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Vestibular damage in chronic ototoxicity: A mini-review

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ABSTRACT

Ototoxicity is a major cause of the loss of hearing and balance in humans. Ototoxic compounds include pharmaceuticals such as aminoglycoside antibiotics, anti-malarial drugs, loop diuretics and chemotherapeutic platinum agents, and industrial chemicals including several solvents and nitriles. Human and rodent data indicate that the main target of toxicity is hair cells (HCs), which are the mechanosensory cells responsible for sensory transduction in both the auditory and the vestibular system. Nevertheless, the compounds may also affect the auditory and vestibular ganglion neurons. Exposure to ototoxic compounds has been found to cause HC apoptosis, HC necrosis, and damage to the afferent terminals, of differing severity depending on the ototoxicity model. One major pathway frequently involved in HC apoptosis is the c-jun N-terminal kinase (JNK) signaling pathway activated by reactive oxygen species, but other apoptotic pathways can also play a role in ototoxicity. Moreover, little is known about the effects of chronic low-dose exposure. In rodent vestibular epithelia, extrusion of live HCs from the sensory epithelium may be the predominant form of cell demise during chronic ototoxicity. In addition, greater involvement of the afferent terminals may occur, particularly the calyx units contacting type I vestibular HCs. As glutamate is the neurotransmitter in this synapse, excitotoxic phenomena may participate in afferent and ganglion neuron damage. Better knowledge of the events that take place in chronic ototoxicity is of great interest, as it will increase understanding of the sensory loss associated with chronic exposure and aging.

1. Introduction

Impaired function of the vestibular system causes vertigo, loss of balance and loss of gaze fixation during movement, often accompanied by dizziness and nausea. In humans, one cause is the toxicity of some pharmaceuticals, including aminoglycoside antibiotics, anti-malarial drugs, loop diuretics, and the chemotherapeutic agent cisplatin (Rybak (2007); Rybak and Whitworth, 2005; Schacht et al., 2012; Yorgason et al., 2006). Workplace chemicals with potential inner ear toxicity include several solvents such as toluene, styrene and trichlorethylene and synthetic intermediates such as cis-2-pentenenitrile (Fechter et al., 1998; Hoet and Lison, 2008; Perrine and Dominique (2008); Pouyatos et al., 2002; Saldaña-Ruíz et al., 2012; Campo et al., 2013). These and other compounds are ototoxic, that is, toxic to both the vestibular and auditory sensory systems. The main targets of toxicity are the hair cells (HCs), which are the mechanosensory cells responsible for the transduction of sound waves and of head accelerations, including gravity and those resulting from linear and rotational movements of the head. Their name refers to the apical bundles of specialized microvilli, known as stereocilia, that contain the molecular machinery for mechano-electrical transduction. Mature vestibular HCs also have one single cilium, named a kinocilium, while auditory HCs lose their kinocilium during maturation.

Animal studies with acute or short-term repeated exposure models have clearly demonstrated that ototoxic compounds may cause permanent disability due to degeneration of most or all of the HCs, because these cells cannot regenerate, or do so to a very limited extent in most mammalian species (Forge and Schacht, 2000; Groves, 2010; Llorens et al., 1993; Rubel et al., 2013). However, on many occasions, vestibular dysfunction appears progressively as a result of a mild but persistent stress to the system, as may occur in chronic aminoglycoside treatment. If the stress is removed, as for instance by halting drug use, the symptoms may fully persist, or decrease up to either complete or incomplete recovery (Black et al., 2001, 2004). The events taking place during the progressive injury that causes slowly appearing symptoms of ototoxicity, which may be partly or fully reversible, are scarcely established. A deep understanding of these processes

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is of great interest for several reasons. First, the slow damage process may be a target for therapeutic intervention aimed at blocking its progression before irreversible events take place. Second, the recovery process may be a target for therapeutic intervention aimed at shortening the time of recovery or at ameliorating the final outcome of the process, i.e., turning partial recovery into better or full recovery. Third, knowledge of the events that take place during damage and repair will undoubtedly shed light on the basic processes involved in the physiology and homeostasis of the system. Fourth, slow damage mechanisms are probably involved in the sensory loss commonly associated with aging. While presbycusis (age-related hearing loss) is a widely recognized phenomenon, age-related loss of vestibular function is much less known, but has a similar high incidence. It may affect as much as 65% and 85% of people over 60 and 80 years respectively, and it constitutes a significant risk factor for falls (Agrawal et al., 2009; Ishiyama, 2009). Loss of sensory functions is one major determinant of the deterioration of quality of life during aging, and to what extent chronic neurotoxicity is responsible for this functional decline is an important open question. As in neurodegenerative diseases, sensory decline was once assumed to be an unavoidable consequence of natural aging. However, it is increasingly accepted that it is the end result of null or limited capacity for regeneration combined with the damaging consequences of different insults, including toxic insults, which may be avoidable, at least in part.

The main purpose of the present paper is to review the scarce data available on the cellular and molecular events that operate in slowly progressing damage in the mammalian vestibular system resulting from chronic toxic exposure. To give a more comprehensive view of the field, the data on HC degeneration from other exposure models and in other related epithelia are also briefly reviewed, but an extensive presentation of these aspects is beyond the scope of the present review. Other recent reviews are available that cover the best-known aspects of HC degeneration following ototoxic exposure (Cheng et al., 2005; Forge and Schacht, 2000; Guthrie, 2008; Li and Steyger, 2009; Op de Beeck et al., 2011; Schacht et al., 2012; Warchol, 2010; Xie et al., 2011; Yorgason et al., 2011).

2. Vestibular sensory epithelia

There are five vestibular sensory epithelia in each ear: three cristas, one utricle and one saccule. All of them contain two morphological types of HCs, known as type I (HCI) and type II (HCII) (Fig. 1). In the auditory system, a single sensory epithelium, known as the organ of Corti, contains two types of HCs, outer HCs (OHCs) and inner HCs (IHCs). In all HCs, deflection of the stereocilia opens the mechano-electrical transduction channels at the tips of the stereocilia, allowing a cation current to flow and depolarize the cell. Vestibular HCs are presynaptic to afferent terminals of the vestibular ganglion neurons, and depolarization leads to neurotransmitter release at the basolateral membrane of the cell. The neurotransmitter is glutamate and this makes the post-synaptic afferent terminals a candidate target for excitotoxic damage. HCII are contacted by button afferent terminals, and these synapses are surrounded by supporting cells that express EAAT1 (excitatory amino acid transporter 1, also known as GLAST) for glutamate clearance (Takumi et al., 1997). The contact between the HCI and their afferent terminals is a very unique structure. The cell has an amphora-like shape, and the terminal has a calyx shape that envelope the cell up to its neck. Growth of the calyx afferents during development depends on trophic signals secreted by the HCs, including BDNF acting through TrkB/PLCγ (tyrosine kinase receptor B/phospholipase C gamma) signaling in the nerve terminals (Sciarretta et al., 2010). Scaffolding, cell adhesion, extracellular matrix proteins, and ion channels have now been identified and shown to form several microdomains within the calyx membrane (Lysakowski et al., 2011). The HCI-calyx ending contact is a very unique setting with regard to excitotoxicity potential, because the calyx separates the synaptic cleft from the neighboring supporting cells, and this makes it impossible to remove glutamate by EAAT1. Recent data (Dalet et al., 2012) indicate that HCs express the excitatory aminoacid transporters EAAT4 and EAAT5, whose particular kinetics may match the exceptional arrangement of this synaptic contact with regard to the regulation of glutamate concentrations in the synaptic cleft.

3. Experimental models in ototoxicity research

Experimental research into ototoxic damage has largely focused on clinically important drugs, such as the aminoglycosides and the chemotherapeutic drug cisplatin. Data from the temporal bones of patients exposed to these drugs indicated that HCs are the main target and that persistent hearing or balance loss after exposure to these compounds is usually associated with loss of HCs. However, other effects, such as damage to the stria vascularis and loss of the spiral and vestibular ganglia neurons, have also been observed. Animal studies in a variety of species have corroborated these findings (reviewed by Guthrie, 2008; Schacht et al., 2012).

In many species, ototoxic drugs have other toxic effects that compromise survival, such as renal toxicity, and this makes it difficult to establish good animal models to study ototoxicity. Rats and mice are comparatively resilient to aminoglycoside-induced HC toxicity, whereas guinea pigs are more susceptible to this toxicity and have the advantage of a large inner ear; so this last species has frequently been chosen for this research (Forge and Schacht, 2000; Li et al., 1995). Another particularly sensitive species is the chinchilla, and a number of studies have been carried out on this species (see McFadden et al., 2002; Yorgason et al., 2011). As well as some studies in other mammalian species (see Yorgason et al., 2011), many studies of avian species have also been published (Mangiardi et al., 2004), as have studies of the zebrafish more recently (Chiu et al., 2008). Since the work by Wu et al. (2001), significant efforts have been devoted to establishing systemic rat and particularly mouse models of ototoxicity with the twofold aim of reaching a better understanding of ototoxicity in vivo, and easily causing reproducible lesions to develop protection, repair and regeneration strategies. Using repeated administration of selected aminoglycosides in selected strains, cochlear and vestibular toxicity are achieved (Wu et al., 2001; Murillo-Cuesta et al., 2009, 2010). Alternatively, partial lesions of the cochlea, usually sparing the vestibular epithelia, are obtained by acute co-administration of an aminoglycoside and a loop diuretic (Oesterle et al., 2008; Taylor et al., 2008). A similar model has been developed by co-administering cisplatin and furosemide to mice (Li et al., 2011). Other compounds, notably the nitriles, have been discovered to cause ototoxic effects in a variety of species including rats and mice with no or limited associated mortality (Balbuena and Llorens, 2001; Llorens et al., 1993; Llorens and Rodríguez-Farré, 1997; Saldaña-Ruíz et al., 2013; Soler-Martín et al., 2007).

As discussed below, repeated exposure models have provided some new information on the molecular events that may be involved in ototoxicity, although these models remain sub-optimal in terms of ease of use, flexibility and the presence of other toxic effects. Although many studies have been published that provide data on ototoxicity, few canonical toxicological studies are available that use a range of doses that produce from no effect to complete lesions, or that compare different time exposure conditions (acute, sub-acute, sub-chronic and chronic).

To circumvent the systemic toxicity problem, ototoxins have on many occasions been studied by local application to the middle ear

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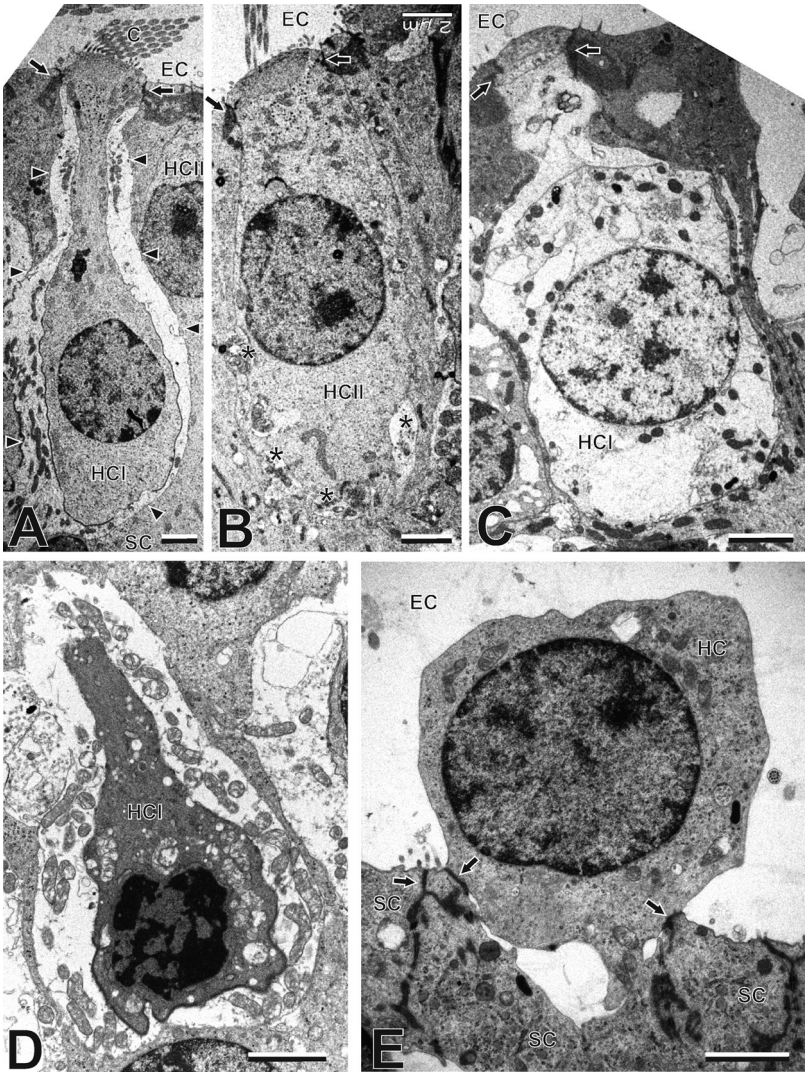

Fig. 1. Organization of the mammalian vestibular epithelia (A and B) and modes of demise of sensory hair cells (HCs) (C–E). (A and B) Vestibular epithelia from control rats. These contain two types of HCs, which extend their bundles of cilia (C) into the endolymphatic cavity (EC). Supporting cells (SC) surround the HCs. Tight junctions (arrows) between HCs and SCs and among SCs close the apical part of the epithelium to exclude the potassium-rich endolymph from the epithelium. Type I HCs (HCI) are contacted by calyx afferents (arrowheads in A) that envelope the cell, while type II HCs (HCII) are contacted by button afferents (* in B). (C) A degenerating HCI in a vestibular epithelium from a rat acutely exposed to 3,3′-iminodipropionitrile (IDPN), showing features of cell necrosis, nuclear swelling and cytoplasm vacuolization and swelling. (D) A degenerating HCI showing features of apoptosis, cell shrinkage, and cytoplasm and chromatin condensation, in a vestibular epithelium from a rat sub-acutely exposed to IDPN. (E) Extruding HC (type undetermined) in a vestibular epithelium from a rat chronically exposed to IDPN. The integrity of the epithelium, which is located toward the bottom of the image, is preserved by the SCs that keep their tight junctions (arrows) among them and with the HC, until the extrusion of the cell into the EC is completed. In this chronic ototoxicity model, there is a striking scarcity of major signs of damage in the cytoplasm, nucleus or mitochondria of the extruding HCs. Scale bars: 2 μm.

of rodents, from where they diffuse into the inner ear causing auditory and vestibular toxicity with little systemic toxicity (Bauer and Brozoski, 2005; Dupont et al., 1993; Heydt et al., 2004; Lyford-Pike et al., 2007; Sera et al., 1987). Using this approach, rats and mice are as susceptible to aminoglycoside and cisplatin ototoxicities as guinea pigs are. All these studies correspond to acute toxicity.

Ototoxicity has also been studied in vitro, in explant cultures of the organ of Corti or the vestibular epithelia (Cunningham, 2006; Forge and Li, 2000; Kotecha and Richardson, 1994; Matsui et al., 2004). Those studies provide valuable information on the response of the HCs to ototoxic stress, but have many important limitations with regard to the understanding of chronic ototoxicity. Among other differences, the explanted epithelia lack the endolymphatic compartment and innervation.

Ototoxic drugs typically damage both the vestibular and the auditory system. However, it is important to keep in mind that research findings in one of the systems may or may not be valid in the other. While many cellular and molecular components are similar in both systems, important differences also exist. The amount of data available on auditory toxicity is far greater than that available on vestibular toxicity, and many questions that have been investigated in the cochlea remain unexplored in the vestibular system.

4. Apoptosis and necrosis of HCs in ototoxic damage

The mechanisms responsible for the HC loss caused by ototoxic exposure have been studied in a variety of experimental models. In several acute or repeated exposure in vivo studies (Lenoir et al., 1999; Li et al., 1995; Llorens and Demêmes, 1994; Seoane et al., 2001a; Vago et al., 1998) transmission electron microscopy data identified mostly apoptotic, but also necrotic, patterns of HC degeneration. Ultrastructural evidence of HC apoptosis was also obtained from utricular explant cultures (Forge and Li, 2000). One specific feature of HC degeneration, which seems to be part of

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the apoptosis program of the cells, is the severing of its neck and the release of its apical end, including the cuticular plate and ciliary bundle, toward the endolymphatic space. Simultaneously, supporting cells close the forming gap and preserve epithelial integrity by forming characteristic scars that impede the exposure of the basolateral membranes of the epithelial cells to the potassium rich endolymph (Forge, 1985; Leonova and Raphael, 1997).

The molecular pathways involved in the apoptosis of mammalian HCs induced by ototoxic insults have mainly been studied in the organ of Corti and utricular explants exposed to either aminoglycosides or cisplatin. The data available (reviewed by Casares et al., 2012; Cheng et al., 2005; Guthrie, 2008; Op de Beeck et al., 2011; Schacht et al., 2012; Warchol, 2010) support the hypothesis that generation of reactive oxygen species in the HC mitochondria is the key event that precipitates apoptosis. In the case of the antibiotics, the formation of iron-aminoglycoside complexes which are redox-active seems to be the main factor contributing to the oxidative stress, although enzymatic pathways may also participate. The way by which cisplatin generates reactive oxygen species is less clear but may involve their generation by NOX3 (a superoxide-generating isoform of NADPH oxidase) and by xanthine oxidase. For both cisplatin and aminoglycosides, apoptosis depends on activation of the effector caspase-3 preceded by activation of the upstream caspase-9. One major link between oxidative stress and caspase activation that has been identified in the aminoglycoside studies is the c-jun N-terminal kinase (JNK) signaling pathway. For cisplatin, it has been suggested that JNK activation participates in HC repair rather than HC death, while activation of the p53 tumor suppressor has been identified as a major upstream pathway for caspase activation. Nevertheless, multiple signaling pathways for apoptosis may activate simultaneously.

Molecular evidence of a role of apoptosis in ototoxic HC loss in vivo is also abundant, but the emerging picture is a complex one, and it is quite clear that the modes of HC demise may vary from one exposure model to another, and that they may include non-apoptotic modes. That HC apoptosis occurs in both the cochlea and the vestibular epithelia during ototoxic damage was indicated early on by biochemical evidence, such as DNA fragmentation (Lenoir et al., 1999; Nakagawa et al., 1997; Seoane et al., 2001a; Usami et al., 1997). As found in vitro, several different pathways have been identified in in vivo studies. For instance, in chinchilla, activation of the initiator caspase-9 has been identified as a mediator of the ototoxicity caused by co-exposure to gentamicin and ethacrynic acid (Ding et al., 2010) while activation of the initiator caspase-8 has been identified instead for the cisplatin/ethacrynic acid combination (Ding et al., 2007). However, caspase-independent pathways of HC cell death have been found to predominate in other models. Thus, following kanamycin administration, mouse auditory hair cells showed EndoG translocation and activation of μ-calpain and cathepsin D, but no markers for classic apoptotic pathways (cytochrome c, caspase-9, caspase-3, JNK and TUNEL) (Jiang et al., 2006). In that study, ultrastructural features of HC necrosis were observed along with apoptotic HC figures; the results suggest that chronic aminoglycoside treatment may trigger multiple cell death pathways, including those leading to necrosis (Jiang et al., 2006). This would be in contrast to the predominant role of apoptosis found in vitro and in acute models (see above).

From the data reviewed in the previous paragraph, one may be tempted to conclude that HC apoptosis is associated with acute modes of ototoxicity while necrosis predominates in more chronic models. However, this hypothesis is not supported by the data obtained using the 3,3′-iminodipropionitrile (IDPN) model in the rat vestibular system (Seoane et al., 2001a). In that model, necrosis predominates according to ultrastructural criteria after high acute doses, while apoptosis predominates following more progressive repeated exposure. This observation is in agreement with data from the field of neuronal degeneration, which show necrosis at high intensities of damaging stimuli that cause apoptosis at lower intensities (Nicotera et al., 1999). One factor that may be involved in the differential response of HCs to ototoxic chemicals is their pharmacokinetics. It is well known that aminoglycosides readily enter HCs and then undergo biphasic clearance with a second half-life of longer than 30 days (Schacht et al., 2012). This probably accounts for the progression of ototoxicity events even long after the end of the exposure period. It could also facilitate higher HC concentrations being reached following chronic dosing, which in turn may condition the cell death pathways being activated. In the cochlea, the interaction with noise may also be a conditioning factor (Li and Steyger, 2009).

5. HC extrusion in ototoxic damage

In epithelia, cell demise can proceed by extrusion of the cell from the luminal surface. The best-known example is the extrusion of cells from the intestinal mucosa, which is part of the continuous renewal of this epithelium. The extrusion of cells in the apical parts of the villi balances the generation of new epithelial cells in the deep parts of the intestinal crypts (Stappenbeck et al., 1998). Although there are data indicating that HCs extrusion can occur in the mammalian cochlea (Seoane and Llorens, 2005; Whitworth et al., 1999), this phenomenon is not well-documented in this epithelium and the common finding is apoptotic intraepithelial degeneration (Forge, 1985). In contrast, there is no doubt that extrusion operates in the mammalian vestibular epithelia and in the auditory and vestibular epithelia of non-mammalian vertebrates following ototoxic exposure. In one of the first studies that specifically addressed this question, two patterns of HC demise were identified by scanning and transmission electron microscopy in the vestibular sensory epithelia of guinea pigs exposed to gentamicin (Li et al., 1995). These were apoptosis of HCs within the sensory epithelia and extrusion of apparently live cells toward the endolymphatic cavity. Evidence of this form of HC demise was already available in mammals (e.g., Wërsall et al., 1973) and had long been recognized as the main form of HC loss in non-mammalian vertebrates following a variety of insults (Corwin et al., 1991; Cotanche, 1987). In some particular intoxication models, HC extrusion is the only observed form of cell demise in the mammalian vestibular epithelia (Seoane et al., 2001a,b). Despite this prominent occurrence of HC extrusion, its physiological significance and the molecular mechanisms involved remain insufficiently characterized. One widely accepted hypothesis is that HC extrusion and apoptosis both allow the preservation of tissue integrity by minimizing damage and inflammation in the local environment (Hirose et al., 2004; Hordichok and Steyger, 2007; Li et al., 1995; Mangiardi et al., 2004; Seoane et al., 2001a). One less clear aspect is the relationship with the intoxication rate. It is well known that many toxic compounds may cause cell necrosis or apoptosis depending on the concentrations, with necrosis observed at higher concentrations (Ankarcrona et al., 1995; Bonfoco et al., 1995; Gwag et al., 1999; Nicotera et al., 1999). In a comparison of acute, sub-acute and sub-chronic exposure to IDPN, Seoane et al. (2001a) observed that necrosis predominated following acute high-dose exposure, while apoptosis predominated following sub-acute exposure, and sub-chronic low-dose exposure caused extrusion of most HCs. It was concluded that the predominant mode of HC demise depends on the intensity of the damaging stimulus and that extrusion is the predominant form associated with the low intensity, persistent insult caused by chronic low-dose ototoxic exposure. Thus, extrusion would be a finely controlled way of eliminating damaged HCs, and would be

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caused by the more progressive forms of HC damage. Although there is no independent confirmation of these statements, some data support the conclusion that HC extrusion is a major factor in chronic ototoxicity (Granados and Meza, 2005), in contrast to acute ototoxicity (Llorens and Demêmes, 1994).

The relationship between HC apoptosis and extrusion remains to be better understood. In the auditory system of chicks exposed to gentamicin, Mangiardi et al. (2004) found expression of molecular markers of apoptosis, including activated caspase-3, in the extruding HCs. The observation that extruding cells undergo apoptosis at the same time had been reported in the intestinal epithelia (Rosenblatt et al., 2001). However, no evident ultrastructural or biochemical signs of apoptosis were observed in the extruding mammalian vestibular HCs (Li et al., 1995; Seoane et al., 2001a). Interestingly, live cell extrusion, rather than apoptotic cell extrusion, has recently been characterized as a major mechanism operating in a number of mammalian and non-mammalian epithelia to maintain cell numbers (Eisenhoffer et al., 2012). To our knowledge, the molecular events triggering HC extrusion in ototoxically damaged mammalian vestibular epithelia have not been studied.

6. Ganglion cells and afferent terminals in ototoxic damage

Although there is ample consensus that ototoxic compounds are defined by their toxicity upon the HCs, it is also well known that afferent dendrite terminals and the corresponding ganglion neurons may also be damaged (Dau and Wenthold, 1989; Koitchev et al., 1982; Ylikoski et al., 1974). In fact, several reports have found that the terminals are damaged before the HCs. These include studies of human auditory systems that apparently show primary loss of auditory neurons following ototoxic exposure (Hinojosa and Lerner, 1987; Sone et al., 1998). These results differ from the more common finding of auditory HC degeneration preceding neuronal degeneration, which is usually interpreted as secondary degeneration of the ganglion neurons due to loss of trophic support after the HC degeneration that would be the primary ototoxic event (e.g. Ernfors et al., 1996; reviewed by Schacht et al., 2012). In animal studies, the common view of primary HC loss followed by secondary neuronal degeneration (Ladrech et al., 2004; Schacht et al., 2012) is nevertheless accompanied by evidence that direct damage to the terminals may also occur (e.g. Ruan et al., in press). Little data is available on the molecular mechanism involved in spiral ganglion neuron degeneration, but calpain and protein kinase C activation have been reported to occur and may have damage-mediating and protective roles, respectively (Ladrech et al., 2004). In both the auditory and vestibular ganglia, aminoglycosides increased the expression of transient receptor potential cation channel superfamily V (TRPV) and of mitochondrial uncoupling proteins 2 and 3, and this was interpreted as activation of defense mechanisms against the ototoxic insult (Kitahara et al., 2005a,b).

In the vestibular system, a decrease in the number of vestibular ganglion neurons has been recorded in specimens from humans affected by aminoglycoside ototoxicity (Ishiyama et al., 2005). According to the authors, the findings were compatible both with a secondary consequence of HC loss and with a direct toxic effect of the antibiotics on the vestibular neurons. In animal studies, vestibular ganglion neurons have been found to survive longer than spiral ganglion neurons after HC loss (Dupont et al., 1993; Jensen, 1983), but available data also support the hypothesis that ototoxic compounds may have a direct toxic effect on the ganglion neurons in addition to their HC effect (Sera et al., 1987). One particular view is offered by studies with IDPN in the rat, as this ototoxic compound offers unique flexibility in dosing regimes. Following acute high-dose exposure, exquisite preservation of the vestibular afferent terminals was observed at short times after exposure when the HCs were degenerating through necrotic and apoptotic patterns (Llorens and Demêmes, 1994). In contrast, in the chronic IDPN model that predominantly causes HC extrusion (Seoane et al., 2001a), the initial evidence for HCI extrusion was preceded by calyx afferent detachment, and this was followed by retraction and fragmentation of the calyces (Seoane et al., 2001b). The known effects of IDPN on neurofilaments (Chou and Hartmann, 1964; Griffin et al., 1978; Llorens and Rodríguez-Farré, 1997), leading to loss of NF in the vestibular afferents (Seoane et al., 2003) as in the motor endplates (Soler-Martín et al., 2012), may have a role in the pathology of the afferents after chronic exposure (Seoane et al., 2003). However, this may be a common response to chronic ototoxicity.

As indicated above, the HC synapses on the ganglion neurons are glutamatergic, so these neurons are exposed to excitotoxicity. This has been investigated through trans-tympanic injection of glutamate agonists, which results in acute excitotoxic damage to the cochlear and vestibular afferents that in mild conditions can involve reversible swelling only (Brugeaud et al., 2007), while stronger stimuli will cause complete degeneration (Raymond et al., 1988). Acute excitotoxic damage to the afferent terminals is also a key event in ischemia-induced inner ear damage (Pujol et al., 1992), and is believed to have a major role in noise-induced auditory neuron damage (Kujawa and Liberman, 2009). In the case of reversible damage to the terminals, functional evidence indicates the temporary loss of synaptic transmission, known as "synaptic uncoupling" (Brugeaud et al., 2007; Puel et al., 1995). The occurrence of excitotoxicity in the inner ear synapses may explain the observations of primary afferent or neuronal damage by ototoxic compounds that in other experimental settings show high selectivity for the HCs. The explanatory hypothesis would be that HCs under toxic stress have a limited capacity for regulating glutamate release and reuptake, which leads to chronic excitotoxic aggression to the afferents. If this is true, slowly evolving afferent damage would be the most relevant event in the early stages of any chronic ototoxic exposure. As noted above, the synapse between calyx afferents and HCIs may be particularly sensitive to deregulation of glutamate homeostasis.

7. Conclusion

Ototoxic compounds that cause HC loss in both the vestibular and auditory systems are probably quite selective for these cells, where they often activate apoptotic pathways, although they also activate other cell demise pathways, including necrosis. The ganglion neurons are also a target of this toxicity and the extent to which ganglion neuron damage is due to direct toxicity or a secondary consequence of HC loss may depend on the particular exposure model. The data available from chronic low-dose exposure models indicate that more complex patterns of HC demise and afferent neuron damage may occur, and these are scarcely understood. The effects of chronic low-dose exposure are more relevant to human populations and to the possible role of ototoxic exposure on the sensory loss observed in aging. Thus, research efforts are required to understand the physiological role and molecular mechanism of HC extrusion and the role of excitotoxic processes in chronic ototoxicity.

Conflict of interest

J.L. is a member of the Scientific Advisory Board of Sensorion Pharmaceuticals, a company that produces treatments for vestibular disorders.

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