New aspects of inner ear research

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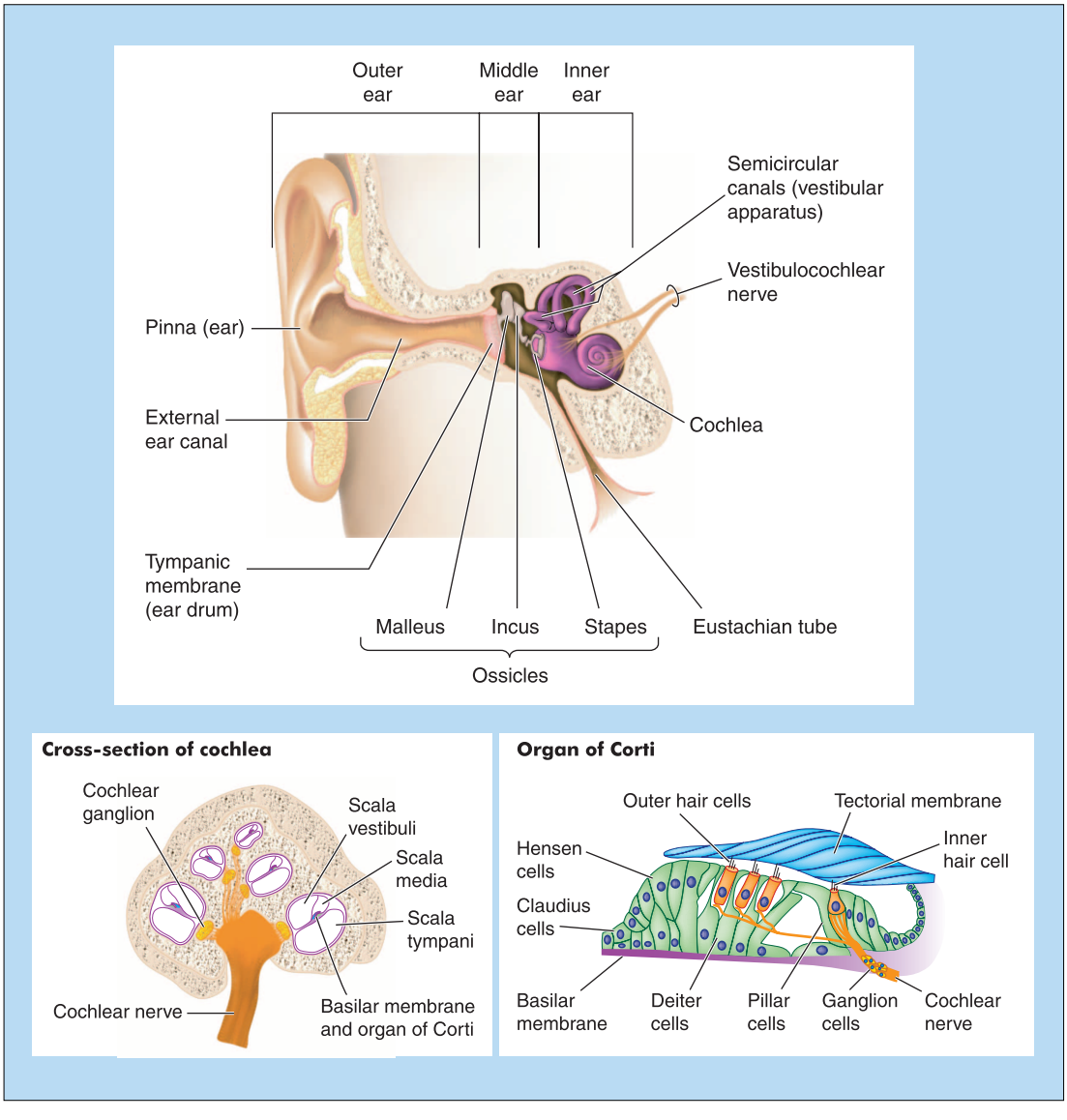
At birth, one in 850 babies are profoundly deaf, and hearing loss affects more than 50% of all people over 60 years of age. While hearing loss caused by disease of the external and/or middle ear is treatable, hearing loss as a result of damage to and loss of hair cells and/or auditory neurons can only be alleviated using prosthetic devices such as hearings aids or cochlear implants.

The causes of hair cell and neuronal cell loss within the inner ear (*Figure 1*) are diverse. Noise, ototoxic drugs and ageing are among the most prominent causes. While noise damage can be prevented using protective devices such as earmuffs, ageing and certain ototoxic drugs used to treat life-threatening illnesses cannot be avoided.

Figure 1. Schematic illustration of human inner ear anatomy.



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In humans, hair cells develop during the first trimester of gestation and are expected to survive for the whole life of the individual. Since loss of auditory hair cells in adult mammals is irreversible, therapeutic efforts are directed at either preventing hair cell loss (protection) or, if hair cells are already lost, at finding a way to generate new cochlear hair cells to replace the lost ones.

PROTECTION OF HAIR CELLS

To develop strategies to protect hair cells requires knowledge of the molecular events involved in damage and death of the auditory cells. Protective strategies could be used before the application of ototoxic drugs, e.g. aminoglycosides or cisplatin. It may also be possible to start protective therapy immediately after an event that can lead to hair cell damage and loss. In the last few years, progress has been made in the understanding of the molecular events involved in sensorineural hearing loss (Ryan, 2000).

Both ototoxic drugs and acoustic overstimulation result in the production of reactive oxygen species within the organ of Corti (Schacht, 1999). It has also been demonstrated that stress pathways operate in hair cells and that programmed cell death, so-called apoptosis, is involved in hair cell damage and death (Pirvola et al, 2000). Finally, it has been shown that neurotrophic factors are important for the developing auditory sensory epithelium (Fritzsch et al, 1998). Based on these observations three major agents have been investigated for preventing hair cell loss resulting from ototoxic and acoustic damage: antioxidants, inhibitors of intracellular stress pathways leading to apoptosis and finally neurotrophic factors. It may also turn out that a combination of different agents will be used in future therapeutic efforts.

Antioxidants

For aminoglycoside toxicity it is a well-established fact that free radicals are formed in the organ of Corti via the formation of iron complexes, and different free radical scavengers have proven to be effective at reducing hair cell loss. In addition, it has been shown that acoustic overstimulation as well as cisplatin toxicity could be reduced by agents which enhance the antioxidant defence of the cochlea. A variety of compounds have been tested as antioxidant agents, including vitamins C and E, and aspirin.

Inhibitors of intracellular stress pathway leading to apoptosis and inhibitors of the apoptotic machinery

Different studies suggested that hair cell loss resulting from a variety of different kinds of stress was initiated by stress signalling pathways leading to caspase activation, the key enzymes in the apoptotic pathway. It was rational to test whether inhibitors of the apoptotic cell death machinery were able to prevent hair cell death following trauma. Not surprisingly, a general caspase inhibitor (Z-VAD-FMK) protected hair cells from cisplatin toxicity (Liu et al, 1998). Interestingly, inhibitors of stress signalling pathways, such as an inhibitor of the rac/cdc42 family of small G-proteins, as well as inhibitors of the JNK signalling pathway, also protected hair cells from aminoglycoside-induced cell death (Pirvola et al, 2000; Bodmer et al, 2002). Even membrane proteins might play a role in aminoglycoside-induced apoptotic cell death as it has been demonstrated that the N-methyl-D-aspartate (NMDA) receptor, a membrane protein that is activated upon glutamate binding, is downregulated in organ of Corti explants soon after aminoglycoside exposure. Combined application of an NMDA antagonist and neurotrophin-3 (NT-3), a neurotrophic factor, has proven to be effective even in vivo: it preserved hair cell morphology and decreased threshold shifts in auditory brainstem responses when given before noise or amikacin application compared to control animals (Duan et al, 2000).

Role of neurotrophic factors for hair cells

Neurotrophic factors are usually small peptides that serve different roles in their target tissue, e.g. tissue maintenance and cell survival. They have been primarily studied for their effects on neuronal cells and their role in hair cells has remained elusive. However, it has also been demonstrated that NT-3 as well as brain-derived neurotrophic factor (BDNF) are expressed by hair cells. In addition, different neurotrophic factors, such as NT-3, BDNF and glial-derived neurotrophic factor, have been found to be effective against trauma-induced hair cell loss.

GENERATION OF NEW COCHLEAR HAIR CELLS

Because the inner ears of mammals show very limited regenerative capacity, deafness caused by loss of auditory hair cells is irreversible. Therefore, it would be of great interest to replace the lost hair cells. In principle, there might be three possible ways of generation of new hair cells for therapeutic application:

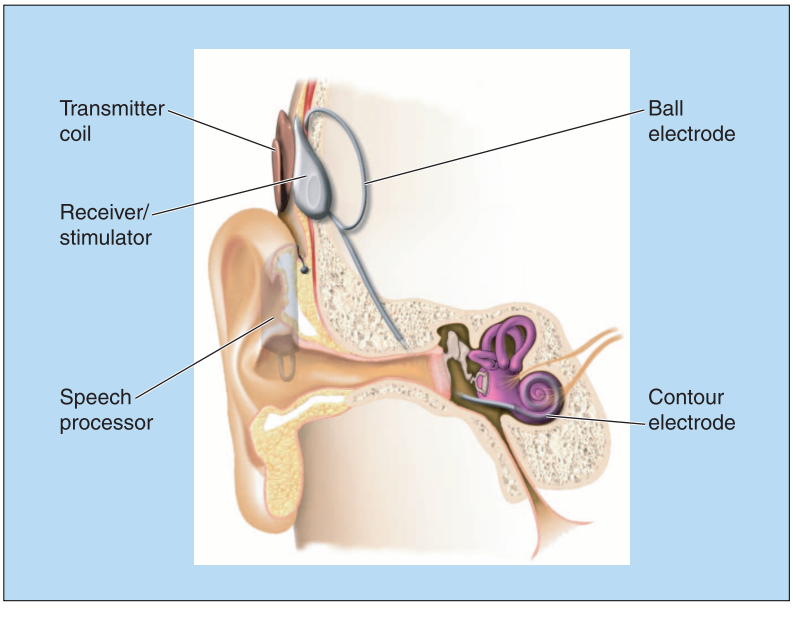
1. Generation of auditory hair cells by mitosis from supporting cells
2. Conversion of supporting cells into hair cells
3. Implantation of cells into the cochlea with the ability to become hair cells (stem cells).

Data from the avian inner ear have already shown that supporting cells are able to change their phenotype and become new hair cells (Ryals and Rubel, 1988). In addition, it has been demonstrated that supporting cells can generate new hair cells by transdifferentiation (Raphael, 1992) or by conversion of the phenotype into a hair cell phenotype without cell division. However, to further develop strategies for regeneration of new auditory hair cells, it is crucial to understand the normal development of hair cells.

It has been discovered that the transcription factor Math1 is essential for generation of hair cells (Bermingham et al, 1999) and that overexpression of Math1 in cultures of immature rat cochleas resulted in the production of ectopic hair cells (Zheng et al, 2000). Even viral-mediated gene transfer of Math1 to non-sensory epithelial cells in the mature cochlea lead to the development of new cochlear hair cells, some of which attracted auditory neurons. Cell cycle regulators may also play an important role in future efforts to generate hair cells postnatally. In a study conducted by Chen and Segil (1999) mice deficient for the cell cycle inhibitor p27 displayed hair cell overproduction in the organ of Corti, however, a massive degeneration followed the initial overproduction.

Stem cells, with their ability to develop into different cell types depending on local environmental cues, have received considerable attention. Neural stem cells have been successfully transplanted into the mammalian inner ear where they integrated and some cells appeared to take on the morphology of hair cells (Ito et al, 2001). Also, embryonic stem cells were able to differentiate into hair cell-like cells in vitro (Li et al, 2003a). In a second study, the same group has succeeded in isolating stem cells from the inner ear of the adult mouse (Li et al, 2003b), although those cells were found in the vestibular part of the inner ear (utriculus) and not in the organ of Corti.

Figure 2. Schematic illustration of a cochlear implant system.



LOST OF SPIRAL GANGLION NEURONS

When hair cells are lost from the adult organ of Corti, spiral ganglion (SG) dendrites retract and are possibly lost. Moreover, the neurons of the SG are progressively lost over a period of months and years, presumably as a result of lack of trophic support. Total loss of hair cells can result in the loss of most cochlear neurons.

Role of neurotrophins, extracellular matrix and ephrins for cochlear neurons

The production of tonotopically precise connections between SG neurons and hair cells is a central feature of cochlear development. The factors that control the targeting of SG dendrites within the osseous spiral lamina and organ of Corti are poorly understood. However, it is likely that many factors interact to produce appropriate connections. Among these are neurotrophic factors and extracellular matrix (ECM) molecules. Studies also suggest that ephrins might play a role during cochlear development (Brors et al, 2003).

Neurotrophins, ECMs and their receptors are present in the inner ear during the period of innervation of the organ of Corti, and play a functional role in innervation. NT-3, BDNF and fibroblast growth factor-1 are expressed by cells of the developing organ of Corti, while the corresponding thyrosine kinase receptors, trkC and trkB, are expressed by developing and adult SG neurons (Ernfors et al, 1994). Deletion of either neurotrophin or trk receptor genes results in deficits in SG neurons (Ernfors et al, 1994; Fritzsch et al, 1997). While neurotrophic factors clearly support the survival of SG neurons, they also provide guidance cues to developing neurites. The ECM factors fibronectin and laminin are present in the developing osseous spiral lamina, while fibronectin is present in the developing organ of Corti. It is possible that SG neurites use tracts of ECM molecules as guidance pathways.

NEW DEVELOPMENTS OF COCHLEAR IMPLANTS

Cochlear implantation is increasingly used for the treatment of profound hearing loss and deafness. The cochlear implant (*Figure 2*) activates surviving cochlear neurons with electrical stimu-

lation, and higher levels of neuronal survival have been hypothesized to increase performance of cochlear implants (Marzella and Clark, 1999). Moreover, because of the loss of cochlear dendrites, the stimulating electrodes are located some distance from the neurons. One result of this difficulty is activating neurons in discrete groups, even with multiple electrode arrays. A consequence of this is relatively poor spatial resolution of stimulation, resulting in a limited number of independent channels for information transmission. A second consequence of poor spatial resolution is a severely restricted dynamic range.

To improve the poor resolution of electrical stimulation efforts have been made to reduce the distance between stimulating electrodes and SG neurons. This has led to the design of cochlear implant electrodes that can be positioned close to the modiolus and to the development of perielectrode positioners in the scala tympani (Cords et al, 2000). Another strategy being studied is stimulating neurite growth toward the electrode. Direct interactions between SG neurites and implants could be fostered by appropriate treatment with neurotrophic factors, ECMs or ephrins. This might lead to improved efficacy of cochlear implant systems.

CONCLUSIONS

Sensorineural hearing loss is associated with damage to cochlear hair cells or neurons. Different in-vitro and in-vivo studies demonstrated the prevention of hair cell loss caused by ototoxic and acoustic damage by the use of antioxidants, inhibitors of the intracellular stress pathways and neurotrophic factors. New approaches focus on the replacement of lost hair cells in the cochlea by generation of auditory hair cells by mitosis from supporting cells, conversion of supporting cells into hair cells and implantation of stem cells into the cochlea.

Cochlear implantation is increasingly used as a treatment for profound hearing loss and deafness. Since the cochlear implant activates surviving cochlear neurons with electrical stimulation, the knowledge of factors supporting the survival of these neurons is essential. NT-3 and BDNF have been proved to be the key molecules for this process. In the future the efficacy of cochlear implants might be improved by stimulating the growth of SG neurites towards the cochlear implant electrode. HM

Conflict of interest: none.

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KEY POINTS

- Hair cell loss as a result of ototoxic and acoustic damage could be prevented by antioxidants, inhibitors of intracellular stress pathways leading to apoptosis and neurotrophic factors.
- Lost hair cells could be replaced by generation of auditory hair cells by mitosis from supporting cells, conversion of supporting cells into hair cells and implantation of stem cells into the cochlea.
- Neurotrophic factors support the survival of spiral ganglion neurons and also provide guidance cues to developing neurites.
- Stimulating spiral ganglion neurite growth toward a cochlear implant electrode by appropriate treatment with neurotrophic factors, extracellular matrix molecules or ephrins might lead to improved efficacy of cochlear implant systems.