Review of Hair Cell Synapse Defects in Sensorineural Hearing Impairment

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Objective: To review new insights into the pathophysiology of sensorineural hearing impairment. Specifically, we address defects of the ribbon synapses between inner hair cells and spiral ganglion neurons that cause auditory synaptopathy.

Data Sources and Study Selection: Here, we review original publications on the genetics, animal models, and molecular mechanisms of hair cell ribbon synapses and their dysfunction.

Conclusion: Hair cell ribbon synapses are highly specialized to enable indefatigable sound encoding with utmost temporal precision. Their dysfunctions, which we term auditory synaptopathies, impair audibility of sounds to varying degrees but commonly affect neural encoding of acoustic temporal cues essential for speech comprehension. Clinical features of auditory synaptopathies are similar to those accompanying auditory neuropathy, a group of genetic and acquired disorders of spiral ganglion neurons. Genetic auditory synaptopathies include alterations of glutamate loading of synaptic vesicles, synaptic Ca²⁺ influx or synaptic vesicle turnover. Acquired synaptopathies include noise-induced hearing loss because of excitotoxic synaptic damage and subsequent gradual neural degeneration. Alterations of ribbon synapses likely also contribute to age-related hearing loss. Key Words: *Sensorineural hearing impairment—Synapses.

Otol Neurotol 00:00–00, 2012.

Mechanisms of Sensorineural Hearing Impairment

Sensorineural hearing impairment encompasses various pathologies of the cochlea and auditory nerve. Based on human temporal bone histology Schuknecht and Igarashi (1) proposed a nosology for slowly progressing sensorineural hearing loss. He distinguished conditions affecting stria vascularis (disrupting cochlear ion homeostasis and energetics), organ of Corti (disrupting hair cell function), and neurons (disrupting transmission of auditory information to the brain). Recent advances in the identification of human deafness genes and their physiological characterization in mouse models have helped to elucidate specific cellular mechanisms contributing to sensory and neural hearing loss. Combining genetic, physiologic, and psychophysical approaches to human sensorineural hearing loss one aims to differentiate primary defects of cochlear ionic homeostasis and endolymph production cause a global dysfunction of the cochlea. For example, the most common hereditary deafness caused by mutations in the gene coding for Connexin 26 impair the endocochlear potential (2,3), which is a prerequisite for the function of hair cells. Defects of outer hair cell electromotility or loss of outer hair cells altogether disrupt cochlear amplification and present primarily with loss of audibility, abnormal loudness gain (recruitment), and impaired frequency discrimination (4,5). Otoacoustic emissions (OAEs), acoustic signals produced by outer hair cell amplification of sound-induced vibrations in the cochlea, are reduced or absent. However, suprathreshold stimuli still evoke synchronized neural potentials in auditory nerve and brainstem pathways identified as auditory brainstem responses (ABRs). These subjects typically have impairments of speech reception affecting mainly consonants and their performance benefit from hearing aids.

Disorders of inner hair cell synapses—auditory synaptopathies—cause evoked potentials of the early auditory pathway to be absent or abnormal (6,7). However, as cochlear amplification is functional, at least initially, OAEs and/or cochlear microphonic potentials are
often present (7–10). Psychophysical findings in auditory synaptopathy vary from normal pure tone audiograms to complete deafness (6,8–14). Still, even when audibility is normal or minimally affected, speech comprehension is impaired and is often not improved by hearing aids (15). Defects of the auditory nerve (16) have similar findings as auditory synaptopathies rendering their differentiation difficult (15,16).

“Synaptopathy” is a recently introduced term for a long-known nosological concept. Myasthenic disorders such as Myasthenia gravis and Lambert-Eaton syndrome are long established synaptopathies of the neuromuscular junction (17–20). Recently, synaptic dysfunction has received much attention as a potential disease mechanism in neuropsychiatric diseases such as Huntington’s disease (21) and autism spectrum disorders (22,23). Although evidence indicates an important role of synaptic alterations in the pathophysiology of major brain diseases, their relevance as primary disease mechanism is an active topic of research (24).

Strong alterations of neuromuscular junction and synapses of the central nervous system are not compatible with life (e.g., ref. (25,26)). This is very different for ribbon synapses formed by sensory cells in the ear and retina. They are molecularly and structurally specialized and, to some extent, distinct from other synapses, such that mutations can specifically affect hearing and/or vision by impairing ribbon synapse function while sparing other synapses. The synaptic ribbon is an electron-dense structure that extends into the cytosol and tethers a halo of synaptic vesicles (Fig. 1B). Depending on the position of an inner hair cell along the tonotopic cochlear axis, it forms between 5 and 20 ribbon synapses (27) with the unbranched peripheral axons of spiral ganglion neurons (28). The exact role of this multi-protein nanomachinery is subject of current studies (29–32). It is hypothesized to support a large pool of readily releasable vesicles and its replenishment after exocytosis. Its main molecular constituent is the protein Ribeye (33) (Fig. 1C) that is specific to ribbon synapses and thought to build the ribbon in a brick-stone like manner interacting with itself.
(34) and other proteins such as bassoon (35). Bassoon is a big scaffold protein (36), common to many synapses, and organizes the active zone of photoreceptors (37) and hair cells (29,32). While sharing some of the common scaffold proteins of the active zone, the hair cell ribbon synapse seems to otherwise employ different proteins than most other synapses (38–43) (Fig. 1C), some of which have been shown to be affected in hereditary synaptopathic hearing impairment.

GENETIC SYNAPTOPATHIES

Defects of Presynaptic Calcium Influx Into Inner Hair Cells

Unlike in other synapses, hair cell ribbon synapses use CaV1.3 L-type Ca2+ channels for stimulus-secretion coupling (44–46). Their active zones cluster tens of CaV1.3 L-type Ca2+ channels (32,47–51) (Fig. 2) that activate rapidly already at hyperpolarized potentials (52) and show only mild inactivation during ongoing stimulation (53,54). These functional properties arise from the unique molecular composition of the channel complex that involves interaction with numerous other proteins such as Ca2+-binding proteins (55–57) (Fig. 2A). Recently, a loss of function mutation in the CACNA1D gene has been identified in a family with congenital deafness and bradycardia, signifying the importance of CaV1.3 for hearing and atrial pacemaking (12). This channelopathy is likely only the first of several other deafness mutations in the various genes controlling hair cell synaptic Ca2+ influx. In fact, we know for photoreceptors that mutations in the genes coding for the poreforming α1F (CACNA1F) subunit (58,59) of the presynaptic CaV1.4 Ca2+ channels, the auxiliary α2δ4 subunit (60) and the interacting Ca2+ binding protein 4 (61) (CaBP4) cause human retinal disease such as night blindness probably by disturbing synaptic transmission at the photoreceptor ribbon synapses.

The human phenotype related to the loss of function CACNA1D mutation (12) is very closely resembled in Cacna1d knock-out mice, displaying both deafness and bradycardia (44,46). The mouse model allows for analysis of Ca2+ influx and the ensuing exocytosis in inner hair cells, which were both reduced by 90% (45) (Fig. 2C, D). This defect of hair cell transmitter release readily explains the lack of ABRs (Fig. 2E). The dramatic reduction of presensory and sensory afferent neural activity leads to substantial neurodevelopmental alterations in the auditory pathway (62–64) and to a progressive loss of hair cell afferent synapses, hair cells and spiral ganglion neurons (62,65), respectively. Interestingly, neither affected humans nor mice seem to have vestibular disorders. This is

FIG. 2. Molecular physiology and pathology of hair cell calcium influx. A, Top: a defect in Ca2+ influx disrupts stimulus-secretion coupling, bottom: domain structures of the subunits forming the hair cell CaV1.3 Ca2+ channel: pore-forming α1D subunit, auxiliary δ2, α2δ, and γ subunits (adapted from Caterall, Pharmacol Rev 2005). B, Left: nanoanatomy of presynaptic CaV1.3 Ca2+ channel clusters resolved by STED microscopy after immunolabeling (taken from Frank et al., Neuron 2010); right: 5 presynaptic Ca2+ microdomains visualized as fluorescence hotspots of Fluo-5N indicator at the ribbon-occupied active zones (marked by a fluorescent Ribeye-binding peptide; taken from Frank et al., PNAS 2009). C, Representative Ca2+ currents and (D), membrane capacitance increments (∆Cm, reflecting exocytic fusion of vesicles to the plasma membrane) of a normal IHC (black) and an IHC lacking the CaV1.3 Ca2+ channel (gray): near-complete block of Ca2+ influx and exocytosis (taken from Brandt et al., 2003). E, Deafness of CaV1.3 deficient mice is indicated by lack of ABRs (representative recordings in response to 100 dB clicks).
consistent with the finding of a sizable remaining \( \text{Ca}^{2+} \) current in vestibular hair cells of \textit{Cacna1d} knock-out mice (46).

**Genetic Alteration of Vesicular Glutamate Uptake in Hair Cells Disrupt Hearing**

The glutamatergic ribbon synapses of hair cells use the transporter VGLUT3 to load their synaptic vesicles with glutamate (41,42,66), whereas all other glutamatergic synapses studied so far use VGLUT1 or 2 (67,68). Instead, in the CNS VGLUT3 is used by monaminergic and cholinergic neurons that co-release glutamate. Genetic ablation of Vglut3 function caused deafness in mice (41,42) and zebrafish (66) because of abolition of glutamate release (Fig. 3). Hair cell synapses remained surprisingly intact. They display robust \( \text{Ca}^{2+} \) influx and exocytosis of glutamate-devoid vesicles (42) (Fig. 3B), and spiral ganglion neurons exhibited robust postsynaptic receptor currents in response to application of exogenous glutamate (41). Loss of synapses, hair cells and spiral ganglion neurons proceeded at relatively slow pace (weeks rather than days as found for otoferlin mutants, see below) perhaps because of preserved release of trophic factors. Interestingly, no overt vestibular dysfunction was observed in \textit{Vglut3} knock-out mice.

First efforts toward virus-mediated transfer of \textit{Vglut3} DNA into inner hair cells of \textit{Vglut3} knock-out mice have yielded promising results: normal thresholds were restored
for several weeks following viral injection into the cochlea (69). Vglut3 knock-out mice and heterozygotes littermates showed EEG abnormalities indicative of a neocortical hyperexcitability, but myoclonic activity has not been detected (41). The human hearing impairment DFNA25 was first described in 2003 (13) and was then linked to a VGLUT3 mutation in 2008 (42). Affected subjects become progressively hearing impaired starting during...
adolescence (Fig. 3D) and apparently lack other symptoms. Future studies are needed to address the precise mechanism of the progressive synaptopathy DFNA25.

Mutations in OTOF Cause Prelingual Deafness DFNB9 and Temperature-Sensitive Synaptic Hearing Impairment

Mutations in the OTOF gene coding for otoferlin—a member of the ferlin family of transmembrane multi-C2- proteins (70,71), which is expressed in hair cells (72)—cause the prelingual deafness DFNB9 (6,8,10,73,74) and a temperature-sensitive hearing impairment (75,9,14) (Fig. 4). Since its identification, more than 50 pathogenic mutations of vesicle protein Vglut3 (78) (Fig. 4B). Ablation of distribution in hair cells is similar to that of the synaptic found by immuno-electron microscopy (72) and its general cell biological functions have been proposed based on protein interaction studies and the broad distribution of otoferlin in hair cells also outside the presynaptic active zones (85–87).

 Noise-Induced and Age-Related Hearing Loss

Recent findings indicate that cochlear synaptic mechanisms may contribute to these 2 most common forms of hearing impairment. Changes in synapse number and structure have been implied in noise-induced (88–90) and age-dependent hearing loss (91). Interestingly, a human association study suggests polymorphisms in the gene coding for the metabotropic glutamate receptor mGlur7 to contribute to susceptibility for age-dependent hearing loss (92). Excitotoxic synaptic and neural damage is a key candidate mechanism for noise-induced and age-dependent hearing loss (Fig. 5A). It may result from excessive presynaptic release of glutamate, which has long been discussed for noise-induced hearing loss (see below) and has recently been implied for a human progressive hearing loss caused by mutations in the gene GIPC3 (93,94). Susceptibility to excitotoxic damage could also arise from abnormally high numbers or sensitivity of postsynaptic glutamate receptors (95), alterations of efferent innervation (96) and from interference with glutamate uptake (97,98), but the relevance of these mechanisms for human disease has not yet been demonstrated.

Excitotoxic synaptic damage due to excessive presynaptic release of glutamate has long been indicated to contribute to noise-induced hearing loss (88–90). Immunohistochemical quantification of ribbon synapse number (27,29) has now been used to establish the loss of ribbon synapses during noise exposures (99,100). Strikingly, even noise exposures that caused only temporary threshold loss were accompanied by a permanent loss of approximately 50% of the hair cell synapses and subsequent slow degeneration of spiral ganglion neurons in the high frequency region of the cochlea (Fig. 5C, D, F, G). The morphologic damage was reflected by a reduced spiral ganglion compound action potential. Measured as Jewett wave I of the auditory brainstem responses, a permanent reduction was found (Fig. 5E), despite full recovery of the physiologic threshold (Fig. 5B). One possible hypothesis explaining this discrepancy of functional findings is that the noise-induced insult hits the low-sensitivity spiral ganglion neurons, which signal loud sounds, but spares the

Otology & Neurotology, Vol. 00, No. 00, 2012

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high-sensitivity neurons, which are responsible for sound perception near threshold. This hypothesis can well explain the finding of poor speech recognition in noisy background. Not surprisingly synaptic insult occurs also during noise exposures that cause a permanent threshold increase (99).

Current research aims to understand the presynaptic and postsynaptic changes that occur during noise damage. Moreover, studies explore the reasons why excitotoxic synapse loss is not followed by de novo synapse formation during the weeks after the insult when the disconnected inner hair cells and spiral ganglion neurons are still present. The extent, irreversibility, and functional consequences of excitotoxic synapse loss had not yet appreciated and now require studies of the relevance of this disease mechanism for human noise-induced hearing loss. If comparable to the animal findings, which is likely the case, noise exposure is much more dangerous than we have assumed. We will then have to acknowledge that noise induces synapse and progressive neuron loss and thereby impairs speech reception in noisy environments. We will need to revise noise exposure guidelines, diagnostic procedures and clinical evaluation of occupational hearing loss. In summary, excitotoxic synaptic damage is likely a disease mechanism of noise-induced and possibly also of age-dependent hearing loss (100,101).

**FIG. 5.** Excitotoxic irreversible loss of IHC ribbon synapses during noise-induced temporary threshold loss. A. Cartoon illustrating excitotoxic synaptic insult: loud noise induces excessive presynaptic glutamate release that causes overexcitation and massive sodium influx into the postsynaptic terminal of the SGN. The ensuing osmotic load causes swelling and finally disruption of the terminal. Work by Kujawa and Liberman (2009) in animals suggests that the SGN do not re-establish synaptic connections with IHCs after the insult and are finally lost. B. Induction and recovery of ABR threshold loss following a 100 dB octave band noise for 2 h. C. Irreversible loss of half of the synaptic ribbons in high-frequency IHCs in the same mice, despite threshold recovery after 2 weeks. D. Representative projections of confocal images of the immunolabeled IHC ribbons in control and noise-exposed mice: reduction of ribbon number. Long-term percentage reduction of (E) the amplitude of ABR wave 1 reflecting the loss of synchronously firing SGN, (F) ribbon synapse number in high frequency IHCs and (G) SGN somata: simultaneous loss of synapses and synchronously firing neurons, delayed physical loss of SGN. (B-G) were taken with permission from Kujawa and Liberman, J Neurosci 2009.

**DISCUSSION**

**Identification and Characterization of Auditory Synaptopathy**

Auditory synaptopathy-impaired synaptic sound encoding has only recently been appreciated as a disease mechanism of both genetic and acquired hearing impairments. The similarity of clinical expression to auditory neuropathy (15,16): preserved otoacoustic emissions and/or cochlear microphonic potentials reflecting cochlear outer hair cell function but absent or abnormal auditory brainstem responses due to impaired sound coding led to the initial denomination as auditory neuropathy or auditory neuropathy spectrum disorder. This review describes specific disease mechanisms,
focusing on presynaptic alterations at the inner hair cell synapse. Human genetics has uncovered that monogenic defects and complex genetic diseases also affect sound encoding at the hair cell synapses. Starting with the identification of otoferlin (10), an increasing number of defects in genes that code for synaptic proteins and ion channels have been identified, and the list is expected to still increase. Molecular physiology in genetically manipulated mice has provided insights into gene function at the synapse and the synaptic mechanisms underlying the human disease. These studies unambiguously demonstrate the synapse as a primary site of lesion and hence support the use of auditory synaptopathy as the precise nosologic category. However, severe auditory synaptopathy sooner or later leads to degeneration of the spiral ganglion neurons and, thus, has a common final path with primary neural disorders such as hereditary motor and sensory neuropathy.

Understanding Mechanisms and Phenotypes of Auditory Synaptopathies Based on Detailed Analysis of Mouse Models

Mouse models serve as powerful tools for dissecting the precise disease mechanisms, for predicting onset and progression of degeneration and for devising therapeutic approaches. Different from the described mouse models of human auditory synaptopathy, other “synaptic” mouse mutants allow one to study the consequences of more subtle synaptic deficits for auditory systems function. Genetic disruption of the presynaptic protein Bassoon causes a mild synaptic hearing impairment (29,102) because of a reduction in the number of releasable synaptic vesicles and Ca2+ channels (32). ABR are present but display a massive reduction in wave 1 amplitude (29,102) because of reduced auditory nerve fiber spiking rates and increased jitter of first spike latency (102). Although no human mutations have been described so far, this mouse line has gained considerable interest as a model for auditory synaptopathy.

Otoferlin: Synergistic Research on Human Subjects and Animal Models Advance Our Understanding of Otoferlin Function and Dysfunction

The genetics, structure, and function of otoferlin in the context of hearing and deafness define a hot topic of auditory research. After identifying OTOF about a decade ago as the gene defect underlying autosomal recessive, nonsyndromic profound deafness DFNB9 (10), work now encompasses molecular, cellular, and systems level approaches. The presence of human subjects with temperature-sensitive OTOF mutations enables advanced electrophysiologic and psychophysical studies and promises to contribute to our understanding of otoferlin-related hearing impairment and auditory synaptopathy in general. Genetic manipulations in mice combined with comprehensive structural and functional analysis will continue to contribute. In particular, these studies will help to further test the Ca2+ sensor of vesicle fusion and vesicle replenishment hypotheses.

Presynaptic and Postsynaptic Mechanisms of Synaptopathy

Here, we have reviewed exemplary presynaptic and postsynaptic mechanisms of synaptic hearing impairment with much emphasis on the presynaptic dysfunction. Future research will reveal further genetic and acquired synaptopathies, which will likely also include other alterations of postsynaptic function. Combining specific clinical and genetic testing will likely help to distinguish primarily presynaptic and postsynaptic dysfunctions.

Acknowledgments: The authors thank Nicola Strenzke, Martin Canis and Charles M. Liberman for the comments on the manuscript. The authors also thank Regis Nouvian and Linda Hsu for contributing graphical illustrations.

REFERENCES

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