Commentary

Mutant ion channel in cochlear hair cells causes deafness

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The sensation of balance and hearing is initiated by the conversion of the movement of stereocilia in hair cells of the inner ear into electrical signals in nerve fibers leading to the brain. Driven by pressure waves that are generated by sound, head movement, or gravity, this transformation of energy occurs in structures of exceptional delicacy and intricacy, where movements of atomic dimensions result in perception (1). Not surprisingly, these sensory modalities may be easily and irreversibly damaged by, for example, chemical agents, noise, or head trauma. Because sensory transduction requires the performance of single molecules, it is also sensitive to mutation, and there is now a growing list of genetic loci for deafness that have been successfully traced to genes coding for membrane, regulatory, or structural proteins of the inner ear. In this issue of PNAS, Kharkovets *et al.* (2) report the expression pattern of a novel potassium channel, KCNQ4, in subtypes of hair cells and neurons of the auditory and vestibular systems. As mutations in the gene encoding KCNQ4 appear to cause one form of nonsyndromic dominant deafness, the results of this paper may lead to a refined molecular understanding of transduction, of the central processing of signals generated by hair cells, and of treatment of deafness and balance disorders.

Although the mechanism of mechanotransduction is largely the same throughout the inner ear, hair cells are used in remarkably diverse ways (1). The apex of the hair cell features a bundle of stereocilia, which, when moved in a preferred direction, causes the opening of ion channels in the tips of the stereocilia and the consequent depolarization of the hair cell. A single row of inner hair cells in the organ of Corti, a structure that runs along the middle of the cochlea (Fig. 1), is arranged to transduce sound-driven movements of the cochlear basilar membrane into the release of the neurotransmitter glutamate onto the dendrites of spiral ganglion cells, resulting in signals in the auditory nerve. In mammals, the movement of the basilar membrane is "tuned," so that progressively higher pitches cause maximal movement of more basal regions of the cochlea; in this way, select populations of inner hair cells are excited by specific frequencies of sound. This mechanical tuning is thought to be critically improved or amplified by a feedback mechanism involving several rows of outer hair cells. Movements of their stereocilia, and depolarization of their cell bodies, result in contractile movements of the outer hair cells, which are widely believed to feed back into movements of the organ of Corti, thus enhancing the resonant behavior of the entire structure. Although the inner hair cells receive afferent innervation, the outer hair cells are innervated by cholinergic efferent axons that cause damping of hair cell activity, a protective response to intense noise. In the vestibular hair cells of the utricular and saccular maculi, and the crista ampularis of each semicircular canal, position and movement of the head causes deflection of hair cell stereocilia, resulting in release of transmitter onto dendrites of the vestibular ganglion neurons. Here, too, there are two varieties of hair cell, Type I and Type II, but the functional significance of this distinction is unclear. Unlike Type II cells, the Type I hair cell has a remarkable amphora-like shape and is almost entirely encased by the huge calyciform dendritic terminal of the ganglion cell (Fig. 1).

Ion channels of specialized composition figure critically in the performance of the auditory and vestibular systems. As noted, the purpose of hair cell stereocilia is to activate mechanosensitive ion channels. The flow of ions through these channels is driven by an elevated potassium concentration in the endolymph, the fluid overlying the hair cells. Once depolarized, calcium and potassium channels in the hair cells may mediate tuned electrical oscillations, and the release of neurotransmitter (3). Efferent feedback onto the outer hair cells utilizes a novel acetylcholine receptor subunit, α-9, which gates a channel of exceptionally high calcium permeability (4). In the central nervous system (CNS), specialized neural circuits extract specific aspects of auditory and vestibular information. The precise complement of ion channels they express enables these neurons to preserve temporal aspects of electrical signals coming from the inner ear (5). For example, spherical bushy cells and octopus cells of the ventral cochlear nucleus express a low-threshold potassium channel, probably composed of the Kv1.1 and -1.2 subunits, which is essential for their ability to respond with microsecond precision. Transmission through these circuits often relies on an unusually fast-gating glutamate-activated channel containing GluR4-flop subunits.

Clearly, mutations in genes encoding specific ion channels could have highly selective effects on sensory function. A wide variety of inherited deafness disorders have been described, and recent efforts have determined many of the loci and gene products involved (see http://dnalab-www.uia.ac.be/dnalab/hhh/ for a comprehensive description of deafness loci). One family of potassium channel, termed KCNQ, figures in syndromic and nonsyndromic deafness disorders, suggesting that these channels are both susceptible to mutation and important in sensory transduction. For example, mutations in KCNQ1, or an accessory protein KCNE1, may lead to lethal distortions in cardiac action potentials, causing long QT syndrome (LQTS); when additional mutations are present, patients may also show congenital deafness, collectively termed Jervell and Lange-Nielsen syndrome (6). Loss of potassium channel function in this disorder may inhibit the generation of a potassium-rich endolymph, leading to hearing impairment.

In 1999, Jentsch and colleagues reported the determination of the gene responsible for another form of hereditary deafness, DFNA2 (7). DFNA refers to the class of nonsyndromic autosomal dominant deafnesses. The gene mapped to this locus was KCNQ4, a new member of the KCNQ family of potassium channel, which in DFNA2 families features a missense mutation, G285S, that eliminates

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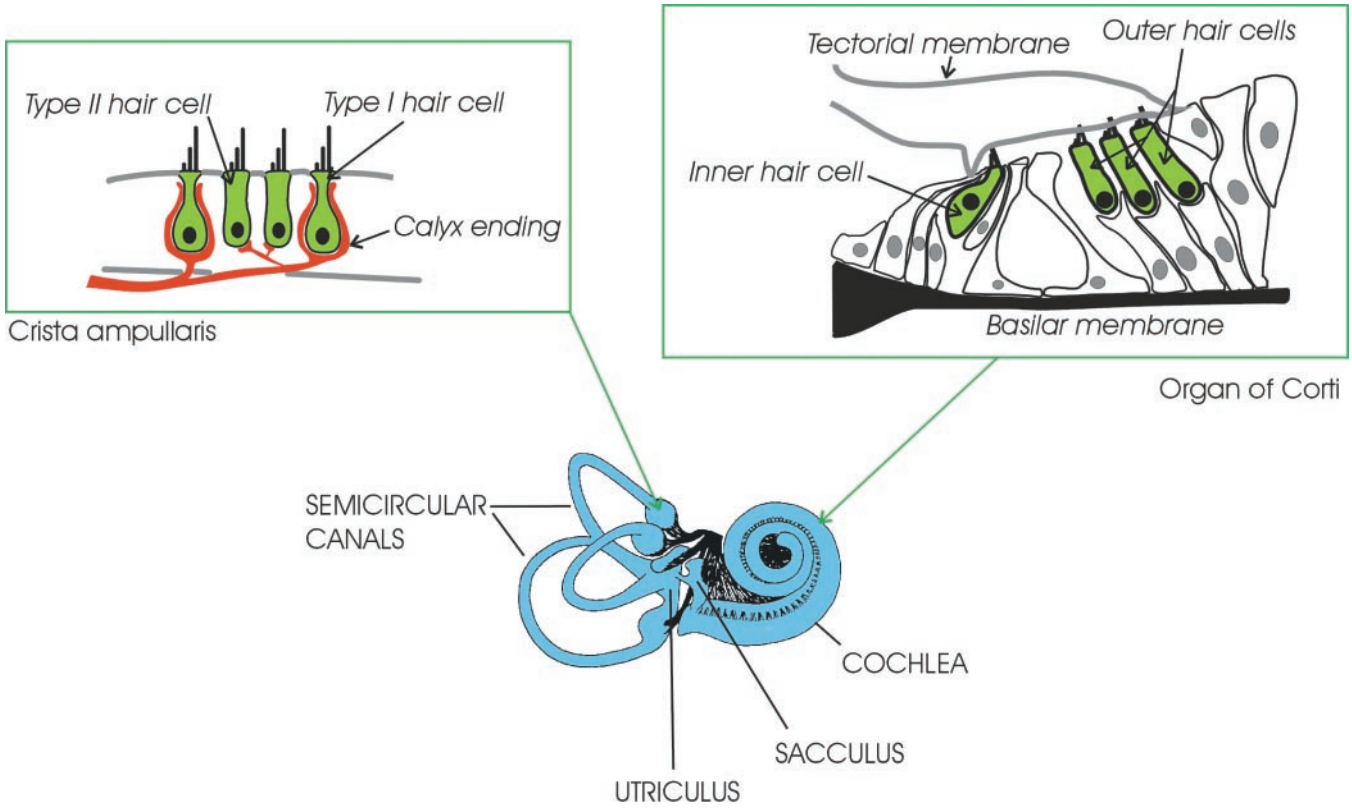

Fig. 1. Some key structures of the inner ear. In blue is the layout of the auditory (cochlea) and vestibular organs. The latter consists of the layer of hair cells in the utriculus and sacculus, and in the crista ampullaris, lying at the end of each semicircular canal. Vestibular hair cell epithelia contain Type I and II hair cells (green), with a characteristic pattern of innervation (red), as shown on the left. The auditory hair cell epithelium in the organ of Corti (right) contains inner and outer hair cells (green) with a distinct arrangement.

channel function. Moreover, expression of the mutant channel with normal KCNQ4 also suppresses function, consistent with a dominant-negative action in heterozygous individuals. The latest report from this group describes in detail the remarkable cellular distribution of KCNQ4. Using antibodies raised against KCNQ4, immunohistochemical methods revealed the channel protein in the basal membrane of outer hair cells and Type I vestibular hair cells, but not in other hair cell subtypes.

What can we learn from this cellular distribution about the function of the channel? It is critical first to identify which ionic currents are unique to the subset of cells that contain KCNQ4 protein. Analysis of the complement of ionic currents in different hair cells have provided one answer. Both outer hair cells and Type I vestibular hair cells express an unusual potassium selective ''leak'' current, termed $I_{K,N}$ and G_{KL} (or G_{KI}), respectively, which is notable both for its large size and for its being active at extremely negative membrane potentials (8–10) (Fig. 2). Curiously, the size and activation voltage of the leak current are such that it would be expected to strongly oppose sensory or synaptic signals in the hair cells. Does KCNQ4 generate this current? Kharkovets *et al.* argue that it does, based on solid, but indirect, arguments: The distribution across the cochlea, the subcellular distribution, and the developmental time course of expression for the leak

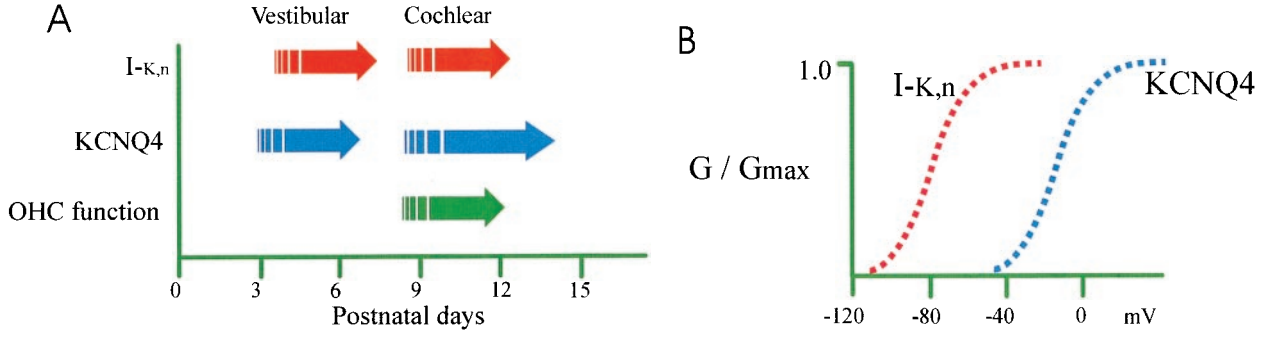

Fig. 2. Similarities and differences between leak current (here termed $I_{K,n}$) and KCNQ4 channels. (*A*) Both channels show similar developmental onset in the cochlear and vestibular organs and its appearance in the cochlear coincides with the onset of outer hair cell function (OHC). (*B*) The membrane potentials needed for activation of the two channels differ markedly, with 50% activation of I_{Kn} (red) at −80 mV (8) and KCNQ4 channels (blue) at −10 mV.

current and KCNQ4 protein match precisely and coincide with the onset of outer hair cell function (Fig. 2). No other channel has yet been identified that is unique to these two types of hair cell. KCNQ4 is also sensitive to linopirdine, which blocks the leak current. Molecular identification of this channel may help clarify its function. A proposed role for this channel in Type I hair cells is to accumulate potassium in the synaptic cleft of the calyx terminal (11). However, as KCNQ4 has been now localized in the postsynaptic membrane of the calyx, as well as in the hair cell itself (2), potassium may flow freely between pre- and postsynaptic cells, minimizing its accumulation and perhaps promoting ephaptic transmission (11).

Yet, there are conspicuous differences between KCNQ4 and the leak current in the membrane potential they require for activation (Fig. 2) and in the speed of channel activation, with KCNQ4 activating more slowly. Resolving whether the leak channels contain KCNQ4 may come with electrophysiological analysis of knockouts of KCNQ4. But if the leak current is indeed produced by KCNQ4, understanding why they behave differently will be revealing. As noted by Kharkovets *et al.* (2), the patch clamp studies of leak current may have somehow altered the channel. This would mean that KCNQ4 channels are extremely sensitive to cellular metabolic state and thus might be easily modulated. Although this may be the case, typical leak currents have been observed in cells having unperturbed cytoplasm, using the perforated patch technique (12). Alternatively, the native channel may normally function as the leak current, suggesting that KCNQ4 may be modified *in vivo* or that leak channels may also contain other subunits. Indeed, Jentsch and colleagues previously showed that both KCNQ3 and -4 are co-expressed in the inner ear and may co-assemble (7), and other studies showed that β subunits KCNE1–3 may modify the function of some KCNQ-containing channels (13–15). Finally, if genetic alteration of the KCNQ/leak current results in deafness, what role does the channel play in hearing? Surprisingly, DFNA2 families show no apparent balance disorders, despite the higher expression of both KCNQ4 and leak current in vestibular hair cells. Again, analysis of auditory and vestibular physiology in animal models of DFNA2 promises to be particularly revealing. For example, if Type I hair cells express other subunits or channels that contribute to the leak current, they may be better able to tolerate mutations in KCNQ4.

Kharkovets *et al.* (2) add to the intrigue about KCNQ4 function by demonstrating expression of the channel subunit in regions of the brain associated with auditory and vestibular function. Could the electrical response profiles of neurons in these areas be altered in DFNA2? Indeed, this possibility suggests that the differences between auditory and vestibular impairments in DFNA2 might actually lie in the altered function of the brain and not the inner ear. Although not identifying the cell types involved, the localization of KCNQ4 in these regions will likely spur further efforts to determine ionic mechanisms that control the response patterns of cells involved in these neural circuits. Thus, DFNA2 and other genetic loci for sensory disorders offer new potential for determining how subtypes of ion channel, and the neural circuits that express such channels, are engaged in processing specific components of sensory information in the brain. From this, and an understanding of the pharmacological modification of these channels, new treatments may be developed that are selective for a sensory modality, and perhaps even for the quality of perception.

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1. Hudspeth, A. J. (1989) *Nature (London)* **341,** 397–404.
2. Kharkovets, T., Hardelin, J.-P., Safieddine, S., Schweizer, M., El-Amraoui, A., Petit, C. & Jentsch, T. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 4333–4338.
3. Fettiplace, R. & Fuchs, P. A. (1999) *Annu. Rev. Physiol.* **61,** 809–834.
4. Vetter, D. E., Liberman, M. C., Mann, J., Barhanin, J., Boulter, J., Brown, M. C., Saffiote-Kolman, J., Heinemann, S. F. & Elgoyhen, A. B. (1999) *Neuron* **23,** 93–103.
5. Trussell, L. O. (1999) *Annu. Rev. Physiol.* **61,** 477–496.
6. Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Faure, S., Gary, F., Coumel, P., Petit, C., *et al.* (1997) *Nat. Genet.* **15,** 186–189.
7. Kubisch, C., Schroeder, B. C., Friedrich, T., Lutjohann, B., El-Amraoui, A., Marlin, S., Petit, C. & Jentsch, T. J. (1999) *Cell* **96,** 437–446.
8. Marcotti, W. & Kros, C. J. (1999) *J. Physiol. (London)* **520** (3), 653–660.
9. Rennie, K. J. & Correia, M. J. (1994) *J. Neurophysiol.* **71,** 317–329.
10. Housley, G. D. & Ashmore, J. F. (1992) *J. Physiol. (London)* **448,** 73–98.
11. Goldberg, J. M. (1996) *Ann. N.Y. Acad. Sci.* **781,** 474–488.
12. Ricci, A. J., Rennie, K. J. & Correia, M. J. (1996) *Pflugers Arch.* **432,** 34–42.
13. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M. & Romey, G. (1996) *Nature (London)* **384,** 78–80.
14. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L. & Keating, M. T. (1996) *Nature (London)* **384,** 80–83.
15. Schroeder, B. C., Waldegger, S., Fehr, S., Bleich, M., Warth, R., Greger, R. & Jentsch, T. J. (2000) *Nature (London)* **403,** 196–199.