region and 76 ± 8 HC's in the extrastriolar region. Similar densities were obtained with animals receiving streptomycin treatment alone. Animals that received saline alone had 75 ± 3 in the striolar region and 153 ± 3 HC's in extrastriolar region. These results suggest that caspase inhibitors promote hair cell survival following systemic treatment with streptomycin.

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262 Prevention of Aminoglycoside-induced Hearing Loss by Aspirin: Preliminary Data from a Clinical Study

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Aminoglycosides are still among the most commonly used antibiotics worldwide but no clinical therapy exists to prevent their ototoxic and nephrotoxic side effects. Animal experiments (in guinea pigs and mice) have shown that various antioxidants or metal chelators may reduce the magnitude of aminoglycoside-induced auditory and vestibular damage, including salicylate. This latter finding (Sha and Schacht, 1999) suggested that aspirin, whose active metabolite is salicylate, might become an antidote to aminoglycoside-induced ototoxicity in patients.

195 patients were evaluated in a double-blind randomized placebo-controlled study. Patients hospitalized for infections and diagnosed to receive short-term (5 to7 days) treatment with gentamicin were additionally given either a placebo dosing or 3 x 1 gm of aspirin per day during and for one week following the gentamicin treatment. Audiological evaluation at frequencies of up to 8 kHz were given before the first dose of gentamicin, again at day 7 (day of discharge form the hospital) and at a follow-up session 5 to 7 weeks later. The patient groups matched for age and total dose of gentamicin received and pretreatment thresholds were also similar in both groups. The criterion for gentamicin-induced "hearing loss" was set as a threshold shift of > 15dB at both 6 and 8 kHz in the same ear. By this criterion, there were significantly fewer adverse events in the group receiving aspirin than in the placebo group. The results give hope that antioxidant treatment in general and aspirin in particular may be beneficial in attenuating hearing loss in patients receiving aminoglycoside therapy.

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263 The Role of Lateral Wall Pathology in Hearing Loss of Aged Animals.

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Age-related hearing disturbance (presbycusis) is one of the most common causes of human hearing loss. Although the factors involved in presbycusis have not yet been clarified, there is some evidence to suggest that disturbance of inner ear fluid homeostasis may be related to auditory dysfunction. Disruptions to homeostasis may be caused by pathologic changes within the lateral wall of the cochlea. Increased oxidative stress, decreased heat shock protein (Hsp) levels and other pathologic changes in stria vascularis and spiral ligament may be important causative factors. The aim of the present study is to investigate the pathophysiology of cochlear lateral wall that may cause age-related hearing loss in animals.

In the present study, expression of inducible nitric oxide synthase (iNOS) and Hsp70 in young and aged animals was compared by immunohistochemistry. The effect of sodium nitroprusside (SNP) on intracellular Ca^{2+} in cultured marginal cells was assessed using fluorescence ratio imaging. Hearing threshold changes in chinchilla after application of SNP through round window was also assessed. Functional changes in young and aged animals were compared by measuring auditory brainstem response(ABR).

Intracellular Ca^{2+} in cultured marginal cells increased. In immunohistochemical staining for Hsp 70, positive staining was observed in the stria vascularis of both young and aged rats. Immunoreactivity was stronger in the young rats compared to aged rats. For iNOS, positive immunoreactivity was seen in stria vascularis of aged rats. After SNP application to the round window of the chinchilla, ABR change was observed

In conclusion, these findings indicate that lateral wall components may play an important role in the maintenance of homeostasis in the inner ear, and may be an important determinant in the pathophysiology of age-related hearing loss.

264 The Mouse Ahl Gene May Not Affect the Cochlear Lateral Wall, and Does Not Lead to Reduction of the Endocochlear Potential (EP).

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Ahl is a gene that causes age-related hearing loss (ARHL) in at least 10 mouse strains (Johnson et al. 2000). In C57BL/6 mice, where it is best characterized, it has been associated with progressive hair cell loss, neuronal loss, and degeneration of stria vascularis/spiral ligament (Spongr et al. 1997; Ichimiya et al. 2000; Hequembourg and Liberman, 2001). Ahl therefore appears to lead to a 'mixed' form of ARHL, according to Schuknecht's criteria (Schuknecht, 1974), and has important implications for how this condition arises. If it is to be useful for our understanding of human ARHL, the 'essential' phenotype associated with Ahl must be determined. This issue can be addressed by direct comparison of different inbred mouse strains that carry Ahl. We examined cochleas of 5 such strains: C57BL/6, BALB/c, C57BR/cd, BUB/Bn, and 129S6/SvEv. ABR thresholds, hair cell counts, EP recordings (lower basal turn), and mid-modiolar sections were obtained in mice up to 17 mos of age. In keeping with previous reports (Johnson et al. 2000), each strain showed progressive hearing loss, particularly at high frequencies. Although histological analyses are ongoing, C57BL and BALB mice were found to differ with regard to the extent of degeneration of the lateral wall. In contrast to C57BL, both stria and spiral ligament appear normal in BALBs to at least 13 mos of age. Moreover, EP measures in 3 Ahl strains (C57BL, BALB, 129S6) show no reduction for ages up to 17 mos, despite severe ARHL. Average EPs by strain were: 106±9 mV in C57BL (n=7); 109±2 mV in 129S6 (n=4); and 100±6 mV in BALB (n=13). Based on our results to date we propose the following: 1) Degeneration of stria vascularis/spiral ligament is not necessarily associated with Ahl. 2) Age-related degeneration of these structures in C57BL mice may arise from gene(s) other than Ahl. 3) Degeneration of stria vascularis/spiral ligament in C57BL mice is not severe enough to account for their hearing loss.

265 An Augmented Acoustic Environment Delays Age-Related Hearing Loss in C57BL/6J Mice as Revealed by DPOAEs and Cochlear Histopathology

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Willott and Turner (Hear Res, 1999) reported that an augmented acoustic environment (AAE) delayed age-related hearing loss (AHL) in C57BL/6J (C57) mice. The present study assessed cochlear function at

the outer hair cell (OHC) level by measuring distortion-product otoacoustic emissions (DPOAEs) in both AAE and control mice. These results were subsequently correlated with cochlear histopathology. Beginning at 25 d of age, C57 mice were exposed nightly, for 12 h, to a 70-dB SPL broadband AAE (200-ms pulses at 2/s), centered at 10 kHz. DPOAEs were recorded in the form of DP-grams (DPOAE level as a function of primary-tone frequency), with geometric–mean (GM) frequencies ranging from 5.6-48.5 kHz (f_2=6.3-54.2 kHz), in 0.1-oct steps. DP-grams were collected at three primary-tone levels (L_1=L_2=55, 65, 75 dB SPL). ABRs were recorded at 6, 8, 12, 16, 24, and 32 kHz to confirm the AAE effect. At 6 mo of age, both cochleas from 4 control and 3 AAE mice were post-fixed in OsO4, dehydrated, embedded in plastic and dissected as flat preparations. Hair-cell, supporting-cell and nerve-fiber losses were determined from apex to base and cytocochleograms prepared for all cochleas. Function-structure correlations were made by overlaying the final DP-gram on the cytocochleogram. By 4 mo of age, clear differences between AAE and control DPOAEs were observed. Control C57s showed average DPOAEs that were near noise-floor levels between GM frequencies of 25-48.5 kHz, while the AAE ears exhibited moderately reduced DPOAEs over a restricted range of GM frequencies between 25-35 kHz. The functional data matched well with the histopathology in all ears. As a group, the controls had worse function and more hair-cell loss than the AAE mice. However, in both groups, there was large variability across mice. The ears of the AAE mice were more variable than the controls and 2/3 had pronounced asymmetries between ears.

266 Aging, DPOAEs Across f2/f1 Ratio, and Ultra-high Frequency Hearing

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Human auditory aging studies often match hearing sensitivity (8kHz and below) of young & older subjects to control for peripheral hearing loss. OAEs and ultra high frequency (UHF) hearing sensitivity may provide more sensitive representations of cochlear status. Age-related reductions of DPOAE amplitudes in subjects with normal hearing thresholds have been reported. However, no study has examined the status of UHF thresholds while studying the effects of f2/f1 ratio on the same, normal-hearing, younger and older human subjects. Two normal-hearing, gender-balanced groups of subjects were used: young (18-30 years) and b), older (60-74 years). DPOAEs were measured with the ILO-88 (v.5.6). Primary-tone ratios ranged from 1.05 to 1.8, with L1 = 70 and L2 = 60 dB SPL Primary-tones ranged from 1 to 6.3 kHz. Hearing thresholds were obtained for the range of .25-20 kHz using a Grason-Stadler 61 audiometer (TDH-50 and Sennheiser HDA 2000 earphones). Results: Older subjects, with normal thresholds below 8 kHz, had elevated thresholds above 8 kHz. Few had measurable thresholds above 12.5 kHz, and mean thresholds were >60 dB higher than young subjects at 12.5 kHz. DPOAEs for both age groups showed the largest amplitude responses for f2/f1 ratios of 1.2 to 1.3, and both groups showed very small DPOAE amplitudes for ratios of 1.1 and below and above 1.4-1.5. For the ratios where DPOAEs were largest, mean DPOAE amplitude was larger for the young adults. Although many of the mean f2/f1 functions peaked at a ratio of 1.2-1.3, the difference in amplitude between young and older adults was often greatest at a ratio just above where the functions peaked. It appears that normal hearing thresholds in the frequency range of 8 kHz and below is insufficient proof of normal cochlear function in older human subjects.

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267 Expression of DNase I, an Endonuclease, in the Stria Vascularis

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The stria vascularis (SV) is a highly specialized tissue compartment responsible for the generation of the endocochlear potential (EP). A reduction in the EP associated with degeneration of the SV occurs with age as well as in mice suffering from a variety of genetic mutations. Analysis of gene expression in the lateral wall of W^v/W^v mutant mice, which lack intermediate cells and have a dysfunctional SV, and their wild-type (WT) background strain (C57BL/6J) has revealed differential expression of mRNA for a Ca^{2+}, Mg^{2+}-dependent endonuclease, DNase I. This endonuclease was originally identified as a secretory product involved in digestion, but more recently has been shown to play a role in apoptosis in both digestive and nondigestive tissues.

Here, the expression of DNase I was investigated immunohistochemically in the inner ear of various age groups of W^v/W^v mutant and WT mice. In young WT mice, immunoreactivity was restricted to vascular smooth muscle and strial marginal cells. Reaction product in the marginal cell occupied a perinuclear location and extended apically toward the scala media. In the W^v mutants, turns lacking intermediate cells failed to stain for DNase I. Enzyme expression varied in different turns of aged WT mice, apparently in relation to strial degeneration, being markedly reduced to absent in regions of severe atrophy.

Although DNase I has been shown to be involved in the digestive process and apoptosis, it likely serves a different role in normal marginal cells. DNase I is also known to bind monomeric actin, which inhibits polymerization as well as enzymatic activity. Since many ion transport proteins including pumps and channels are linked to and regulated by cytoskeletal components such as actin, it is probable that DNase I functions in this capacity in strial marginal cells, which are highly specialized for transporting ions.

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268 The effects of chronic furosemide on spontaneous rates of auditory-nerve fibers in young gerbils

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The activity of low-spontaneous rate (SR) fibers with high characteristic frequencies (CF) is reduced in quiet-aged gerbils compared to young controls (Schmiedt et al. J. Neurophysiol. 76:2799-03, 1996). Recently, we indirectly demonstrated a similar result in ears treated chronically with furosemide through the use of recovery functions of the compound action potential (Schmiedt et al., ARO Abstr. 23:80, 2000). In this study, the effects of furosemide on fiber populations were counted directly. Ten young gerbils were implanted with osmotic pumps containing 5 mg/ml furosemide for one week. The furosemide was applied directly to the round window (RW) via a cannula. The spontaneous activities of 188 auditory-nerve fibers were recorded along with CF and threshold information and compared to a group of 438 fibers obtained from 24 young controls. Low-SR fibers (SR $\leq$ 18 spikes/s) with CFs $\geq$ 6 kHz made up 47.5% and 17.6% of the control and furosemide-treated ears, respectively. The difference was significant ($p<0.01$). At CFs < 6 kHz, low-SR fibers consisted of 23.6% of the population in controls, and 16.9% in the treated ears;a difference that was not significant. Endocochlear potentials (EP) were measured in control and treated ears. Mean control EPs in the three cochlear turns (T1, T2, T3) were 95±8, 89±6, and 87±6 mV, respectively. Corresponding EPs in the furosemide treated ears were 56±8, 62±2, and 58±3 mV, demonstrating that the EP in the treated ears was reduced approximately 30 mV from that in the controls. These results show directly that chronic changes of the EP, similar to those