ABSTRACTS OF THE TWENTY-NINTH ANNUAL
MIDWINTER RESEARCH MEETING

ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

February 5-9, 2006

Baltimore Marriott Waterfront
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Editor

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important roles for many organs in the regulation of endothelial proliferation and differentiation. They function as progenitor cells. They synthesize and secrete a wide variety of vasoactive autoregulating agonists and synthesize and releasing structural constituents of the basement membrane and extracellular matrix. Little is known about the distribution and actions of pericytes in the cochlea. In this study, we investigated the distribution and change of pericytes in the capillaries of the cochlear lateral wall in the control and noise exposure mice with fluorescent immunohistochemistry method and confocal microscope. Experimental groups of mice were exposed to broadband noise at 120 dB SPL for 2 days (4h/d). Pericytes were identified using monoclonal anti-α -smooth muscle actin and anti-desmin antibody. Endothelial cells were visualized with FITC conjugated to anti-mouse CD45. Vasculature was visualized with anti-collagen type VI whole tissue preparations. We found in control tissues that the distributions of pericytes were occurred on capillaries of both the stria vascularis and spiral ligament. The pericytes wrapped around capillaries in close contact with endothelial cells. Their long processes extended along and around the surface of micro-vessels. The ratio of pericytes to endothelial cells in the cochlear microvessels is around 1:2. Noise stimulation caused the regional increase in the population of pericytes on the vessels of the stria vascularis. The results indicated that pericytes are dynamic cellular population that may play a key role in the change of cochlear blood flow caused by loud sound.

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30 Expression of Monocyte Chemoattractant Proteins (MCPs) in Mouse Cochlea Exposed to Acoustic Trauma

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Acoustic injury results in cochlear monocyte/ macrophage migration into the spiral limbus and spiral ligament of the mouse cochlea, but the signals that induce monocyte migration into the cochlea are unknown (Hirose et al, 2005). Chemokines are small secreted peptides that mediate leukocyte recruitment and activation during inflammation. Based on studies conducted in knockout mice, the chemokine monocyte chemoattractant protein (MCP)-1 (Ccl-2 in the systematic terminology) plays a critical role in monocyte migration to the brain, whereas in the cochlea, Ccl-2 is not necessary for monocyte migration (Sautter and Hirose, abstract ARO 2006). This study was designed to examine gene expression of other MCPs that might exert redundant function with Ccl-2 for monocyte migration to the noise-exposed cochlea. We exposed 8-9 week old Ccl-2 knockout mice to 112dB octave band noise for 2 hours, using non-noise-exposed littermates as controls, harvested cochleas at 24, 48, and 96 hours post noise exposure and analyzed gene expression of MCP-2/Ccl-8, MCP-3/Ccl-7, and MCP-5/Ccl-12 by quantitative real time RT-PCR. Ccl-7 was substantially upregulated in the Ccl2$^{-/-}$ mice, peaking at 48 hours post exposure. Ccl-8 expression was slightly upregulated, again peaking at 48-hour post exposure. There was no change in Ccl-12 expression between 24 and 96-hour post exposure. Our data point towards shared mechanisms for upregulation of Ccl-2 and Ccl-7, but not Ccl-8 or Ccl-12 following acoustic trauma and this issue can be addressed in part by comparing gene expression in wild-type and Ccl-2$^{-/-}$ mice. Our results leave uncertain what role MCPs play in response to acoustic trauma: Ccl-2 does not appear implicated in monocyte recruitment but may be involved in macrophage activation. The function(s) of Ccl-2 and Ccl-7 can be examined in knockouts for the common MCP receptor, CCR2.

31 Chemokine Upregulation Following Acoustic Trauma

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Following noise-induced trauma, inflammatory cells are present in the inner ear. To examine potential mechanisms for active recruitment of cells, gene array analysis and reverse transcriptase polymerase chain reaction (RT-PCR) were performed on cochlear tissue. NIH-Swiss mice were exposed to 118 dB octave band noise for two hours and sacrificed at 2, 18, and 48 hours post-exposure. Gene array (SuperArray, Inc.) analysis indicated increased expression of several members of the CC chemokine family including monocyte chemoattractant protein 5 (MCP-5), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1-beta (MIP-1b). The cell adhesion molecule ICAM was also upregulated. RT-PCR was then performed using primers for the individual mRNA sequences. This confirmed the increased expression of MCP-1, MCP-5, MIP-1b, and ICAM relative to non-exposed control mice. This study demonstrates that several members of the CC chemokine family and the cell adhesion molecule ICAM are upregulated following acoustic trauma. The early nature of this expression implies chemokine production by cochlear tissue rather than infiltrating inflammatory cells. These data provide evidence in support of the idea that a cochlear inflammatory response is initiated in response to acoustic trauma and involves the recruitment of circulating leukocytes to the inner ear.

32 Cochlear Distribution of Small Focal Lesions Following Exposure to a 4-kHz or a 0.5-kHz OBN

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An octave band of noise (OBN) delivers fairly uniform energy over a specific range of frequencies (3-6 kHz for 4-kHz OBN & 0.375-0.750 kHz for 0.5-kHz OBN). Above & below these ranges, energy is at least 50 dB less than that in the OBN. Hair-cell loss often occurs outside the exposure OBN. The frequency location of hair-cell loss is evident when the % location of small focal lesions is analyzed. Data sets were assembled from our permanent collection of noise-exposed chinchillas using the following criteria: 1) The sum of exposure duration & recovery time was less than or equal to 10 days; 2) The exposure level was less than or equal to 108 dB SPL; & 3) Focal lesions were less than 1.5 mm in size. The data sets included a variety of exposures ranging from those that were high-level, short duration to those that were moderate-level, moderate duration. The % location of the center of each focal lesion was determined. Means, SDs & medians were calculated for lesion size for each OBN. Histograms were then constructed from the % location data using 2.0% bins & the counts were graphed relative to total number of lesions. For the 4-kHz OBN, 94% of the lesions were in the basal half of the OC & 6% were in the apical half. For the 0.5-kHz OBN, 29% of the lesions were in the apical half of the OC & 71% were in the basal half. The mean lesion size was 1.48% & 0.68% for the 4-kHz & 0.5-kHz OBN, respectively, with medians of 1.10% & 0.50%. The mean lesion size (in mm) for the 0.5-kHz OBN was less than half that for the 4-kHz OBN. For the 4-kHz OBN, a histogram of the % location of lesions showed that most occurred in the 5-7-kHz region, at & just above the upper edge of the OBN. Clusters of lesions were also found around 8 & 12 kHz. A cluster was present at & just below the lower edge of the OBN, as well as in the 1.5-kHz region. For the 0.5-kHz OBN, a histogram of the % location of lesions showed clusters at 0.25, 0.75 & 1.5 kHz in the apical half. In the basal half, the pattern was very similar (Pearson's r=0.69) to that seen with the 4-kHz OBN. The distribution of basal-turn lesions suggests that the 4-kHz & 0.5-kHz OBN are damaging that region of the cochlea in the same way.

33 Histopathological Changes in the Cochlea Following Exposure to Low-Frequency Noise

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Thirteen chinchillas were exposed for 24 h to a 0.5-kHz OBN at 95 dB SPL. The cochleae of 8 animals were fixed at 0-d post-exposure; 5 were fixed after 1-2 wks of recovery. To keep cellular debris from washing away, all cochleae were plastic-embedded before being dissected into flat preparations. By phase-contrast microscopy, hair-cell losses were determined from apex to base. Damage consisted of scattered loss of OHCs in the apical half of the organ of Corti (OC) & small focal lesions (i.e., > 50% hair-cell loss over at least 0.03 mm) in the basal half. These specific patterns of loss suggest that noise damaged the apex & base by different mechanisms. In order to estimate the timing of cell loss, differential counts of missing & severely injured cells were performed. The presence of immature phalangeal scars & necrotic, oncotic & apoptotic hair cells indicates a recent loss while mature phalangeal scars indicate a long-standing loss. In the apical half of the OC in both groups of animals, many of the phalangeal scars replacing the missing OHCs were immature. Cellular debris was seen in the OC fluid spaces beneath these scars. By TEM, the debris was found dispersed in the Nuel spaces & consisted of vesicles of various sizes, small granules, shrunken & swollen mitochondria &, rarely, fragments of plasma membrane. TEM also revealed the presence of cellular debris in the endolymphatic space near the reticular lamina. This latter finding indicates that the barrier function of the reticular lamina broke down temporarily when the hair cells degenerated, before phalangeal scars formed. Debris in the Nuel spaces was not surrounded by plasma membrane as would be the case if the hair cells had been apoptotic before they died & then formed apoptotic bodies. In the basal half of the OC, focal lesions were found in 2 of eight 0-day & 3 of five 1-2 wk animals. In the 0-day ears, oncotic OHCs were found at the edges of the basal-turn lesions. In the 1-2 wk ears, the basal-turn lesions primarily consisted of immature phalangeal scars. The appearance of debris in the apex & base suggests that many of the OHCs were oncotic rather than apoptotic before they disappeared.

34 Intense Noise Causes Damage to the OHC Lateral Wall Leading to Hearing Loss

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The cochlear active process results in a 40-60 dB cochlear amplification. Outer hair cells (OHCs) constitute an important part of cochlear micro-mechanics and are believed to be the driving force of the cochlear active process by way of their electromotility. The OHC membrane skeleton, consisting of F-actin and spectrin, maintains the unique OHC cylindrical shape and provides stiffness to the cell. In addition to its well known basic functions, the OHC plasma membrane is a main contributor to OHC axial stiffness. Prestin, a membrane protein, has been recently recognized as the OHC motor protein. Changes in the OHC lateral wall may affect OHC electromotility and/or cochlear micromechanics and, subsequently cochlear sensitivity. In this report OHC membrane fluidity (by laser bleach approach), gene expression of beta-actin, beta-spectrin, and prestin were determined after noise exposure. The noise exposure caused a reduction of OHC membrane fluidity and a time-dependent gene expression of the proteins in the OHC lateral wall. The noise-induced changes were associated with permanent threshold shifts. The motor protein appeared to be the most sensitive to noise trauma among the three proteins. The data suggest that non-lethal injuries in the OHC lateral wall may cause loss of the OHC electromotility or the cochlear micromechanics leading to a reduction of cochlear amplification and then cochlear sensitivity.

35 Mechanisms of Oxidative Stress in the Potentiation of Noise-Induced Hearing Loss by Acrylonitrile

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Acrylonitrile (ACN), one of the top 50 chemicals produced in the world, is a very powerful pro-oxidant compound whose metabolism leads to a profound glutathione (GSH) depletion and to a production of cyanide (CN) which, in turn, can inhibit superoxide dismutase (SOD). ACN, by itself, is not ototoxic, but we have shown that it can strongly promote noise-induced hearing loss (NIHL), even at noise levels that do not produce auditory impairment. The mechanism by which ACN renders the cochlea more