

Cochlear Trauma, Protection and Repair

Apoptosis (controlled cell death)

signs include cell shrinkage, nuclear fragmentation, plasma membrane blebbing

1. **receptor mediated**

Out of an extensive family of tumor necrosis factor (TNF) receptors, 7 have a “death domain” (DD) intracellular component. When DD receptors bind their respective ligands the death domain is altered and attracts a number of intracellular proteins that assemble and activate “initiator” procaspases, e.g., Procaspase-1, -8, or -9. Activated initiator caspases activate “executioner” caspases (caspase-3, -6, -7) that initiate apoptosis by cleaving essential proteins and nucleic acids.

2. **internally mediated**

Many apoptotic signals are generated within cells. Common signals are damaged proteins, DNA, and/or lipids that render the cell incapable of functioning normally. Damage can be caused by microbial or environmental toxins, by osmotic, physical or oxidative stresses. Perhaps the most common stresses are reactive oxygen species (ROS)(highly reactive radicals that are by products of imperfect oxidative metabolism) such as $-\text{OH}$, $-\text{O}_2$ (superoxide), and hydrogen peroxide. There are intrinsic mechanisms for controlling these compounds. Often apoptosis is the result of the intrinsic mechanisms’ failure to adequately prevent damage to cellular components by ROS.

3. **stress pathways**

Most stress pathways initiate apoptosis by controlling efflux of cytochrome c and other enzymes from mitochondria. These enzymes trigger assembly of Apaf-1 (apoptotic protease activating factor) in to heptameric scaffolds (apoptosomes) that attract procaspase-9 and activate it by dimerization and allosteric alterations. Activated caspase-9 is then capable of activating caspase 3, which initiates apoptosis.

4. **anti-apoptosis** is achieved by 1) decoy receptors that bind TNF family receptors but lack a death domain; 2) compounds that bind elements of the apoptotic pathway and inhibit their activity e.g., viruses, HSPs; 3) IAP (inhibitor of apoptosis) proteins that inhibit caspases; 3) up-regulation of anti-apoptotic members of the Bcl-2 family of proteins that act to either inhibit caspase activity and/or control stress-induced release of mitochondrial enzymes.
5. Few specifics of the apoptotic and anti-apoptotic pathways within the cochlea have been described. It is nevertheless clear that there are surely multiple redundant anti-apoptotic mechanisms active within the cochlea because of its post mitotic state and the fact that hearing usually endures for a lifetime.

Necrosis (uncontrolled cell death) (failure of apoptosis) This occurs when cells swell and burst, spilling their contents into the extracellular space. Unlike apoptosis, necrosis results in inflammatory reactions. Necrosis occurs in the cochlea when noise intensity causes physical disruption of the epithelium.

Genes controlling cochlear survival.

One difficulty in the study of acoustic trauma and ototoxicity has always been that of great differences between individuals in their vulnerability. It has recently been found that individual variability can be reduced in animal studies by the use of inbred strains of mice. This indicates that there is a large genetic component in the outcome of given manipulations but the specific genes that are involved remain to be identified. Identification of the genes that are responsible for given outcomes can be addressed by several approaches. One approach is to do traditional breeding experiments using strains of animals with known traits, e.g., 129/SvEv, or C57Bl6. Another approach is to select and test candidate genes based upon manipulations that involve known molecular pathways. There are a number of manipulations

that are known to induce a state of protection of the cochlea from insults due to excessive noise and/or ototoxic drugs. These include prior mild, non-traumatic stress to the ear such as noise exposure, or systemic stress, such as heat stress, restraint stress, or surgical stress. Little is known about the specifics of molecular pathways underlying these protected states of the cochlea but good hints regarding those pathways is available from the literature on ischemia protection. Pathways involved in ischemia protection are well studied. Manipulations that induce cochlear protection also induce ischemia protection in other tissues so it is likely that there are many similarities in the molecular bases of cochlear protection and ischemia protection in other tissues. This idea is supported by the fact that drugs which are known to induce cochlear protection from noise trauma and ototoxic drugs are effective in some cases of ischemia protection.

Drugs that have been shown to protect the cochlea from noise and ototoxic drugs include anti-oxidants and those that block reactive oxygen species such as allopurinol, d-methionine, glutathione, and L-NAME (a NOS inhibitor), as well as anti-inflammatory drugs, caspase inhibitors, calpain inhibitors, and growth factors, such as FGF, GDNF, BDNF, and neurotrophins. Some of these compounds provide protection from ototoxic drugs and noise trauma, while others are effective for selective traumas. This indicates that there are multiple signaling pathways involved in protection of the ear from different kinds of trauma. None of the signaling pathways are well described in cochlear tissue.

Although most drugs are given before administration of noise or ototoxic drugs, some drugs are effective in reducing permanent cochlear damage if they are given after the noise or ototoxic challenge, which shows that sequelae to initial insults can determine the ultimate outcome. This indicates that learning more about the underlying processes may eventually make it possible to minimize permanent damage in individuals who have been exposed to traumatizing conditions. Clearly there is a need for delineating stress responsive molecular pathways within the cochlea. Doing so should make it possible to devise better drug treatments for trauma. In addition, knowledge of the relevant pathways should make it possible to identify and perhaps treat individuals with genetic traits that make them especially vulnerable to trauma from noise exposure or drugs.

Acoustic trauma

TTS vs. PTS

Anatomical correlates of TTS are subtle. They may include stria edema, nerve fiber swelling, HC swelling, and HC organelle disruption. There may be high K⁺ levels in perilymph. There may be toxic levels of amino acid-like agonists present in the perilymph. Glutamate agonists, such as kainic acid mimic effects of acoustic trauma.

The slope describing the relation of acoustic energy and subsequent PTS is steep (12-16 dB).

Anatomical correlates of PTS are many: stereocilia disrupted, ciliary rootlets may be broken, organelles within HCs are disrupted, HC loss (OHC-1 > 2, 3, IHC), organ of Corti destruction, Schwann cell disruption, limbal cell loss, ligament cell loss, nerve fiber loss accompanies IHC loss and probably also supporting cells loss, CNS degeneration (shrinkage, ? cell loss) The most extreme effect of trauma in the cochlea is the de-differentiation of surviving cells to form a squamous epithelium.

With minimal disruption the OC the most common site of anatomical lesions and of hearing loss are usually half octave higher than traumatizing frequency. The hook region may be even more vulnerable than the half octave site.

Following impulse or low pass noise humans have a predilection for a loss at 4 kHz. The 4 kHz

notch in humans has an acoustic basis in the middle ear. (See handout.) Wideband stimuli often produce a narrowband lesion. The lesion spreads with time, perhaps due to early damage of non-sensory structures at sites flanking the hair cell loss. Loss of high frequency sensitivity results in inability to extract signals, e.g., speech, from noise.

Tinnitus accompanies TTS and PTS. There are no known anatomical bases for tinnitus. There probably are many.

Ototoxic drugs The most common ototoxic drugs are aminoglycosides (e.g., neomycin, gentamicin), which are important tools for treating gram negative bacterial infections, and platinum compounds, (e.g., cisplatin) which are among the most effective anti-cancer drugs. As is true for noise-induced cochlear trauma, almost all cochlear cells are affected by toxic doses. It is naive to suppose that toxic effects are due to selective effects upon hair cells. The effects of ototoxic drugs can be reduced by many of the same manipulations that are effective for reducing noise-induced trauma. This suggests that many of the stress pathways and apoptotic pathways are shared by acoustic and ototoxic stresses. This implies that better knowledge of these pathways could lead to local treatments (e.g., perilymphatic administration) that could spare the cochlea from ototoxic side effects of drugs that are given systemically to save lives and which might otherwise damage the cochlea.

Ototoxic antibiotics usually do not produce nerve degeneration. Patients with this condition are good implant candidates. Cochlear implants are currently the method of choice for treating such patients.

Middle ear administration of gentamicin is currently a widely used treatment for otherwise untreatable symptoms of Meniere's syndrome.

Regeneration

Regeneration of hair cells destroyed by acoustic trauma and ototoxic drugs occurs in non-mammals by division of remaining supporting cells. All of the signals that maintain the post mitotic state of the mammalian cochlear are not known. Disabling genes that block mitosis in the organ of Corti, e.g., *Ink4d*, causes hair cells to re-enter the cell cycle but they die from apoptosis without producing progeny to replace them. For there to be regeneration of mammalian hair cells it must be determined how to return the tissue to a mitotic state without making it more vulnerable. (A large conceptual step in this field would be the discovery of why mammalian cochlear cells are post mitotic.) It has been shown that treatment with *Math1* can induce interdental cells and other non-sensory epithelial cells to be converted to hair cells. This suggests that it may be possible to treat cochleas that have lost hair cells from acoustic trauma or ototoxic drug treatment and regenerate hair cells. This prospect is exciting but there are some fundamental problems that must be solved before this concept is realized. These include: 1) devising a means of inducing hair cells to form in places where they could be activated acoustically. 2) Getting the newly formed hair cells innervated. 3) Getting the cells to survive. It has been shown that knocking out genes that are expressed in supporting cells (*connexin 26*, *connexin 30*, *KCC4*, *p27*, *pendrin*) leads to hair cell degeneration. This indicates that hair cells will not survive if the supporting cells are not functioning properly. This means that it will be necessary to regenerate the entire organ in order to restore function because supporting cells are destroyed by the same insults that destroy hair cells. Because the rules that govern development of the cochlea from an undifferentiated primordium are not known, it seems doubly difficult to learn how to induce formation of a cochlea when the starting point is a de-differentiated remnant that previously was an organ of Corti. Recently embryonic stem cells have been found to be present in the adult mouse utricle. Rebuilding a cochlea with these, rather than with *Math 1* application seems no less daunting.

Suggested reading

Adams, J. M. (2003). "Ways of dying: multiple pathways to apoptosis." Genes Dev **17**(20): 2481-95.

Bhardwaj, A. and B. B. Aggarwal (2003). "Receptor-mediated choreography of life and death." J Clin Immunol **23**(5): 317-32.

Saunders, J. C., S. P. Dear and M. E. Schneider (1985). "The anatomical consequences of acoustic injury: A review and tutorial." J. Acoust. Soc. Am. **78**(3): 833-860.

Lieberman, M. C. and N. Y. Kiang (1978). "Acoustic trauma in cats. Cochlear pathology and auditory-nerve activity." Acta Otolaryngol Suppl **358**: 1-63.

Maison, S. F., A. E. Luebke, M. C. Liberman and J. Zuo (2002). "Efferent protection from acoustic injury is mediated via alpha9 nicotinic acetylcholine receptors on outer hair cells." J Neurosci **22**(24): 10838-46.

Lieberman, M. C. (1987). "Chronic ultrastructural changes in acoustic trauma: serial-section reconstruction of stereocilia and cuticular plates." Hear Res **26**(1): 65-88.

Henderson, D., S. L. McFadden, C. C. Liu, N. Hight and X. Y. Zheng (1999). "The role of antioxidants in protection from impulse noise." Ann N Y Acad Sci **884**: 368-80.