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Ion Channel Defects in Hereditary Hearing Loss

Minireview

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Approximately 1 in 1000 children is born with a significant hearing deficit, which critically affects language development. By old age, nearly 1 in 3 of us will have impaired hearing and the resulting difficulty in communicating with family and friends. Some portion of both the congenital and the progressive hearing loss has a basis in genetic defects. Fortunately, there has been extraordinary progress in identifying genes defective in hearing and balance disorders: nearly 40 such genes have been described, with 10 of them appearing in just the past year (see G. Van Camp and R. J. H. Smith, Hereditary Hearing Loss Homepage at http://dnalab-www.uia.ac.be/dnalab/hhh/). These are exhilarating times for a field that has sometimes felt like a quiet backwater of neuroscience.

Some of these hearing and balance disorders are syndromic, in which deafness occurs along with additional symptoms, such as blindness. Others are nonsyndromic, presenting only with loss of hearing and/or balance. Hearing loss can be recessively inherited (these are most often congenital and more severe), dominantly inherited (usually progressive), or X linked. Mitochondrial mutations often produce hearing loss as well, and six such mutations have been identified. Different mutations in the same gene can cause both syndromic and nonsyndromic forms of hearing loss, or dominant and recessive disorders, with several recent examples of each.

The mammalian hearing apparatus, housed in the snail-shaped cochlea (Figure 1), relies on a unique system of ion transport among fluid spaces. The scala tympani and scala vestibuli contain perilymph, with an ionic composition much like typical extracellular fluid (low K^+, high Na^+). However, the scala media contains an endolymph with an ionic composition almost like cytoplasm (high K^+, low Na^+, low Ca^{2+}). Ion flux from the marginal cells of the stria vascularis maintains these concentrations and sets the endolymphatic potential to about +80 mV. Any drop in this potential diminishes sensitivity to acoustic stimuli. The sensory hair cells have their apical, ciliated surfaces facing endolymph and their basolateral surfaces bathed by perilymph. When the sensory cilia of hair cells are deflected by acoustic vibrations, nonselective cation channels in the cilia open and allow K^+ to pass from the endolymph into the hair cell cytoplasm. Efflux from the hair cell basolateral membrane passes the K^+ to the perilymph; from the perilymph, K^+ must somehow get back to the endolymph. Kikuchi et al. (1995) suggest that the K^+ is taken into the supporting cells, then diffuses through two syncytial networks back to the marginal cells of the stria vascularis, and is pumped by marginal cells to the endolymph. With such a complex and regulated flux of K^+, it should perhaps come as no surprise that five of the recently identified deafness genes encode ion channels and that most of these channels are expressed by specific components of the K^+ recycling pathway.

KCNQ1 *and* KCNE1

Defects in two related K^+ channel genes occur in Jervell and Lange-Nielsen Syndrome (JLNS; see references in OMIM *192500 and *176261 at http://www.ncbi.nlm.nih.gov/omim/). This recessively inherited disorder is characterized by congenital bilateral deafness and by cardiac abnormalities, including a prolonged QT interval and arrhythmia that can lead to sudden death. Perhaps the first description of a JLNS patient was recorded in 1856 by Meissner, involving a deaf girl who was called before the director of her school for a reprimand and fell dead. One can imagine the director trying to explain the circumstances to the parents, but the parents were not surprised: they had lost two other deaf children under similar circumstances of stress. The genetic basis, the deafness, and the arrhythmia are all apparent in this incident.

In some families with JLNS, mutations were found in the K^+ channel gene *KCNQ1* (also known as *KvLQT1*). *KCNQ1* is expressed in the heart, and mutations in this gene had previously been found in families with long QT syndrome without deafness. In the inner ear, the KCNQ1 channel is expressed in the apical surface of the marginal cells of the stria vascularis and in similar cells of the vestibular system (Shen and Marcus, 1998, and references therein). The channel is tonically active and is thought to pass K^+ into the endolymph.

Associated with *KCNQ1* is a smaller subunit encoded by *KCNE1* (a.k.a. *minK*, *IsK*), that does not form part of the conducting pore (Kaczmarek and Blumenthal, 1997). Mutations in *KCNE1* have recently been found in other JLNS families that do not have a defect in *KNCQ1.* Moreover, a null mutation in the mouse ortholog of *KCNE1* causes a loss of K^+ secretion by the stria vascularis of the cochlea and related cells of the vestibular epithelium, eventual death of the hair cells, and concomitant loss of both hearing and balance (Vetter et al., 1996). Thus, the cells that generate the high K^+ concentration in endolymph require *KCNQ1* and *KCNE1* to perform that function.

GJB2 *(Connexin 26)*

Another critical element in the pathway for recycling K^+ back to marginal cells has been described by Kikuchi et al. (1995). Electron microscopy reveals an extensive network of gap junctions in two sets of cochlear cells: an epithelial cell system that surrounds the hair cells, and an adjacent connective tissue system of fibrocytes positioned under and around the marginal cells of the stria (Figure 1). Immunostaining demonstrates that the connexin 26 protein (encoded by *GJB2*) is expressed by both sets of cells. Cells of the epithelial system have a very negative resting potential (−100 mV), which would

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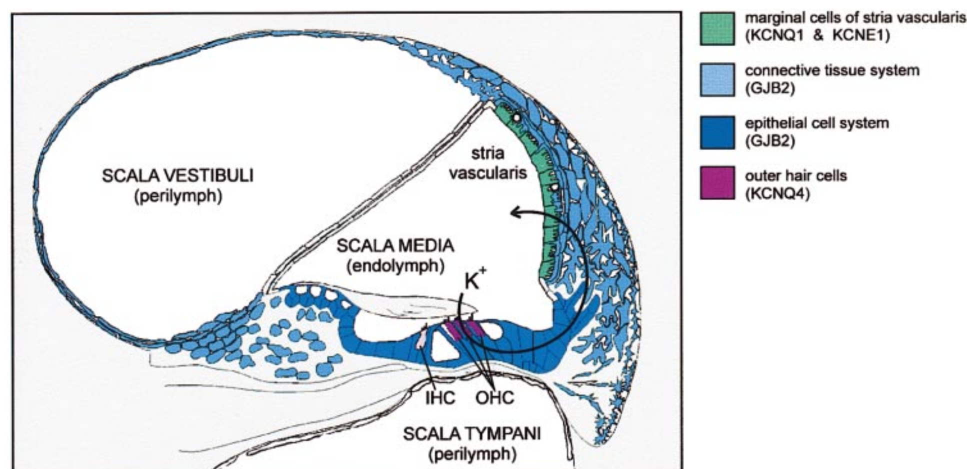

Figure 1. One Turn of the Mammalian Cochlea in Cross Section

Colors indicate the cells expressing different channels genes. IHC and OHC are inner and outer hair cells, respectively. Adapted from Kikuchi et al. (1995).

pull in K^+ from the perilymph around the hair cells. Passive diffusion through the network could then bring K^+ to the stria vascularis, where it could be taken up by the marginal cells using their abundant (Na^+, K^+)ATPase.

Mutations in *GJB2* have now been found in families with the congenital recessive nonsyndromic deafness DFNB1 and also in the progressive dominant DFNA3 (see references in OMIM *121011 at http://www.ncbi.nlm.nih.gov/omim/). Mutations in *GJB2* are extraordinary in three respects. First, in most individuals, the defect is a single nucleotide mutation—the loss of a guanine in a string of six—that is referred to as 30ΔG or 35ΔG. Multiple haplotypes for this mutation suggest it arose spontaneously in different individuals. Second, defects in *GJB2* are extremely common, with a carrier rate in different ethnic groups ranging from 2% to nearly 5%. Thus, this single gene may account for 10% or more of all congenital hearing loss. Third, heterozygotic carriers show subtle abnormalities in a sensitive test of inner ear function, raising the question of whether carriers might be at higher risk for late-onset hearing loss.

GJB3 *(Connexin 31)*

Connexin 26 is but one of a family of gap junction proteins. Another gap junction protein, connexin 31, was shown by RT–PCR to be expressed in the inner ear, but its cellular localization and function within the cochlea are not understood (Xia et al., 1998). The human gene for connexin 31, *GJB3*, has been mapped to the region containing the locus for DFNA2 (see references in OMIM *603324 at http://www.ncbi.nlm.nih.gov/omim/). DFNA2 is a nonsyndromic, dominant, progressive hearing loss with high frequencies preferentially affected that has been linked to chromosomal region 1p34–p35 in five unrelated families. Xia et al. (1998) found two additional families with similar phenotype in which affected individuals had mutations in *GJB3*. Thus, *GJB3* is the gene defective in at least some DFNA2 families; on the other hand, a different channel gene in the same region of chromosome 1 may account for other DFNA2 families (see below).

KCNQ4

The newest deafness gene is also an ion channel, which may bring K^+ out of the hair cells into the perilymph. Looking for channels related to the KCNQ1 channel of the marginal cells, Kubisch et al. (1999) found a novel subunit, KCNQ4. In situ hybridization demonstrated a very strong signal in hair cells, but specifically in outer hair cells and not inner hair cells. *KCNQ4* was mapped to human chromosome 1p34—again, the locus for the nonsyndromic dominant progressive deafness, DFNA2. Sequencing of *KCNQ4* in additional families with the progressive DFNA2 phenotype revealed one in which affected individuals have a point mutation that alters the canonical GYG sequence of the potassium channel pore. A pore mutation in any one subunit of this tetrameric channel renders the channel nonconducting and would explain the dominant inheritance; indeed, similar mutations in *KCNQ1* cause dominant long QT syndrome.

As a first step, the finding of KCNQ4 in hair cells nearly completes the K^+ flux pathway as represented by deafness genes, since KCNQ4 would let K^+ out of hair cells to where it could be taken up by the epithelial cell syncytium. But more perplexing questions remain. If the function of KCNQ4 is K^+ efflux, why is it not in inner hair cells, which also let K^+ in during acoustic stimulation? What differences between inner and outer hair cells might be related to KCNQ4 function? And why would a *KCNQ4* mutation that blocks conductance cause a hearing loss that takes years to develop? Answers may suggest a much more specific and interesting role for KCNQ4.

Both inner and outer hair cells transduce acoustic vibrations that deflect their cilia. Inner hair cells synapse on the eighth nerve fibers that carry the auditory signal to the cochlear nucleus; outer hair cells—with little or no direct connection to the eighth nerve—are thought to use their transduction signal to drive a mechanical amplification of the vibrations, which the inner hair cells can sense. Outer hair cells have an amazing and unique ability to shorten or lengthen when their membrane voltage is changed. Is KCNQ4 the long-sought electromotility

protein? Probably not: although they share three features—specific expression by outer hair cells, abundance, and a roughly similar voltage dependence—the electromotility protein does not conduct, whereas KCNQ4 clearly does.

Could KCNQ4 carry any of the several ionic currents described in outer hair cells? One of these, an outwardly rectifying K^+ current known as I_{Kn}, is expressed by outer (but not inner) hair cells. Type I (but not type II) hair cells of mammalian vestibular organs express a similar current known as I_{KL} (Rusch and Eatock, 1996). I_{Kn} and I_{KL} are active at the resting potential and contribute to the large resting conductance of these cells (Housley and Ashmore, 1992). Like the KCNQ4 channel expressed in oocytes, both activate slowly upon depolarization and do not inactivate. While the KCNQ4 channel has an activation potential much more positive than that for I_{Kn}, suggesting that KCNQ4 is not the channel protein carrying the I_{Kn} current, other recent findings raise the possibility that KCNQ4's activation potential can be modulated. Two other members of the KCNQ family, KCNQ2 and KCNQ3, form a heteromultimeric channel in brain that apparently carries the M current, a current regulated by a number of signaling pathways including the muscarinic acetylcholine receptor (Wang et al., 1998). The M current activation potential has a wide variability that is likely to result from modulation by second messengers (Marion, 1997). Kubisch et al. (1999) found that KCNQ4 can also form heteromultimeric channels with KCNQ3, and found that KCNQ3 is expressed in cochlear and vestibular tissues. It is plausible that a KCNQ4/KCNQ3 channel in hair cells is susceptible to second messenger modulation that shifts the activation range to more negative potentials, where it could match I_{Kn} and I_{KL}. Indeed, Jagger and Ashmore (1999) found that the activation range of I_{Kn} is regulated by cAMP, protein kinase A, and protein phosphatase.

A possible source of such modulation is cholinergic synaptic feedback from brainstem nuclei to the hair cells. Although cholinergic transmission is often excitatory, stimulation of these efferent projections is usually inhibitory. The complex physiology of the efferent synapse, which acts on the time scale of tens or hundreds of milliseconds, was clarified by Fuchs, Fettiplace, and others (reviewed by Fuchs, 1996). However, there are also slower effects of efferent stimulation that occur over tens or hundred of seconds (Sridhar et al., 1997), which reduce the sensitivity of the cochlea but also protect the cochlea from temporary damage that follows acoustic overexposure (Reiter and Liberman, 1995). Removal of efferent innervation altogether greatly increases the vulnerability of the ear to permanent damage from acoustic overexposure (Kujawa and Liberman, 1997). The physiological basis of these slow modulatory effects are not yet understood; here may be a role for KCNQ4. If slow modulation of an M channel formed by KCNQ3 and KCNQ4 somehow protects the outer hair cells, a progressive hearing loss in individuals with mutations in *KCNQ4* could be seen as following from accumulated insults to an unprotected ear. It is interesting in this regard that the slow efferent effects primarily occur at higher frequencies (Reiter and Liberman, 1995), the I_{Kn} current is found preferentially in high-frequency regions (Mammano and Ashmore, 1996), and the DFNA2 phenotype has greater high-frequency hearing loss.

Concluding Remarks

The K^+ recycling pathway outlined by Kikuchi et al. (1995) and others now appears critically important for normal hearing and balance, since mutations at nearly every point of the pathway lead to severe hearing disorders. Other genes involved in K^+ flux—including the elusive transduction channel of hair cells—will doubtless be added to the list. KCNQ4 is part of this pathway but may also mediate subtle modulation of outer hair cell function. The next important step is to understand in detail the normal function of the channels. Adenoviruses infect hair cells and efficiently mediate expression of other potassium channels in vitro (Holt et al., 1999). Because so many of these ion channel deafnesses are dominantly inherited, acute viral introduction of dominant negative constructs may prove particularly useful in elucidating the functions of ion channels in hearing.

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