the shallower slopes and marked reduction in the maximum amplitudes of the response waveform (Hellstrom and Schmiedt 1990, 1991, 1996; Mills et al. 1990; Schmiedt 1993; McFadden et al. 1997b; Schmiedt et al. 2002b).

Given that the furosemide model is obtained in a young gerbil, the populations of auditory hair cells and nerve fibers are assumed to be intact; however, the reduction in the CAP waveform is similar to that of the aged ear (which almost certainly has an age-related loss of primary fibers). Thus either the number of excited fibers or their synchronicity or both are affected both by age and by a decreased EP. Because the EP is instrumental in driving K^+ through the IHC for the transduction process, it is tempting to hypothesize that it is fiber synchrony that is being compromised by increased age and reduced EP. (Note that single-fiber data from quiet-aged gerbils showed no differences in spike rate intensity functions from young control animals; however, synchrony was not examined in those studies [Hellstrom and Schmiedt 1991]). On the other hand, aging and reduced EP may also affect the responsivity of individual populations of afferent fibers. In particular, the activity of the low-spont fiber population drops out with age and lowered EP (Schmiedt et al. 1996). These single-fiber results are similar for furosemide-treated gerbils, suggesting that it is truly a phenomenon of decreased EP alone and not necessarily just of age. Thus metabolic presbycusis may have dramatic consequences to higher-order processing if the low-spont fibers are not functioning properly at high levels of sound intensity. Much of the processing of speech by human listeners is accomplished in the intensity region where the low-spont system is active, so an obvious hypothesis is that the speech understanding problems in the elderly listener may be in part derived from a lowered EP as a consequence of metabolic presbycusis.

A caveat to the above hypotheses concerning the effects of EP changes on the neural response is that the CAP I/O functions of the C57BL/6J mouse after four months of age also show a shallow slope and much diminished maximal response compared with littermates one month of age (Lang et al. 2002). Because the mutation in this phenotype affects the cochlear hair cells and not the EP, the decreased CAP responses in this model may be of different origins than those in the metabolic models. Perhaps in this model, the shrinkage and loss of the SGCs are responsible for the decreased evoked potentials, unlike the metabolic model. Conversely, the cochlear amplifier may indeed be reduced in gain as a result of the scattered OHC loss, thereby yielding results similar to those of metabolic presbycusis and lowered EP. This hypothesis would assume that decreased OHC amplification implicitly results in CAP I/O functions with flattened slopes. However, in animals with OHC loss from noise or drug exposure, CAP thresholds are shifted, but the slopes of the I/O function can be normal or even steeper than normal (Bobbin 1992).

2.5.2.2 Multiple Tones

OAEs are a direct consequence of a properly functioning cochlear OHC amplifier (Probst 1990). As such, they are an excellent tool for the noninvasive investigation of the OHC system (Schmiedt 1986; Boettcher et al. 1995; Mills 2003, 2006; Mills and Schmiedt 2004). Gerbils have very robust OAEs, and they are only somewhat diminished in furosemide-treated animals with a lowered EP as shown in Fig. 2.9, top. OAEs are similarly diminished in quiet-aged gerbils as shown in Fig. 2.9, middle (Mills et al. 1993; Schmiedt et al. 2002b). Note from Fig. 2.9, bottom, that lowered EP effectively mimics the age-related loss in DPOAEs.

Similarly, emissions are diminished but present in non-noise-exposed elderly humans exhibiting signs of metabolic presbycusis. Indeed, the fine structure of OAEs can survive in these subjects (He and Schmiedt 1996). The point is that the OHC amplifier is still active in metabolic presbycusis with a lowered EP, but it is just not as robust as in the young animal. Conversely, cumulative noise and drug injury to the OHCs as found in many, if not most elderly, humans will drastically decrease OAEs. As a result, there are many reports showing significant loss of OAEs with age (e.g., Dorn et al. 1998).

That the OHC amplifier is still functional under quiet-aged conditions is also borne out in single-fiber studies in the auditory nerve that show tuning curve tips that are reduced somewhat in threshold but are still sharp at high characteristic frequencies (CFs; Schmiedt et al. 1990). Moreover, two-tone suppression is present on the low- and high-frequency sides of the tuning curves in quiet-aged animals. Conversely, animals aged in a continuous, low-level noise field have little OHC function remaining, and single fibers affected by the noise show tuning curves with no sharply tuned tips and no suppression (Schmiedt et al. 1990). Thus metabolic presbycusis is not necessarily as traumatic to the OHC system as sensory presbycusis where the hair cell loss is significantly higher or in noise trauma where the functionality of the stereocilia and OHC motor may be compromised.

2.6 Neural Presbycusis

Neural presbycusis seems to be a universal finding among all aging models, including those exhibiting sensory and metabolic characteristics. As illustrated in Fig. 2.10, top, there is an obvious shrinkage of the SGCs in Rosenthal's canal. Additionally, the number of ganglion cells typically decrease ~15–25% along the entire cochlear duct (Mills et al. 2006a; Fig. 2.10, bottom). Why this neuropathy comes about with age is still unknown, but a model of auditory neuropathy using ouabain is available that may be useful in studying the apoptosis and regeneration of auditory nerve fibers, especially when the hair cells and stria are still intact (Schmiedt et al. 2002a). Ouabain is a cardiac glycoside that irreversibly blocks the activity of Na^+-K^+-ATPase, thereby largely stopping the transport of Na^+ and K^+ across the cell membrane.

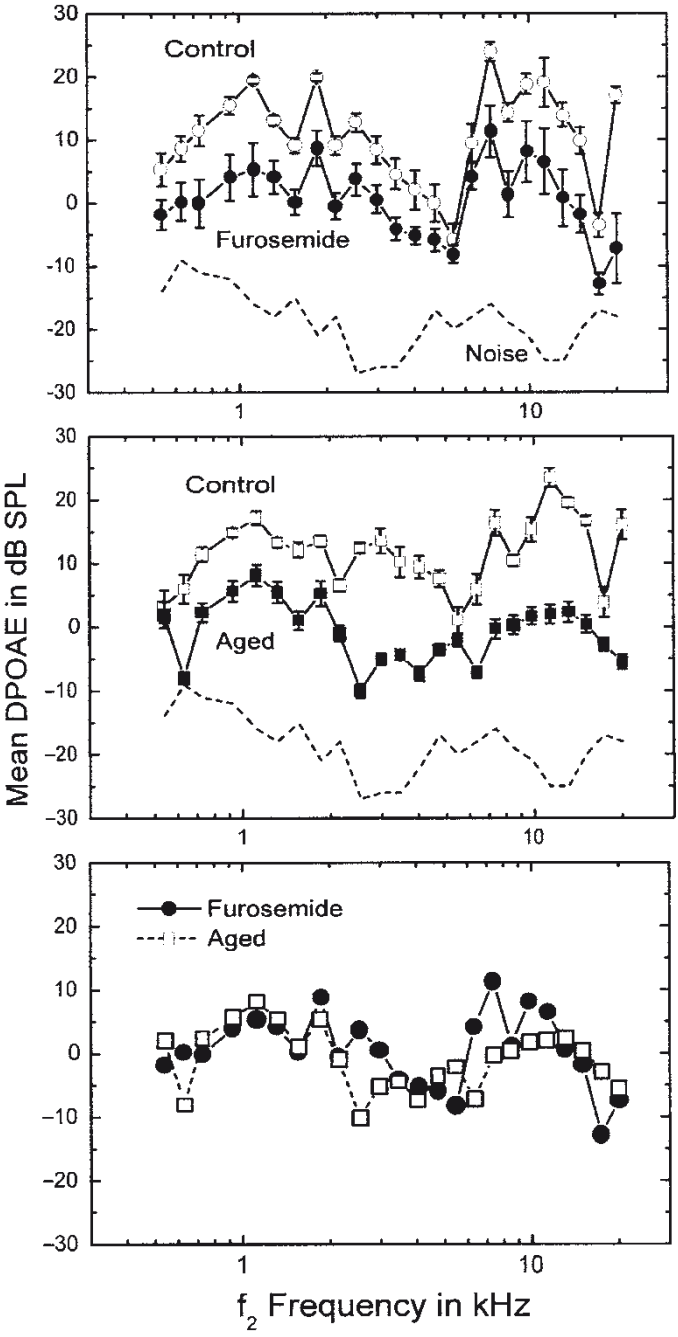

Fig. 2.9 Mean distortion product otoacoustic emission s (DPOAEs) obtained with 50-dB SPL primaries in the gerbil. DPOAEs are reduced but not absent with furosemide treatment (top) or age (middle) as compared with control values. Indeed, the absolute levels of the emissions are almost identical between the quiet-aged and furosemide-treated animals (bottom). Thus whereas the OHC amplifier may be diminished with reduced EP, it is still effective at higher levels with regard to the production of OAEs. (Adapted with permission from Schmiedt et al. 2002b.)

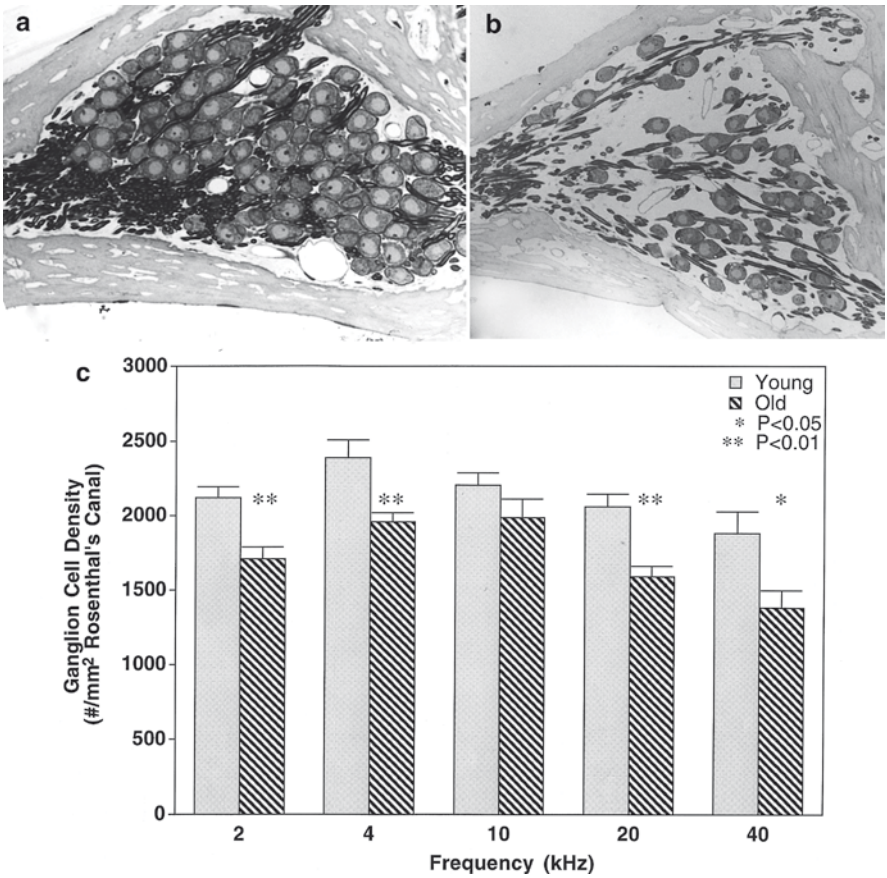

Fig. 2.10 Cross sections of Rosenthal's canal in the first turn of a young adult gerbil (**a**) and a 36-month-old gerbil (**b**). There is a marked reduction in the number and size of the spiral ganglion cells (SGCs) in the aged animal. **c**: SGC counts along the cochlear spiral in young adult and old gerbils. Typically there is about a 20% reduction of SGCs along the spiral at 36 months of age. (Adapted with permission from Mills et al. 2006a.)

In this model, ouabain is applied to the round window niche in gerbils or mice, similar to the furosemide model described previously. Nerve fibers have large amounts of Na^+-K^+-ATPase, and the ouabain selectively targets the type I afferents in the auditory nerve while sparing all hair cells and cochlear lateral wall. All type I afferents undergo apoptosis, whereas the type II fibers going to the OHCs are spared (Lang et al. 2005). This ouabain model also has been used to explore the use of stem cells in the regeneration of auditory ganglion cells (Lang et al. 2006a, 2008). Unfortunately, because all type I afferents degenerate in this model, it is not a good one for neural presbycusis where only 15–25% of the ganglion cells typically degenerate with age.

A very real problem with neural presbycusis is that functionally the neurons in aged animals seem to have grossly similar responses to sound as the neurons in young animals. One of the few studies of single fibers in aged animals revealed the difference between younger and older animals was with regard to the loss of activity of the low-spont system (Schmiedt et al. 1996; Lang et al. 2002). Interestingly, the loss of activity in this fiber group was not related to the shrinkage and disappearance of either the SGCs or the radial fiber population as predicted by another study on cats (Kawase and Liberman 1992; Suryadevara et al. 2001). The only correlation of the loss of the low-spont activity was with decreased EP. Thus aging effects on the EP may have a direct impact on the responsivities of different fiber populations in the auditory nerve. This scenario may possibly associate basal strial degeneration in the cochlea with diminished suprathreshold capabilities in elderly humans.

In another avenue of research, there has been some discussion that the cochlear lateral wall may be trophic (supplies support) to the hair cells, supporting cells, and neurons. This hypothesis arises simply from the fact that in the gerbil model, there was never a case where the hair cells and neurons were still present without survival of some section of the lateral wall. Conversely, whenever there was no lateral wall present in an ear, the hair cells and neurons were completely absent and the ear was functionally dead. Similarly, some new data suggest that certain fibrocytes in the lateral wall may have trophic influences on auditory afferents (Lang et al. 2006b; Adams et al. 2007; Adams 2008). This could be a significant development in our understanding of neural presbycusis.

Among the most difficult aspects in understanding neural presbycusis is that there is still no known functional change given relatively minor fiber losses. One of the best examples are the experiments of Schuknecht and Woellner (1955) where the auditory nerves of cats were partially sectioned before behavioral testing. Most of the treated cats showed little deficit in their thresholds, suggesting that the central nervous system probably needs only a few neurons to detect a sound. The data also speak to the redundancy of the afferent system, especially with regard to threshold loss. On the other hand, older humans show more problems than just their hearing loss, so neural presbycusis could help explain age-related changes in psychophysical performance with suprathreshold stimuli (see Fitzgibbons and Gordon-Salant, Chapter 5). The causes and effects of neural presbycusis are still very much a puzzle at this point.

2.7 Future Directions

2.7.1 Regeneration of Hair Cells

There is now a huge literature of hair cell regeneration (for reviews, see Martinez-Monedero et al. 2007; Stone and Cotanche 2007). These efforts are in

part the result of the somewhat erroneous concept that age-related hearing loss in humans is largely sensory in nature. As this review hopefully has shown, human presbycusis is more likely metabolic than sensory. Certainly, it would be of great advantage to regrow hair cells in ears deafened by noise or ototoxic drugs; however, with regard to pure aging, it is the EP that must be regenerated before replacing lost hair cells. Two ways to reestablish the EP come to mind: using external currents to jump start the cochlear battery or regenerating the cells along the lateral wall that are responsible for generation of the EP.

2.7.2 Current Injection for Metabolic Presbycusis

Glass pipettes filled with a K^+ solution can be inserted into the scala media of quiet-aged and furosemide-treated gerbils with the purpose of passing a positive current to bolster the EP (Schmiedt 1993). When this is done, the EP does indeed increase, with concomitant increases in the slopes and maximum amplitudes of the CAP I/O functions as shown in Fig. 2.11, top. A positive current of ~10 μA can increase the EP by ~10-30 mV along the cochlear spiral. Negative currents will decrease the EP and will decrease the CAP response. Fig. 2.11, bottom, demonstrates that thresholds also improve with positive current application in the quiet-aged ear. Unfortunately, the disadvantage to current injection in this manner is that the K^+-recycling pathways function poorly in aged or furosemide-treated animals (which is why the EP is reduced). Passing a large K^+ current through the electrode into the scala media gradually overwhelms the recycling pathways and after some period of time (about an hour at +10 μA), the K^+ overwhelms the homeostatic mechanisms in the cochlea, whereupon the system dies. Thus, although promising at first, the K^+ buildup in the scala media is problematic.

2.7.3 Cell Regeneration

Another way to approach the problem of increasing the EP in metabolic presbycusis lies in maintaining and regenerating the cells responsible for its production. Studies from several labs have shown that the lateral wall cells, including the intermediate cells in the stria vascularis, turn over, i.e., they have a normal cycle of cell renewal (Roberson and Rubel 1994; Lang et al. 2003; Fig. 2.12). If stressed by furosemide, the rate of turnover is greater, whereas aging slows it down. Thus perhaps the most promising way to approach the amelioration of metabolic presbycusis is by understanding lateral wall cell turnover and why it decreases with age. Most of the lateral wall cells are fibrocytes, so maintaining and regenerating these cells should be somewhat easier than finding ways to regenerate terminally differentiated cells like hair cells.

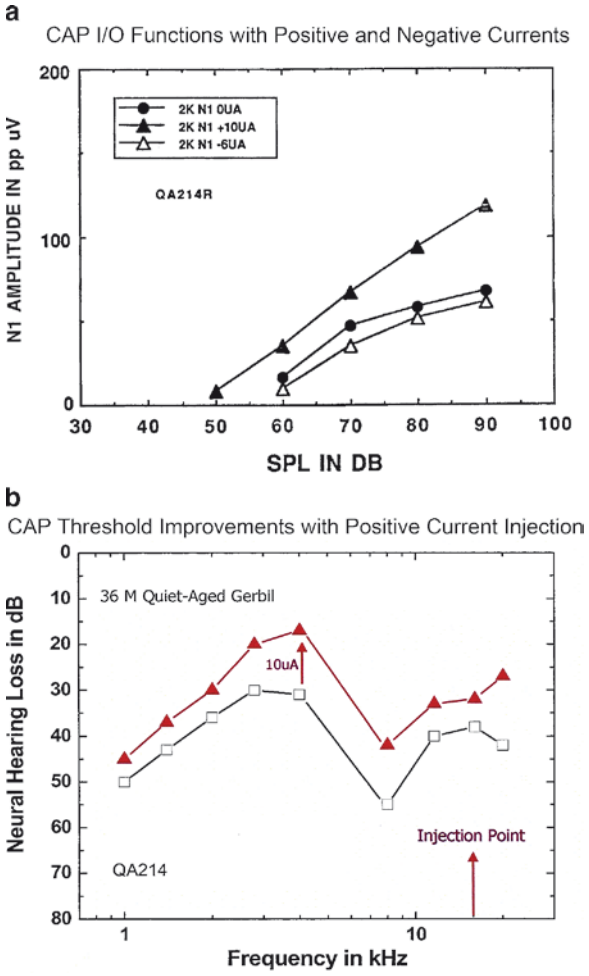

Fig. 2.11 Effects of current injection into the scala media of quiet-aged gerbils. **a**: currents from −6 μA to 10 μA were passed with a constant-current generator through a micropipette filled with 0.1M KCl inserted into the scala media in the basal coil. CAP I/O functions were enhanced with regard to threshold, slope, and maximum amplitudes with positive currents of up to 10 μA, whereas the CAP responses were diminished with negative currents. **b**: effect of current injection on CAP thresholds across frequency in the same animal. Although current was injected into the base, the effect was spread throughout the cochlear spiral (no current, open squares, with current solid triangles). Note that this animal had substantial losses at high and low frequencies before the electrode placement (open squares). Despite these losses, the data suggest that the aged ear is energy starved and boosting the EP would ameliorate much of the age-related hearing loss seen in this model of presbycusis.

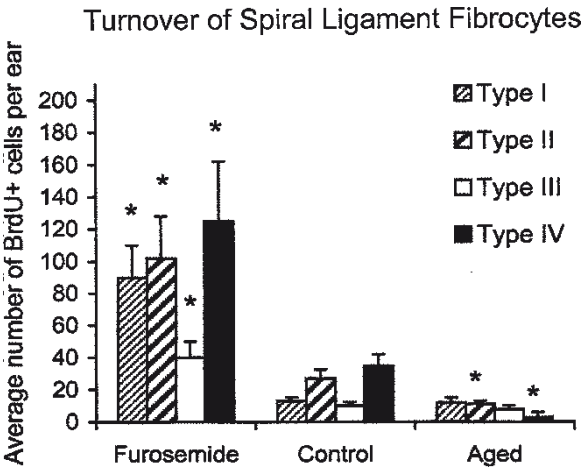

Fig. 2.12 Turnover or proliferation rates of different types of fibrocytes in the cochlear lateral wall. The turnover rates are indicated by the number of counted $BrdU^+$ cells in the four regions of fibrocytes. The rates are increased by cochlear stress (furosemide application) and are decreased with age. There is evidence that these fibrocytes are involved in the recycling of K^+ from the hair cells back to the stria vascularis where the EP is generated. Lateral wall fibrocytes are known to degenerate with age; however, they are continually renewed, probably by endogenous progenitor cells or hematopoietic stem cells from the bone marrow (Lang et al. 2006a, 2008). It may be possible to use various trophic factors or encourage their proliferation in the aged ear to restore proper numbers of fibrocytes with age. (Adapted with permission from Lang et al. 2003.)

2.8 Relating Animal Models to the Human Condition

Given what we now know about the physiology of the normal ear and aging ears with different types of presbycusis, is it possible to predict the underlying cause(s) of hearing loss by examination of the human audiogram? In other words, given what is known from animal models of presbycusis, is there enough information in an audiogram, coupled with suprathreshold hearing tests, to discriminate between sensory, metabolic, and possibly neural presbycusis?

Some simple phenotypical rules would include the following: pure OHC loss should result in a maximum of 50- to 60-dB HL at frequencies above ~2 kHz. Punctate OHC lesions should result in audiometric notches. OHC losses may have fairly steep slopes at frequencies above ~2 kHz. A positive noise history would be expected. Moreover, OHC loss in the base should have little or no effect on low-frequency thresholds. IHC loss should be linked to profound deafness as would a total loss of nerve fibers. Finally, OHC loss should profoundly affect cochlear nonlinearities, including compression phenomena and OAEs.

Conversely, metabolic presbycusis should be characterized by flat low-frequency HLs of between 10 and 40 dB, depending on the amount of EP loss. The flat loss should be coupled to a shallow high-frequency roll-off above ~1-2 kHz with a slope

Table 2.1 Phenotypes of age-related hearing loss as indicated by the audiogram.

			Low Frequency (0.25–1.0 kHz)		High Frequency (1.0–8.0 kHz)	
Category	Noise History	Notch (4-8 kHz)	Range (dB HL)	Slope (dB/octave)	Range (dB HL)	Slope (dB/octave)
Older-Normal	No	No	≤10	−5 to 5	0-20	−5 to 5
Premetabolic	No	No	≤10	−5 to 5	≤25	0-10
Metabolic	No	No	10-40	−5 to 5	30-60	10-20
Sensory	Yes	Yes	≤10	>5	>40	≥20
Metabolic+Sensory	Yes	Yes	10-40	−5 to 5	>40	≥20

of ~10-20 dB/octave. No noise history would be expected. There should be no notches. With regard to suprathreshold phenomena, the OHC amplifier should still be functional, albeit somewhat less robust. Thus compression phenomena and OAEs should still be present but reduced.

Table 2.1 uses the above hypotheses and numbers derived from animal results to discriminate five phenotypes describing age-related hearing loss. Obviously, most human presbycusis involves both sensory and metabolic components in the presence of a universal but poorly understood partial neural degeneration seen with neural presbycusis.

As specified in Table 2.1 and illustrated in Fig. 2.13, older subjects who are classified as "normal" or "premetabolic" would have negative noise histories and thresholds ≤ 10-dB HL from 0.25 to 1.0 kHz and ≤ 25-dB HL at higher frequencies. Subjects classified as "metabolic" (mild to severe) would have negative noise histories, flat hearing loss in the lower frequencies ranging from 10 to 40-dB HL, and gradually sloping hearing loss in the higher frequencies, with slopes ranging from 10 to 20 dB/octave. Subjects classified as "sensory" would have positive noise histories, thresholds in the lower frequencies ≤ 10-dB HL, and steeply sloping hearing loss in the higher frequencies with slopes > 20 dB/octave. Notches may be present. Subjects classified as "metabolic+sensory" would have positive noise histories, characteristics of metabolic presbycusis in the lower frequencies (flat loss ranging from 10- to 40-dB HL), and characteristics of sensory loss in the higher frequencies (steeply sloping loss with slopes > 20 dB/octave). The basic profile of these audiometric templates in Fig. 2.13 should be applicable to both human and animal models of hearing loss and presbycusis and is part of ongoing longitudinal studies of human presbycusis (Lee et al. 2005; Dubno et al. 2008).

Further definition of the phenotypes could be done by identifying audiologic or other characteristics beyond the audiograms that differentiate subjects in the five categories, e.g., OAE amplitudes and I/O functions; upward spread of masking; ABR thresholds, latencies, and amplitudes; and, in the future, genetic variations to discriminate the five phenotypes. This work is currently ongoing (Lee et al. 2005; Dubno et al. 2008).

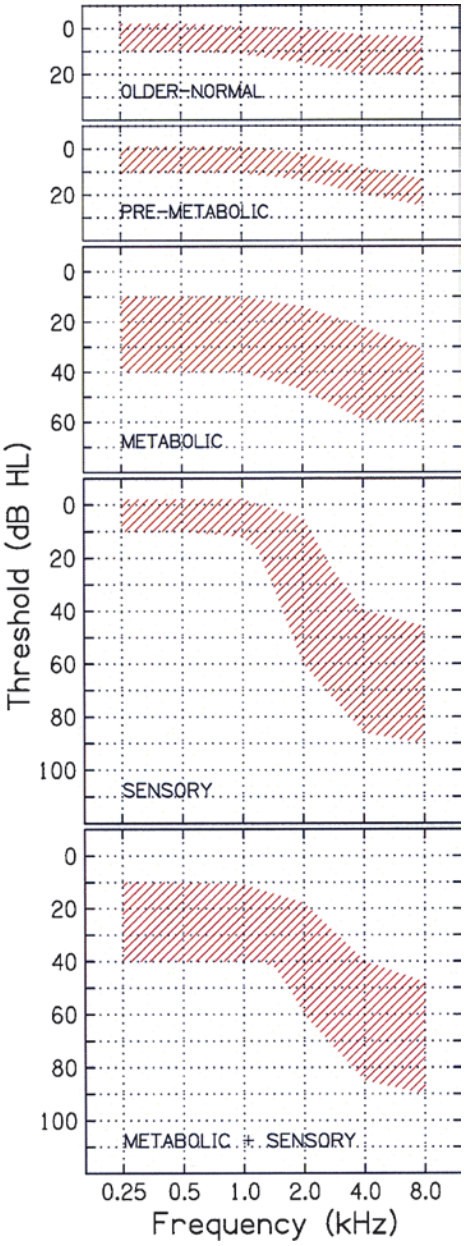

Fig. 2.13 Ranges of audiogram profiles illustrating the five phenotypes of age-related HL in humans. The profiles are schematized from the results of animal models of presbycusis. In general, metabolic presbycusis results in a mild, flat loss at low frequencies coupled with a shallow high-frequency sloping loss above ~1 kHz. The maximum loss is ~60 dB. Sensory loss is typified by steep slopes and notches, with little loss at low frequencies. Maximum loss is ~60 dB if only OHCs are involved; if IHCs are lost, there will be profound deafness. Most humans, particularly men, given their typical noise history, fall under the "metabolic+sensory" profile where the damage is from sensory loss from excess noise exposure coupled with a metabolic loss from aging.

2.9 Summary

Animal models have yielded insights as to the nature of hair cell function, the cochlear amplifier, the generation of the EP, the subsequent recycling of cochlear potassium, and the transduction process. All of those factors have roles in our understanding of presbycusis. It is clear from quiet-aged animal models where noise, drugs, and genetic mutations are strictly controlled, that (1) sensory loss is almost always present, but paradoxically the loss occurs largely at the apex or extreme base, is scattered, and often has little effect on gross auditory nerve thresholds to simple sounds; (2) there is a lowering of the EP as a consequence of the degeneration of the cochlear lateral wall including the stria vascularis; (3) the EP modulates the gain of the cochlear amplifier, yielding an audiometric profile commonly seen in humans and animals; and (4) auditory nerve ganglion cells shrink and are reduced in number throughout the cochlea. Although advances in tissue regeneration and invasive techniques to correct age-related cochlear physiology are of great interest to the research community, a real advance in the amelioration of the effects of presbycusis would be differentiating the types of presbycusis in older adults and relating this improved clinical differential diagnosis to novel biomedical interventions aimed at improving auditory function in aged listeners.

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