Second Place - Resident Basic Science Award 1995

Mitochondrial role in hair cell survival after injury

GREGORY E. HYDE, MD, PhD, and EDWIN W RUBEL, PhD, Seattle, Washington

The role of mitochondrial biogenesis in hair cell survival after injury was evaluated by inhibiting mitochondrial protein synthesis with chloramphenicol and then studying the effects on hair cell survival after exposure to two different types of ototoxins, gentamicin and acoustic trauma. Seven- to 10-day-old chicks were treated with either a single injection of gentamicin (250 mg/kg) or noise (1500 Hz at 120 dB sound pressure level for 14 hours). A subset of the gentamicin- and noise-treated animals also received chloramphenicol (1200 mg/kg during a 24-hour period) through a subcutaneous osmotic pump. A control group received chloramphenicol alone (1200 mg/kg during a 24-hour period). All animals were sacrificed after 5 days, and their basilar papillae (cochleas) were prepared for scanning electron microscopy. Hair cell loss was quantified with stereologic techniques. Animals treated with chloramphenicol alone did not have any evidence of hair cell loss. Gentamicin-treated animals had characteristic hair cell loss beginning at the basal tip and tapering out along the inferior edge more distally. The addition of chloramphenicol to gentamicin treatment significantly increased hair cell loss by 30%, extending the area of hair cell loss into the superior hair cell region at the distal boundary of the lesion. Pure-tone noise exposure characteristically produced hair cell loss along the inferior edge and occasionally included hair cells along the most superior edge. Addition of chloramphenicol to noise exposure significantly increased hair cell loss by 80%, with extension of the lesion across the full width of the sensory epithelium and basally. These results demonstrate that mitochondrial biogenesis is involved in cellular responses to injury. They suggest that mitochondrial function may regulate the probability of survival after metabolic challenges to hair cell integrity. (OTOLARYNGOL HEAD NECK SURG 1995;113:530-40.)

Exposure of a hair cell to an ototoxin or acoustic trauma results in an injury to the cell. After injury there are two potential outcomes. If the injury is sufficiently severe, the hair cell will succumb to the injury and die. On the other hand, if the injury is less severe or has a reversible component, the hair cell may respond to the injury and ultimately survive after the damage is repaired.

An impressive amount of research has been done to try to understand the mechanisms involved in the injury of hair cells by ototoxic drugs and noise and the processes involved in the apoptotic-like death of hair cells. Relatively little is known regarding the cellular mechanisms that allow a hair cell to recover from injury and survive.

Injury places an oxidative stress on the cell. The repair of damaged cellular structures and the correction of altered intracellular ion concentrations that result from injury dramatically increase the energy requirements of the cell. Mitochondria can respond short term to the increased demands by upregulating existing respiratory enzyme complexes.[1] However, with large or continued energy requirements, mitochondria must increase their ability to make adenosine triphosphate by synthesizing additional respiratory enzyme complexes.[2] This process is known as mitochondrial biogenesis.

Mitochondrial biogenesis has been shown to be an

From the Department of Otolaryngology/Head and Neck Surgery, University of Washington School of Medicine.

Funded by an American Academy of Otolaryngology–Head and Neck Surgery resident research grant and National Institute on Deafness and Other Communication Disorders grant no. DC00395.

Presented at the Annual Meeting of the American Academy of Otolaryngology–Head and Neck Surgery, New Orleans, La., Sept. 17-20, 1995; and at the Midwinter Meeting of the Association for Research in Otolaryngology, St. Pete Beach, Fla., Feb. 9, 1995.

Received for publication July 20, 1995; accepted July 20, 1995.

Reprint requests: Edwin W Rubel, PhD, Virginia Merrill Bloedel Hearing Research Center, Department of Otolaryngology/Head & Neck Surgery, RL-30, University of Washington School of Medicine, Seattle, WA 98195.

0194-5998/95/$5.00 + 0 **23/1/67761**

important response to injury in several different tissue types. Oxidative stress or injury to skeletal muscle[3-5] or cardiac muscle[6] results in the stimulation of mitochondrial biogenesis. A similar response has been shown for liver.[7]

In the central auditory system, we have previously shown that the injury that occurs in cochlear nucleus neurons after the removal of afferent input causes a rapid induction of mitochondrial biogenesis.[8] Inhibition of mitochondrial biogenesis after deafferentiation dramatically increases the number of neurons that undergo transneuronal cell death.[9] Bagger-Sjöback and Wersall,[10] in their study of the progressive changes in hair cell mitochondria caused by gentamicin exposure, showed ultrastructural changes consistent with mitochondrial biogenesis.

The purpose of this study is to assess the role of mitochondrial biogenesis in responses of hair cells to the two most well-characterized forms of injury, aminoglycoside toxicity and noise exposure, in a well-characterized animal model. To test the hypothesis that mitochondrial biogenesis is involved in hair cell survival, we compared loss of hair cells in control animals exposed to each of these treatments with animals in which mitochondrial biogenesis was inhibited during the treatment and for the succeeding 24 hours. Mitochondrial biogenesis was inhibited by administering chloramphenicol, an antibiotic. Chloramphenicol binds to the 50S subunit of mitochondrial ribosomes and inhibits the transcription of proteins encoded on the mitochondrial genome without inhibiting transcription of nuclear-encoded proteins.[11] Chloramphenicol has good central nervous system penetrance and has been previously shown to have the ability to inhibit mitochondrial biogenesis in brain stem neurons.[9] We also show that chloramphenicol alone is not ototoxic.

METHODS AND MATERIAL

Animals

We used 7- to 10-day-old White Leghorn chickens as the subjects of our studies. Eggs obtained from a local supplier (H&N International, Redmond, Wash.) were incubated in a forced-draft incubator and hatched at the University of Washington vivarium, a facility approved by the American Association for Accreditation of Laboratory Animal Care. After hatching and during the experimental period, animals were housed in communal brooders with ad libitum food and water. All procedures involving experimental animals were reviewed and approved by the University of Washington Animal Care Committee.

Mitochondrial Protein Synthesis Inhibition

Chloramphenicol was administered at a rate of 1200 mg/kg for a 24-hour period through an osmotic pump (Alza Corp., Palo Alto, Calif.) implanted subcutaneously in the birds' backs. This dosage of chloramphenicol has been shown in previous studies to attenuate mitochondrial biogenesis sufficiently to increase neuronal death after injury.[9] An aqueous solution of chloramphenicol was prepared at an appropriate concentration after the mean weight of the animals in the treatment group was determined. The pumps were filled and primed for 4 hours by submersion in normal saline at 37° C. After inhalational anesthetic (Metofane) was administered, the pumps were implanted in a subcutaneous pouch created over the birds' superior backs. The pumps were designed to be exhausted after 24 hours of delivery. They were left in place for the duration of the study.

Three animals treated with cloramphenicol alone comprised a control group for both the gentamicin and acoustic trauma treatment groups (see below). Four papillae from these animals were examined by scanning electron microscopy to determine whether chloramphenicol alone disrupted hair cell morphology or produced hair cell loss.

Gentamicin Treatment

Five animals were treated with gentamicin (Lyphomed) 250 mg/kg given in one intramuscular injection. Five additional animals were treated with gentamicin (250 mg/kg) plus chloramphenicol (1200 mg/kg). Both gentamicin- and gentamicin/chloramphenicol-treated animals exhibited lethargy for 12 to 24 hours after injection. This was followed by full recovery with appropriate weight gain through the remainder of the survival period.

Acoustic Trauma

Sound exposure was administered in an acoustic chamber. A cylindrical wire cage with partitions was constructed to allow the placement of four birds directly under the speaker with their heads at the same height. Sound level exposure was measured with a 0.5-inch microphone (General Radio [Concord, Mass.] type 1560-P42) and spectrum analyzer (Hewlett-Packard model 3561A, Hewlett-Packard Co., Palo Alto, Calif.). The spectrum analyzer was calibrated to a 114-dB piston phone (General Radio type 1562-A) at 125, 250, 500, 1000, and 2000 Hz. Chamber calibration showed less than 1-dB sound pressure level variation between each of the four sections of the animal cage. Sound exposure levels

were set with the animals in place in the chamber and the microphone suspended at the level of their heads. A 1500-Hz pure tone was generated with a Wavetek Waveform generator (model 186; Wavetek Corp., San Diego, Calif.) and amplified to 120 dB SPL. Animals were exposed for 14 hours. Sound levels were checked two to three times during the exposure. A total of 11 animals were treated with noise alone, and 13 animals were treated with noise plus chloramphenicol.

Specimen Preparation

All animals were euthanized after 5 days' survival from the onset of treatment. Deep anesthesia was induced by intracardiac injection of Nembutal, and the animals were decapitated. The basilar papillae were then fixed by perfusion of the perilymphatic space with 4% paraformaldehyde, 1% glutaraldehyde, and 3% sucrose in 0.1 mmol/L phosphate-buffered saline solution (pH 7.4). The round and oval windows and lagena were opened for the perfusion. Perfusion of both papillae was initiated within 4 minutes after decapitation and continued for 10 minutes. The heads were then immersed in the same fixative overnight. The bone and cartilage overlying the basilar papillae were then dissected away, exposing the tegmentum vasculosum. The individual temporal bones were then harvested and washed in several changes of phosphate-buffered saline solution. The specimens were then osmicated, and the tegmentum vasculosum and tectorial membranes were removed to expose the entire length of the sensory epithelium. The temporal bones were then critical point dried, mounted on stubs, and sputter coated with gold/palladium. Each basilar papilla was viewed with a JEOL 6300 scanning electron microscope (JEOL USA, Inc., Peabody, Mass.) with 15-kV accelerating voltage. Photomontages were made of those papilla that were relatively free of artifact and dissection damage in the hair cell loss region. We attempted to photograph each papilla at a right angle to the plane of the neuroepithelium whenever possible.

Stereologic Analysis of Hair Cell Loss

Photomontages were constructed of the entire papilla for each specimen photographed. The area of hair cell loss was quantified with the Cavalieri point-counting method.[12,13] In brief, transparent point grids were randomly oriented over each frame of the photomontage. The surface area of the region displaying damaged hair cells and the total area of the papilla were estimated by counting the number of points overlying each area, respectively. The point grid spacing was then calibrated to the calibration bar on each photomicrograph, and the surface area in square micrometers was calculated.

In addition to measuring the total area of hair cell damage in each papilla, we measured the length of the region of hair cell loss along both the inferior and superior edges of each papilla. Raw measurements were then converted to micrometers with the photomicrograph calibration bars.

For the noise-exposed animals, we also measured the hair cell numerical density in three regions. The midpoint of the length of damage for each papilla was calculated and marked on the photomontages. The number of hair cells in a 25-μm-wide strip running perpendicular to the inferior edge of the papilla at the midpoint was then counted. The standard deviation of the length of damage in animals treated with noise only was then calculated. Points 1 SD proximal and distal to the midpoint position were then marked on each photomontage, and the number of hair cells in a 25-μm-wide strip was again counted at each of these positions on all montages. Hair cell counts across the width of the papilla were converted into numerical density per 1000 μm^2. All measurements on all animals were performed in a blinded fashion; the experimenter was unaware of the treatment group when performing measurements.

Significant differences between treatment vs. treatment plus chloramphenicol groups in each experiment were detected with a two-tailed, nonpaired *t* test with a criterion level of 5%. No comparisons were made between the gentamicin and acoustic trauma groups.

RESULTS

Chloramphenicol Alone

Photomontages of four papillae from the three animals treated with only chloramphenicol were examined (Fig. 1*A*). We carefully examined the neuroepithelium and looked for disruptions in the orderly hexagonal array over the entire length of the papilla. We were not able to find any disruption or evidence of hair cell loss. We also viewed the hair cells at higher magnification and looked for evidence of injury without hair cell loss (Fig. 1*B* and *C*). The stereocilia were orderly throughout the papilla, and there was no evidence of hair cell swelling or apical blebbing, as has been described with injury.[14,15]

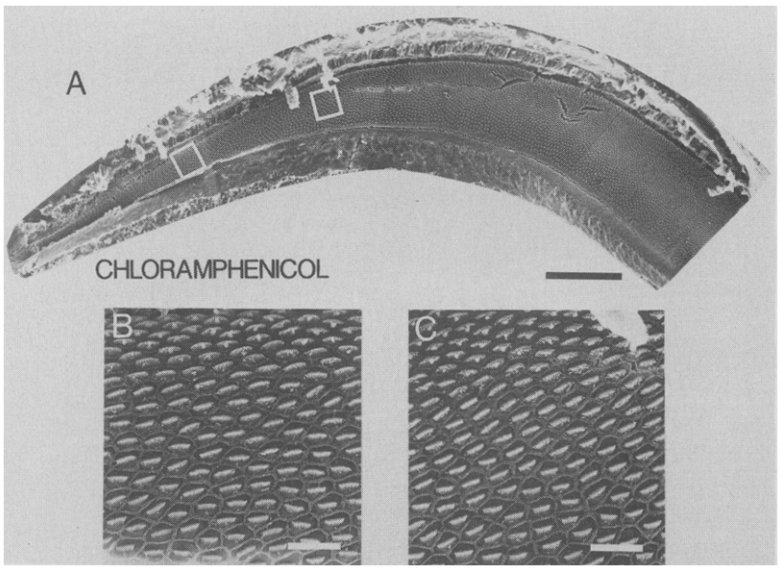

Fig. 1. **A,** Representative photomontage of the proximal two thirds of the basilar papilla from a bird treated with chloramphenicol alone. Apical portion in all photomontage figures was deleted to allow greater magnification. There was no evidence of hair cell loss. (Bar = 200 μm.) **B,** Higher magnification of hair cells 400 μm from basal tip (*proximal inset box,* **A**). Note orderly array and absence of damaged stereocilia or apical blebbing. (Bar = 20 μm.) **C,** Higher magnification of hair cells 800 μm from basal tip (*distal inset box,* **A**). Again, the hair cells appear entirely normal (same magnification as in **B**).

Gentamicin Vs. Gentamicin Plus Chloramphenicol

A total of eight papillae from the five animals treated with gentamicin alone and eight papillae from the five animals treated with gentamicin plus chloramphenicol were photomontaged and quantitatively examined (Fig. 2*A* and *B*).

Gentamicin alone was found to produce a consistent lesion on the proximal end of the papilla (Fig. 2*A*). There was loss of hair cells across the entire width of the papilla at the basal end. As the lesion proceeded more distally, the hair cell loss tapered out along the inferior edge of the papilla, with preservation of an increasingly greater number of the more superior hair cells (Fig. 2*C*). Early regenerating hair cells could be seen within the lesion area (not shown).

When chloramphenicol was given with gentamicin, a consistently larger lesion was produced (Fig. 2*B*). Like the animals treated with gentamicin alone, there was total loss of hair cells across the entire width of the papilla at the basal end. However, as the lesion proceeded more distally the hair cell loss continued to cover the full width of the papilla, with loss of both the superior and inferior hair cells (Fig. 2*D*). Early regenerating hair cells were also seen within the lesion area in chloramphenicol/gentamicin-treated animals (not shown).

The means of the damaged area for the gentamicin- and chloramphenicol/gentamicin-treated papillae were 23,348 μm^2 ($\pm$1496 SEM) and 30,096 μm^2 ($\pm$2538 SEM), respectively (Fig. 3). Thus addition of chloramphenicol resulted in an average increase in the area of hair cell loss by approximately 30%. This difference in damage area between the two groups was statistically significant ($p < 0.05$).

The average length of the region of hair cell loss was measured along both the inferior and superior edges of each papilla (Fig. 4). The average lengths of the inferior damage border for the gentamicin- and chloramphenicol/gentamicin-treated papillae were 875.9 μm ($\pm$36.0 SEM) and 868.4 μm ($\pm$31.9 SEM), respectively. There was no significant difference in the inferior length of damage between the groups ($p > 0.2$). The average lengths of the region of damage along the superior edge for the gentamicin- and chloramphenicol/gentamicin-treated papillae were 650.0 μm ($\pm$37.1 SEM) and 783.0 μm ($\pm$48.0 SEM), respectively. This amounted to a 20% increase in the length of hair cell loss along the superior edge with the addition of chloramphenicol.

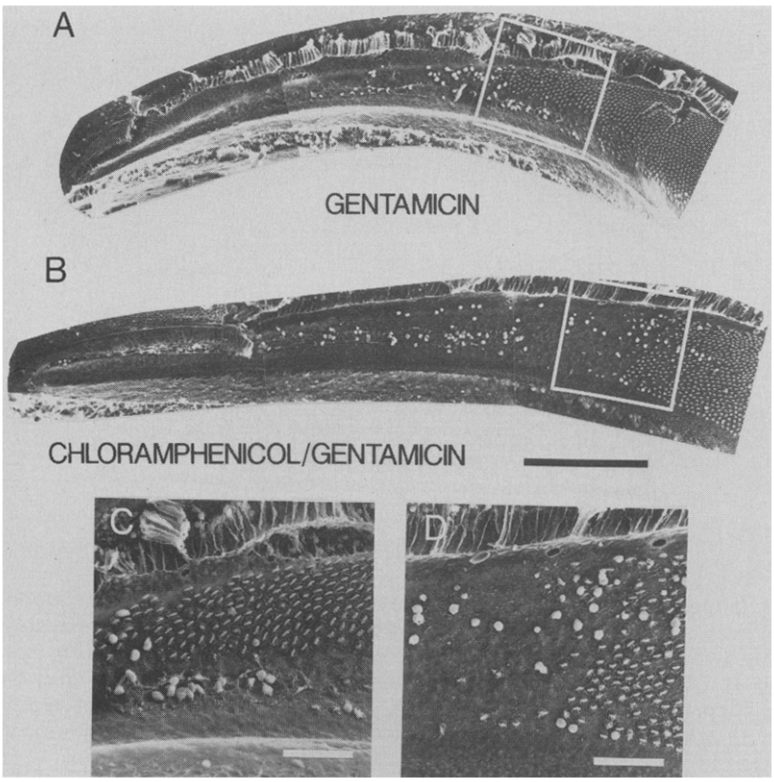

Fig. 2. A, Representative photomontage of proximal papilla from a bird treated with gentamicin alone. Hair cell loss extends the full width at the basal tip and then tapers out along the inferior edge as the lesion proceeds distally, preserving the more superior hair cells. **B,** Representative photomontage of proximal papilla from a bird treated with gentamicin and chloramphenicol. Hair cell loss extends the full width at the basal tip and continues down the papilla, extending the full width of the papilla. (**A** and **B,** Bar = 200 μm.) **C,** Higher magnification of inset box in **A,** showing tapering hair cell loss along inferior edge of papilla. (Bar = 50 μm.) **D,** Higher magnification of inset box in **B** showing full width hair cell loss extending to distal border of lesion (same magnification as in **C**).

This difference was statistically significant ($p < 0.05$). When the superior damage length was normalized to the total length for each papilla, the differences between the two groups remained significant.

Noise Vs. Noise Plus Chloramphenicol

A total of 19 papillae from 11 animals treated with noise alone and 26 papillae from 13 animals treated with noise plus chloramphenicol were photomontaged and quantitatively examined (Fig. 5).

Exposure to a 1500-Hz pure tone at 120 dB SPL for 14 hours produced a consistent crescent-shaped region of hair cell loss along the inferior edge of the papilla, starting approximately 625 μm from the basal tip and extending to approximately 1400 μm (Fig. 5*A*). Occasionally a strip of hair cells along the most superior edge of the papilla was also lost, sparing the more centrally located hair cells. Rarely was there loss of hair cells across the entire width of the papilla in this group.

With the addition of chloramphenicol to noise exposure, there was consistent loss of hair cells beginning an average of 375 μm from the basal tip and proceeding along the full width of the papilla distally to ~1400 μm (Fig. 5*B*). In a few animals in this group, hair cell loss began at the basal tip.

Measurements of the area of hair cell loss revealed an average area of hair cell loss of 48,795 μm^2 (±6890 SEM) and 87,878 μm^2 (±8413 SEM) for the noise- and noise plus chloramphenicol–treated animals, respectively. Thus the addition of chloramphenicol increased the area of hair cell loss by ~80%, which was highly significant ($p < 0.001$).

Measurements of the length of hair cell loss along the inferior edge of the papilla showed an average length of damage of 765.0 μm (±53.5 SEM) for animals treated with noise alone and 1032.8 μm

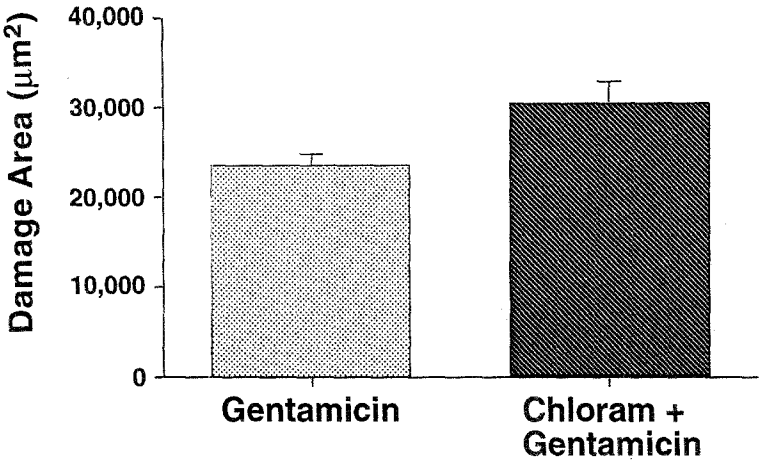

Fig. 3. Graph summarizing the results of damage area measurements. (Error bars = SEM.) There is a 30% increase in the area of hair cell loss with the addition of chloramphenicol, which is statistically significant ($p < 0.05$).

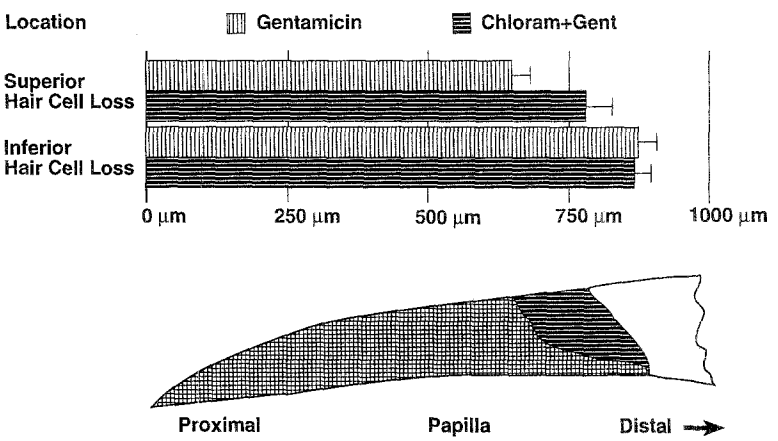

Fig. 4. Graph summarizing the results of length measurements of hair cell loss along superior and inferior edges of the lesion. (Error bars = SEM.) Scale schema below graph shows the pattern of hair cell loss with gentamicin *(vertical stripes)* and gentamicin/chloramphenicol *(horizontal stripes)*. There was no significant change in the length of hair cell loss along the inferior border with the addition of chloramphenicol ($p > 0.02$). There was a significant increase in the length of hair cell loss along the superior border with the addition of chloramphenicol ($p < 0.05$).

(±31.4 SEM) for animals treated with noise plus chloramphenicol (Fig. 6). There was no significant difference in the length of damage distally between the two groups ($p > 0.20$). However, there was a highly significant difference in the basal extent of the damage ($p < 0.01$). Thus addition of chloramphenicol characteristically produces a 35% longer lesion with extension toward the basal end of the papilla. Statistical analyses were repeated after the length of hair cell loss to the total length of the papilla was normalized. The differences between groups for total length of hair cell loss and basal extension of hair cell loss were still highly significant.

To determine the extent of damage to the more centrally located hair cells, we measured the numerical density of the hair cells at the midpoint of the region of hair cell loss and plus or minus 1 SD of the length of damage in noise-exposed animals (Fig. 7). The results of those measurements are summarized in Fig. 7. The difference in the hair cell density between the two groups at the midpoint minus 1 SD was not statistically significant. However, there was a significant difference in hair cell density at the midpoint location and at the midpoint plus 1 SD location.

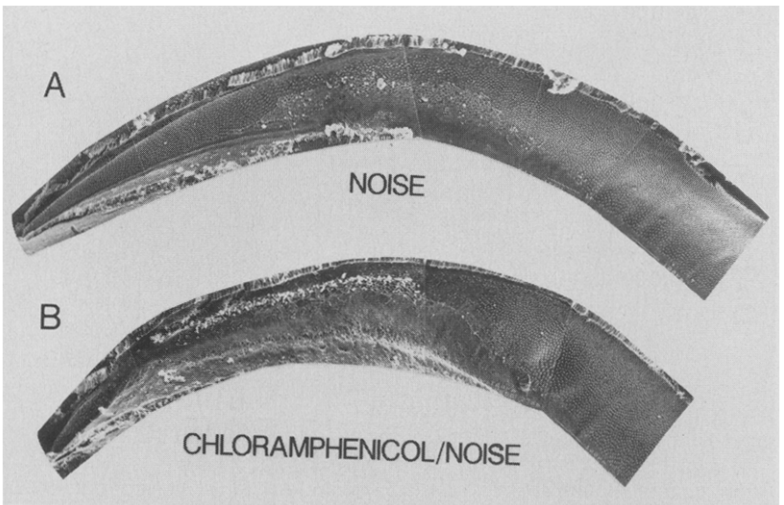

Fig. 5. **A,** Representative photomontage of a papilla from a bird treated with pure tone alone (1500 Hz, 120 dB sound pressure level, 14 hours). Hair cell loss occurs primarily along the inferior edge between 700 and 1400 μm from the basal tip. Occasionally (as in this subject) a strip of hair cell loss was observed along the superior edge as well. The centrally located hair cells were spared. **B,** Representative photomontage of a papilla from a bird treated with the same pure tone and chloramphenicol. Hair cell loss extends across the full width of the papilla and more basally than in **A.** There was an average of an 80% increase in the area of hair cell loss with the addition of chloramphenicol ($p < 0.001$). (Bar = 200 μm.)

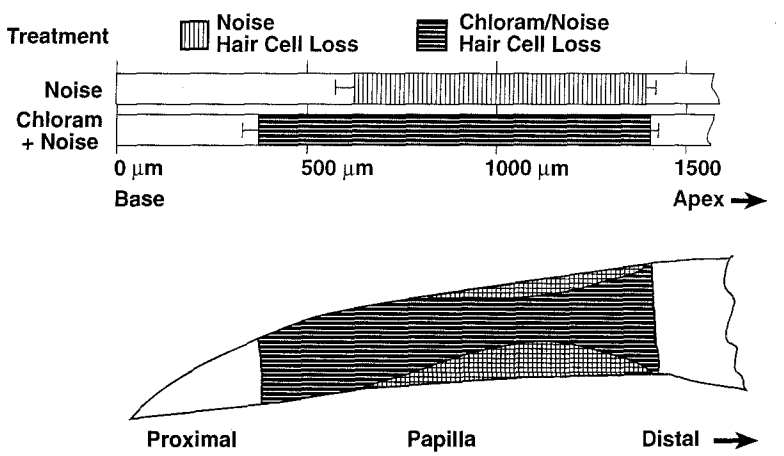

Fig. 6. Graph summarizing the results of length measurements along the inferior border of hair cell loss. (Error bars = SEM.) Scale schema below graph shows the pattern of hair cell loss in noise-treated *(vertical stripes)* and chloramphenicol/noise-treated *(horizontal stripes)* animals. There was no significant difference between the two groups in the location of the distal boundary of hair cell loss. The addition of chloramphenicol caused significant extension of the inferior border of hair cell loss toward the basal tip ($p < 0.01$).

DISCUSSION

In this study we show that mitochondrial biogenesis appears to play a role in the ability of a hair cell to survive after injury. Our findings indicate that mitochondrial biogenesis may be a common response to injury because inhibition of this process significantly increases the degree of hair cell loss after two very different types of injury, gentamicin exposure and acoustic trauma.

It could be argued that we have shown another case of synergistic activity between two ototoxins, chloramphenicol and gentamicin or chloramphenicol

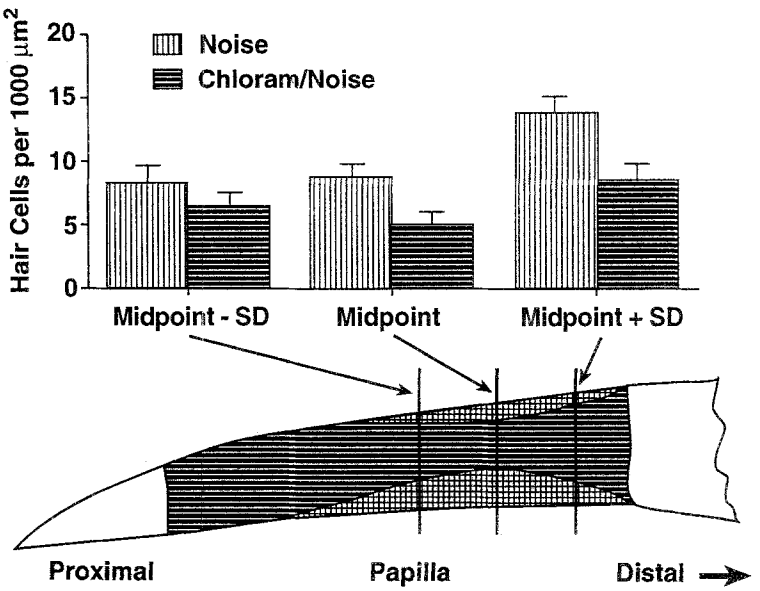

Fig. 7. Graph summarizing the results of hair cell density measurements at the midpoint of the hair cell loss region 1 SD of the length of hair cell loss in noise-treated animals. (See lines on schema showing approximate location of measurements.) There was a significant decrease in hair cell density at the midpoint and midpoint +1 SD locations with the addition of chloramphenicol ($p < 0.05$).

col and noise. We will examine the viability of this alternate interpretation by discussing the effects of ototoxins on mitochondria, reviewing the literature regarding possible chloramphenicol ototoxicity, and examining the role mitochondrial dysfunction plays in sensorineural hearing loss.

Ototoxins as Mitochondrial Toxins

Many ototoxins have been shown to inhibit the respiratory function of mitochondria. Aminoglycosides have been shown to decrease mitochondrial respiration,[16,17] block calcium uptake by mitochondria,[16,18] and stimulate free radical production by mitochondria.[19] Cisplatin also decreases mitochondrial respiration by uncoupling oxidative phosphorylation,[20] decreases calcium uptake by mitochondria,[21] and stimulates the production of free radicals.[22] Salicylates[23,24] and loop diuretics[25] have been shown to be weaker uncouplers of oxidative phosphorylation. Acoustic trauma stimulates mitochondrial swelling,[26] which can be a sign of mitochondrial uncoupling as well.[27]

Combinations of ototoxins have been shown to have a synergistic effect. Cisplatin and noise,[28] aminoglycosides and noise,[29-31] salicylates and noise,[32,33] diuretics and noise,[34] and aminoglycosides and diuretics[35,36] all combine to either increase the size of the lesion produced by a single agent alone or cause hair cell loss when subototoxic levels of both agents are used.

One possible explanation for the synergism of many ototoxins may be a combined mitochondrial toxicity. Additive inhibition of mitochondrial function would limit the hair cell's ability to respond to the oxidative stress of injury. Thus metabolic exhaustion and ultimately death would occur in more hair cells after an injury.

If synergism is additive mitochondrial inhibition, aminoglycosides could be viewed as a synergistic effect in a single agent. Aminoglycosides both inhibit mitochondrial respiration[16] and block mitochondrial protein synthesis,[37] thus doubly limiting the ability of mitochondria to respond to injury.

Is Chloramphenicol an Ototoxin?

Our study failed to show any evidence of hair cell loss in any of the animals treated with chloramphenicol alone. There was also no surface evidence of injury at the time we looked. Because the animals had 4 days to recover after chloramphenicol exposure, it is possible that ultrastructural changes could have developed during chloramphenicol exposure but were then repaired before euthanasia and examination. Our previous work on cochlear nucleus neurons exposed to systemic chloramphenicol at the same dose used in this study failed to show any

changes in the appearance of control neurons when examined during the exposure period by both light and transmission electron microscopy.[9]

A case report of hearing loss in a child who received a prolonged course (26 days) of high-dose (125 mg/kg) chloramphenicol is in the literature.[38] The child was being treated for meningitis, which alone can cause hearing loss. While studying audiogenic seizures, Glenn et al.[39] treated animals with otitis media with oral chloramphenicol and found an ototoxic interaction with noise exposure. Henley et al.[40] reconfirmed this finding but found the most significant synergism only in those animals with otitis media. Animals treated with chloramphenicol without noise exposure did not have any significant change in their cochlear microphonic or N1 component of the eighth nerve action potential.

Chloramphenicol's effect on mitochondria is specific to mitochondrial protein synthesis. Williams and Harlan[11] studied the effects of chloramphenicol on the up-regulation of mitochondrial enzymes in muscle after stimulation. They found chloramphenicol inhibited up-regulation of cytochrome oxidase, a respiratory enzyme partially encoded by the mitochondrial genome. Baseline cytochrome oxidase function was not affected. Also, no effect was found on the up-regulation of nuclear-encoded oxidative enzymes such as the Kreb's cycle enzymes; up-regulation still occurred. In addition, it is doubtful that the chloramphenicol treatment administered in our study had a significant effect on inhibiting baseline mitochondrial function because the half-life of the mitochondria-encoded enzymes exceeds the 24-hour duration of chloramphenicol exposure.[41]

To summarize, although two animal studies and a case report have suggested a synergistic effect between chloramphenicol and ototoxins or noise, an ototoxic effect of chloramphenicol alone has not been established or even suggested. If we are to define an ototoxin as any agent that either alone or in combination with another agent can cause or potentiate the loss of hair cells, chloramphenicol certainly would qualify. However, if our hypothesis that mitochondrial up-regulation and biogenesis facilitates the reparative processes necessary for hair cell survival after injury is correct, any agent or condition that attenuates mitochondrial function should be considered a potential ototoxin. This would include other antibiotics that inhibit mitochondrial protein synthesis such as tetracyclines, neomycin, erythromycin and macrolides, and conditions of impaired mitochondrial function such as hypothyroidism and iron deficiency.

Sensorineural Hearing Loss and Mitochondrial Dysfunction

There are several interesting correlations between mitochondrial function and sensorineural hearing loss that deserve discussion. Hypothyroidism has been associated with hearing loss for some time. Congenital thyroid dysfunction associated with hearing loss was recognized by Pendred in 1896.[42] Batsakis and Nishiyama[43] report that about half of patients with hypothyroidism have hearing improvement on treatment with thyroid extract. Thyroid hormone has been shown to control mitochondrial function by regulating the production of nuclear- and mitochondrial-encoded mitochondrial proteins.[44]

Iron deficiency has been shown to sensitize animals to acoustic trauma.[26] Several key mitochondrial respiratory enzymes, such as the cytochromes, require iron as a cofactor.[41]

Nonsyndromic maternally inherited sensorineural hearing loss has recently been recognized.[45] The defect, which results in increased sensitivity to aminoglycosides, has been traced to a mutation on the mitochondrial genome; this mutation encodes a mitochondrial transfer RNA[46,47] and thus impairs the synthesis of mitochondrial proteins.

Aging results in diminished mitochondrial function.[48] Recently, mitochondrial dysfunction has been recognized in neurodegenerative disorders of aging such as Parkinson's disease,[49] Huntington's chorea,[49] and Alzheimer's disease.[50,51] Degeneration of the auditory system and hearing loss have also been associated with these disorders.[52] These findings raise the possibility of mitochondrial dysfunction playing a role in some forms of presbycusis. Further study is certainly warranted.

Finally, the increasing awareness of mitochondrial dysfunction in a variety of disorders is leading to the development of "mitochondrial medicine," therapeutics aimed at improving mitochondrial function.[53] Treatment with free radical scavengers,[54] calcium channel blockers,[55] and stimulators of mitochondrial biogenesis[56] has been shown to have a positive effect on hair cell survival after injury. All of these treatments may act by enhancing the ability of hair cells to mount an effective survival response to metabolic and/or structural insults. Future research along these lines may be able to identify treatments that could potentially reverse acute hearing loss and protect against the effects of ototoxic agents and noise.

We thank Dale Cunningham for his technical assistance.

REFERENCES

1. Denton RM, Rutter GA, Midgley PJ, McCormack JG. Effects of Ca2+ on the activities of the calcium-sensitive dehydrogenases within the mitochondria of mammalian tissues. J Cardiovasc Pharmacol 1988;12:S69-72.
2. Attardi G, Schatz G. Biogenesis of mitochondria. Annu Rev Cell Biol 1988;4:289-333.
3. Murakami T, Shimomura Y, Fujitsuka N, et al. Enzymatic and genetic adaptation of soleus muscle mitochondria to physical training in rats. Am J Physiol 1994;267:E388-95.
4. Takahashi M, Hood DA. Chronic stimulation-induced changes in mitochondria and performance in rat skeletal muscle. J Appl Physiol 1993;74:934-41.
5. Williams RS, Garcia-Moll M, Mellor J, Salmons S, Harlan W. Adaptation of skeletal muscle to increased contractile activity. Expression nuclear genes encoding mitochondrial proteins. J Biol Chem 1987;262:2764-7.
6. Hood DA, Balaban A, Connor MK, et al. Mitochondrial biogenesis in striated muscle. Can J Appl Physiol 1994;19: 12-48.
7. Nagino M, Tanaka M, Nishikimi M, et al. Stimulated rat liver mitochondrial biogenesis after partial hepatectomy. Cancer Res 1989;49:4913-8.
8. Hyde GE, Durham D. Rapid increase in mitochondrial volume in nucleus magnocellularis neurons following cochlea removal. J Comp Neurol 1994;339:27-48.
9. Hyde GE, Durham D. Increased deafferentiation-induced cell death in chick brainstem auditory neurons following blockade of mitochondrial protein synthesis with chloramphenicol. J Neurosci 1994;14:291-300.
10. Bagger-Sjöback D, Wersall J. Gentamicin-induced mitochondrial damage in inner ear sensory cells of the lizard *Calotes versicolor.* Acta Otolaryngol Stockh 1978;86:35-51.
11. Williams RS, Harlan W. Effects of inhibition of mitochondrial protein synthesis in skeletal muscle. Am J Physiol 1987;253:C866-71.
12. Weibel ER. Stereological methods. London: Academic Press, 1979.
13. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147:229-63.
14. Cotanche DA, Dopyera CE. Hair cell and supporting cell response to acoustic trauma in the chick cochlea. Hear Res 1990;46:29-40.
15. Saunders JC, Cohen YE, Szymko YM. The structural and functional consequences of acoustic injury in the cochlea and peripheral auditory system: a five year update. J Acoust Soc Am 1991;90:136-46.
16. Kaloyanides GJ. Aminoglycoside-induced functional and biochemical defects in the renal cortex. Fundam Appl Toxicol 1984;4:930-43.
17. Weinberg JM, Simmons FJ, Humes HD. Alterations in mitochondrial respiration induced by aminoglycoside antibiotics. Res Commun Chem Pathol Pharmacol 1980;27:521-31.
18. Weinberg JM. The role of cell calcium overload in nephrotoxic renal tubular cell injury. Am J Kidney Dis 1986;8:284-91.
19. Shah SV, Walker PD. Reactive oxygen metabolites in toxic acute renal failure. Ren Fail 1992;14:363-70.
20. Brady HR, Kone BC, Stromski ME, Zeidel ML, Giebisch G, Gullans SR. Mitochondrial injury: an early event in cisplatin toxicity to renal proximal tubules. Am J Physiol 1990;258: F1181-7.
21. Gemba M, Nakatani E, Teramoto M, Nakano S. Effect of cisplatin on calcium uptake by rat kidney cortical mitochondria. Toxicol Lett 1987;38:291-7.
22. Sugiyama S, Hayakawa M, Kato T, Hanaki Y, Shimizu K, Ozawa T. Adverse effects of anti-tumor drug, cisplatin, on rat kidney mitochondria: disturbances in glutathione peroxidase activity. Biochem Biophys Res Commun 1989;159:1121-7.
23. Gutknecht J. Salicylates and proton transport through lipid bilayer membranes: a model for salicylate-induced uncoupling and swelling in mitochondria. J Membr Biol 1990;115: 253-60.
24. Haas R, Parker WD Jr, Stumpf D, Eguren LA. Salicylate-induced loose coupling: protonmotive force measurements. Biochem Pharmacol 1985;34:900-2.
25. Eknoyan G, Sawa H, Hyde Sd, Wood JM, Schwartz A, Suki W. Effect of diuretics on oxidative phosphorylation of dog kidney mitochondria. J Pharmacol Exp Ther 1975;194:614-23.
26. Sun AH, Wang ZM, Xiao SZ, et al. Noise-induced hearing loss in iron-deficient rats. Acta Otolaryngol Stockh 1991;111: 684-90.
27. Wlodawer P, Parsons DF, Williams GR, Wojtczak L. Morphological changes in isolated rat-liver mitochondria during swelling and contraction. Biochem Biophys Acta 1966;128: 34-47.
28. Gratton MA, Salvi RJ, Kamen BA, Saunders SS. Interaction of cisplatin and noise on the peripheral auditory system. Hear Res 1990;50:211-23.
29. Brown J, Brummett RE, Fox K, Bendrick T. Combined effects of noise and kanamycin. Arch Otolaryng 1980;106: 744-50.
30. Pye A, Collins P. Interaction between sound and gentamicin: immediate threshold and stereociliary changes. Br J Audiol 1991;25:381-90.
31. Bhattacharyya TK, Dayal VS. Potentiation of cochlear hair cell loss by acoustic stimulus and gentamicin in the guinea pig. Anat Rec 1991;230:136-45.
32. Carson SS, Prazma J, Pulver SH, Anderson T. Combined effects of aspirin and noise in causing permanent hearing loss. Arch Otolaryngol Head Neck Surg 1989;115:1070-5.
33. Spongr VP, Boettcher FA, Saunders SS, Salvi RJ. Effects of noise and salicylate on hair cell loss in the chinchilla cochlea. Arch Otolaryngol Head Neck Surg 1992;118:157-64.
34. Vernon J, Brummett R, Brown R. Noise trauma induced in the presence of loop-inhibiting diuretics. Trans Am Acad Ophth Otol 1977;84:407-13.
35. Brummett RE, Traynor J, Brown R, Himes D. Cochlear damage resulting from kanamycin and furosemide. Acta Otolaryngol Stockh 1975;80:86-92.
36. West BA, Brummet RE, Himes DL. Interaction of kanamycin and ethacrynic acid: severe cochlear damage in guinea pigs. Arch Otolaryngol Head Neck Surg 1973;98:32-7.
37. Sande MA, Mandell GL. Antimicrobial agents—tetracyclines and chloramphenicol. In: Goodman LS, Gilman A, eds. The pharmacological basis of therapeutics. 6 ed. New York: The Macmillan Co., 1980:1181-99.
38. Gargye AR, Dutta DV. Nerve-deafness following chloromycetin therapy. Ind J Pediat 1959;26:265.
39. Glenn DW, Brown RD, Jobe PC, Penny JE. A comparison of cochlear microphonics and N1 in audiogenic-seizure-susceptible and control rats. Neurolog Res 1980;2:85-100.
40. Henley CM, Brown RD, Penny JE, Kupetz SA, Hodges KB, Jobe PC. Impairment in cochlear function produced by

chloramphenicol and noise. Neuropharmacology 1984;23: 197-202.
41. Luzikov VN. Mitochondrial biogenesis and breakdown. New York: Plenum Press, 1985.
42. Pendred V. Deaf-mutism and goitre. Lancet 1896;2:532.
43. Batsakis J, Nishiyama R. Deafness with sporadic goiter. Arch Otolaryngol Head Neck Surg 1962;76:401.
44. Wiesner RJ, Kurowski TT, Zak R. Regulation by thyroid hormone of nuclear and mitochondrial genes encoding subunits of cytochrome-c oxidase in rat liver and skeletal muscle. Mol Endocrinol 1992;6:1458-67.
45. Hu DN, Qui WQ, Wu BT, et al. Genetic aspects of antibiotic induced deafness: mitochondrial inheritance. J Med Genet 1991;28:79-83.
46. Hutchin T, Haworth J, Higashi K, et al. A molecular basis for human hypersensitivity to aminoglycoside antibiotics. Nucleic Acids Res 1993;21:4174-9.
47. Fischel-Ghodsian N, Fournier P, Prezant TR, Maw M. Mitochondrial tRNA mutation associated with non-syndromic deafness. Presented at the Midwinter Meeting of the Association for Research in Otolaryngology. St. Petersburg Beach, Fla., 1995:192.
48. Schapira AH, Cooper JM. Mitochondrial function in neurodegeneration and ageing. Mutat Res 1992;275:133-43.
49. Schulz JB, Beal MF. Mitochondrial dysfunction in movement disorders. Curr Opin Neurol 1994;7:333-9.
50. Chandrasekaran K, Giordano T, Brady DR, Stoll J, Martin LJ, Rapoport SI. Impairment in mitochondrial cytochrome oxidase gene expression in Alzheimer disease. Brain Res Mol Brain Res 1994;24:336-40.
51. Parker WDJ, Parks J, Filley CM, Kleinschmidt-DeMasters BK. Electron transport chain defects in Alzheimer's disease brain. Neurology 1994;44:1090-6.
52. Sinha UK, Hollen KM, Rodrigues R, Miller CA. Auditory system degeneration in Alzheimer's disease. Neurology 1993; 43:779-85.
53. Luft R. The development of mitochondrial medicine. Proc Natl Acad Sci USA 1994;91:8731-8.
54. Branis M, Burda H. Effect of ascorbic acid on the numerical hair cell loss in noise exposed guinea pigs. Hear Res 1988; 33:137-40.
55. Theopold HM. Nimodipine (Bay 3 9736) a new therapy concept in diseases of the inner ear? [in German]. Laryngol Rhinol Otol Stuttg 1985;64:609-13.
56. Shi QY. An electron microscopic study of thyroxine against ototoxicity of kanamycin [in Chinese]. Chung Hua Erh Pi Yen Hou Ko Tsa Chih [Chinese Journal of Otolaryngology] 1993;28:344-5.