Loss of cochlear HCO$_3^-$ secretion causes deafness via endolymphatic acidification and inhibition of Ca$^{2+}$ reabsorption in a Pendred syndrome mouse model

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Wangemann P, Nakaya K, Wu T, Maganti RJ, Itza EM, Sanneman JD, Harbidge DG, Billings S, Marcus DC. Loss of cochlear HCO$_3^-$ secretion causes deafness via endolymphatic acidification and inhibition of Ca$^{2+}$ reabsorption in a Pendred syndrome mouse model. Am J Physiol Renal Physiol 292: F1345–F1353, 2007. First published February 13, 2007; doi:10.1152/ajprenal.00487.2006.— Pendred syndrome, characterized by childhood deafness and postpuberty goiter, is caused by mutations of $SLC26A4$, which codes for the anion exchanger pendrin. The goal of the present study was to determine how loss of pendrin leads to hair cell degeneration and deafness. We evaluated pendrin function by ratiometric microfluorometry, hearing by auditory brain stem recordings, and expression of K$^+$ and Ca$^{2+}$ channels by confocal immunohistochemistry. Cochlear pH and Ca$^{2+}$ concentrations and endocochlear potential (EP) were measured with double-barreled ion-selective microelectrodes. Pendrin in the cochlea was characterized as a formate-permeable and DIDS-sensitive anion exchanger that is likely to mediate HCO$_3^-$ secretion into endolymph. Hence endolymph in $Slc26a4^{-/-}$ mice was more alkaline than perilymph, and the loss of pendrin in $Slc26a4^{-/-}$ mice led to anacidification of endolymph. The stria vascularis of $Slc26a4^{-/-}$ mice expressed the K$^+$ channel $Kcnj10$ and generated a small endocochlear potential before the normal onset of hearing at postnatal day 12. This small potential and the expression of $Kcnj10$ were lost during further development, and $Slc26a4^{-/-}$ mice did not acquire hearing. Endolymphatic acidification may be responsible for inhibition of Ca$^{2+}$ reabsorption from endolymph via the acid-sensitive epithelial Ca$^{2+}$ channels $Trpv5$ and $Trpv6$. Hence the endolymphatic Ca$^{2+}$ concentration was found elevated in $Slc26a4^{-/-}$ mice. This elevation may inhibit sensory transduction necessary for hearing and promote the degeneration of the sensory hair cells. Degeneration of the hair cells closes a window of opportunity to restore the normal development of hearing in $Slc26a4^{-/-}$ mice and possibly human patients suffering from Pendred syndrome.

pendrin; stria vascularis; $Slc26a4$; $Kcnj10$; $Trpv5$

**Pendred Syndrome** is an autosomal-recessive disorder caused by mutations of the $SLC26A4$ gene that codes for the protein pendrin (10). Childhood deafness, postpuberty goiter, and an enlarged endolymphatic duct are the hallmarks of Pendred syndrome (12, 28, 30, 32). Although deafness is generally profound, it is variable and sometimes late in onset (6, 41). Studies of Pendred syndrome have recently been facilitated by the development of an animal model ($Slc26a4^{-/-}$ mice) and pendrin-specific polyclonal antibodies (9, 33). Expression has been found in the inner ear and thyroid gland consistent with the clinical manifestations of deafness and goiter (10, 11, 32, 44). In addition, pendrin expression has been found in the kidney (33), mammary gland (31), uterus (39), testes (20), and placenta (2). No expression was found in fetal or adult brain, consistent with a peripheral cause of deafness (10, 39).

Pendred belongs to the gene family $SLC26A4$, which contains sulfate transporters, and therefore was initially thought to be a transporter for sulfate (10). Subsequent expression studies in *Xenopus laevis* oocytes, S9 insect, and HEK-293 cells have shown that pendrin functions as an exchanger that transports anions such as Cl$^-$, I$^-$, HCO$_3^-$, and formate but not sulfate or oxalate (34–36). Functional studies in native tissues have only been performed in the murine renal collecting duct, where pendrin is expressed in $\beta$- and non-$\alpha$, non-$\beta$-intercalated cells (33). Isolated, perfused collecting ducts from NaHCO$_3^-$-fed, deoxycorticosterone-treated $Slc26a4^{-/-}$ mice reabsorbed HCO$_3^-$, whereas ducts from similarly treated $Slc26a4^{-/-}$ mice secreted HCO$_3^-$, suggesting that pendrin is involved in HCO$_3^-$ secretion (33). It is conceivable that pendrin is involved in HCO$_3$ secretion in the inner ear. Evidence for HCO$_3$ secretion into endolymph comes from the observations that endolymphatic pH and HCO$_3$ concentrations are relatively high (15, 16). If pendrin would be involved in HCO$_3$ secretion, it would be expected that loss of pendrin leads to an acidification of pH. The first aim of the present study was to determine whether pendrin in the inner ear is functional and whether loss of pendrin affects endolymphatic pH.

Pendred syndrome can be associated with deafness at birth or with progressive hearing loss during childhood (6, 41). This implies that hearing develops in at least some cases but is lost during childhood. Compared with humans, the life span of mice is much compressed. Furthermore, mice are born developmentally more immature than humans. Degeneration of the organ of Corti was found in $Slc26a4^{-/-}$ mice to begin around the time of the normal onset of hearing. Adult mice were found deaf due to a loss of $Kcnj10$ protein expression in the stria vascularis and an ensuing loss of the endocochlear potential, which is the major driving force for sensory transduction (9, 44). The stria vascularis of adult mice was found to degenerate, and this degeneration was found to be associated with an invasion of macrophages (18). Taken together, these findings raised the question of whether $Kcnj10$ expression and endocochlear potential are first developed and then lost, which implies the existence of a window of opportunity to intervene

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and prevent loss of function. Alternatively, it was conceivable that *Kcnj10* expression and the endocochlear potential failed to develop, which may point to a prenatal defect that would be difficult to correct during postnatal life. Thus the second aim of the present study was to determine whether *Slc26a4/−/−* mice develop hearing, express *Kcnj10* in the stria vascularis, and generate an endocochlear potential.

Endolymphatic Ca\(^{2+}\) concentration is unusually low for an extracellular fluid, which is critically important for sensory transduction. Reabsorption of Ca\(^{2+}\) from vestibular endolymph has recently been shown to involve the epithelial Ca\(^{2+}\) channels *Trpv5* and *Trpv6* (*Trpv5* synonyms: ECaCl and CaT2; *Trpv6* synonyms: ECaC2, CaT1) (46). The observations that *Trpv5* and *Trpv6* are inhibited by an acidification of extracellular pH (29, 42) and that transepithelial Ca\(^{2+}\) absorption in *Trpv5*- and *Trpv6*-expressing rat semicircular canal epithelium was inhibited by extracellular acidification (24) raised the hypothesis that loss of pendrin leads to inhibition of sensory transduction via inhibition of Ca\(^{2+}\) absorption and an increase in endolymphatic Ca\(^{2+}\) concentration. Thus the third aim of the present study was to determine whether *Trpv5* and *Trpv6* channel proteins are expressed in the cochlea and whether loss of pendrin leads to a change in endolymphatic Ca\(^{2+}\) concentration that may present a key event in the etiology of deafness.

**METHODS**

**Animal use.** Mongolian gerbils were obtained from a commercial source (Charles River, Wilmington, MA) and housed at Kansas State University (KSU). Adult *Slc26a4/−/−* and *Slc26a4/+/+* mice were obtained either from the colony of Dr. Susan Wall (Emory University) or from a colony at KSU that was established with breeders kindly provided by Dr. Wall. Preweaning mice and *Slc26a4/−/−* mice were solely obtained from the KSU colony. Gerbils were deeply anesthetized with pentobarbital sodium (100 mg/kg ip) and killed by decapitation. Preweaning mice were deeply anesthetized with 4% tribromoethanol (0.013 ml/g body wt ip). Adult mice were deeply anesthetized either with 4% tribromoethanol (0.014 ml/g body wt ip) or with pentobarbital sodium (100 mg/kg body wt ip) and killed by decapitation or transcardial perfusion. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Kansas State University.

**Ratiometric pH measurements.** Temporal bones were extracted from gerbils after death, and lateral wall tissues were obtained by microdissection in Cl\(^{−}\)-free solution. Cl\(^{−}\)-free solution contained (in mM) 150 Na-glucurate, 1.6 K\(_2\)HPO\(_4\), 0.4 KH\(_2\)PO\(_4\), 4 Ca-glucurate, 1 Mg\(_2\)SO\(_4\), and 5 glucose, pH 7.4. Great care was taken to not strip stria vascularis from the underlying spiral ligament. Lateral wall tissues were loaded with BCECF by incubation with 5 μM BCECF-AM for 45 min at 37°C. Tissues were mounted with fine glass needles in a bath chamber installed on the stage of a confocal microscope (PASCAL 5, Carl Zeiss, Jena). For optimal fluorescence imaging, the epithelial side of the tissue faced toward the coverslip. Tissues were initially superfused with 150 Cl\(^{−}\) solution that contained (in mM) 150 NaCl, 3.5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), and 5 glucose, pH 7.4. Cl\(^{−}\) steps from 150 to 15 mM were performed by replacement of 135 mM NaCl with an equimolar amount of Na-glucurate, while increasing the amounts of CaCl\(_2\) and MgCl\(_2\) from 1 to 4 mM to compensate for chelation. Formate (10 mM) and DIDS (1 mM, predissolved in DMSO) were simply added to solutions. Fluorescence originating from surface epithelial cells in the spiral prominence region was recorded without interference of underlying connective tissue or capillary networks. Contributions of connective tissue were excluded since connective tissue cells did not load BCECF under the chosen conditions. Furthermore, contributions of capillary endothelial cells that loaded BCECF were avoided by choice of an appropriate optical section. Fluorescence was imaged in an alternating manner in response to 458- and 488-nm excitation using the same detector operated at a fixed gain, offset, and amplification. The fluorescence ratio was converted to pH according to a calibration performed in droplets of BCECF acid dissolved in HEPES-buffered solutions of varying pH (Fig. 1).

**Electrophysiological pH and Ca\(^{2+}\) measurements.** The endolymphatic pH and Ca\(^{2+}\) concentrations and the endocochlear potential were measured in situ with double-barreled microelectrodes. Procedures were developed by modifying previously described protocols (21). Measurements were made in the basal turn of the cochlea by a round-window approach through the basilar membrane of the first turn. After electrodes were placed in the perilymph, the surgical cavity was covered with liquid Sylgard 184 (Dow Corning, Midland, MI). This maneuver was designed to prevent the measurement of artificially elevated perilymphatic pH values due to the loss of tissue CO\(_2\) into ambient air. Indeed, perilymph values in a preliminary series of experiments that lacked this precaution were significantly higher (7.67 ± 0.04, n = 8 vs. 7.33 ± 0.04, n = 11).

Calibration consisted of taking a reference value and obtaining the slope of the electrode in an agar cup in situ. This method was devised to minimize the contribution of electrode drift and differences between reference electrodes. After cochlear measurements of endolymph and perilymph, the surgical cavity was flooded with pH 7 or 1 mM Ca\(^{2+}\) calibration solutions and the electrode was lifted through the Sylgard layer to rapidly obtain a reference measurement. Subsequently, the slope of the electrode was obtained by placing an agar cup on the exposed neck muscles and connecting it to three inflow lines for calibration solutions and one suction line. The electrode was moved into the cup, and the tip was perfused with calibration solutions (1 min/solution). Agar cups holding ~100 μl were prepared with weakly buffered Ringer solution. Slopes of Ca\(^{2+}\) - and pH-sensitive electrodes were 25.0 ± 0.6 mV/decade concentration (*n* = 19) and 57.1 ± 0.3 mV/pH unit (*n* = 24), respectively.

Double-barreled glass microelectrodes were manufactured from filament-containing glass tubing (1B100F-4, World Precision Instruments, Sarasota, FL) using a micropipette puller (Narishige PD-5, Tokyo, Japan). Before silanization, microelectrodes were baked at 180°C for 2 h to ensure dryness. The longer ion-selective barrel was mounted in the lid of a beaker. The beaker was heated to 210°C and silanized by a 90-s exposure to 0.08 ml dimethyldichlorosilane (Fluka 40136) at room temperature. The shorter reference barrel was protected from silanization by sealing the open end with Parafilm (Alcan Packaging, Chicago, IL). After silanization, microelectrodes were baked at 180°C for 3 h and tips were broken to a final outer diameter of ~3 μm. For pH electrodes, the reference barrel was filled with 1 M KCl and the...
Table 1. Analysis of blood plasma from Slc26a4+/+ and Slc26a4−/− mice

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<th>Na, mmol/l</th>
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<th>Lactate, mmol/l</th>
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<td>(n = 7)</td>
<td>7.18±0.02</td>
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<td>117.1±1.6</td>
<td>2.6±0.5</td>
<td>145±24</td>
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<td>317±3</td>
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<td>(n = 6)</td>
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<td>160.2±1.9</td>
<td>5.1±0.3</td>
<td>116.3±2.6</td>
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When expressed in heterologous expression systems, pendrin has been shown to mediate formate-enhanced and DIDS-sensitive cytosolic alkalinizations in response to reductions in the extracellular Cl\textsuperscript{−} concentration (34, 36). Similar protocols were used here to evaluate whether pendrin expressed in the cochlea is functional. Spiral prominence regions of the gerbil cochlea were isolated, and surface epithelial cells were loaded with BCECF (Fig. 2). Reductions in extracellular Cl\textsuperscript{−} concentration from 150 to 15 mM in the absence of formate resulted in a cytosolic alkalinization by 0.13 ± 0.02 pH units (29 cells in 8 preparations). Addition of 1 or 10 mM formate resulted in an acidification by 0.03 ± 0.01 (9 cells in 2 preparations) and 0.12 ± 0.02 pH units (14 cells in 4 preparations), respectively. Addition of 1 mM DIDS had no significant effect (−0.03 ± 0.02 pH units, 13 cells in 2 preparations). Paired experiments revealed that Cl\textsuperscript{−}-induced alkalinizations were enhanced by 10 mM formate and inhibited by 1 mM DIDS. These observations are consistent with the presence of functional pendrin protein in the cochlea.

*Mice lacking pendrin do not develop hearing.* Inner and outer hair cells in the organ of Corti have been shown to develop normally in *Slc26a4*\textsuperscript{−/−} mice but to begin to degenerate between postnatal day 7 (P7) and P15, which encompasses the onset of hearing at P12 (9). These observations raised the hypothesis that *Slc26a4*\textsuperscript{−/−} mice may develop hearing for at least a brief period. Hearing was evaluated by auditory brain stem response thresholds in *Slc26a4*\textsuperscript{−/−} and *Slc26a4*\textsuperscript{+/−} mice. *Slc26a4*\textsuperscript{+/−} mice began hearing at P12, with the threshold improving daily (Fig. 3). In contrast, *Slc26a4*\textsuperscript{−/−} lacked hearing at all ages tested. The equivalency of develop-
Lack of pendrin leads to loss of \( \text{K}^{+} \text{channel} \) \( \text{Kcnj10} \) and endocochlear potential. We have shown previously that adult \( \text{Slc26a4}^{+/+} \) mice lack protein expression of the \( \text{K}^{+} \) channel \( \text{Kcnj10} \) in the stria vascula and consequently do not generate an endocochlear potential (44). The finding raised the question of whether \( \text{Slc26a4}^{-/-} \) mice never express \( \text{Kcnj10} \) in the stria vascula or whether these mice first express \( \text{Kcnj10} \) and then lose expression. This question was addressed by measuring the endocochlear potential and \( \text{Kcnj10} \) expression in stria vascula before and after the onset of hearing. \( \text{Slc26a4}^{-/-} \) mice developed a small endocochlear potential at \( \text{P10} \) that was progressively lost during further development (Fig. 4). Consistent with this observation, \( \text{Kcnj10} \) was expressed at \( \text{P10} \) and was progressively lost during further development (Fig. 5).

Lack of pendrin causes acidification of endolymphatic pH. The observations that pendrin is expressed in the apical membrane of spiral prominence epithelial cells and that pendrin is an anion exchanger that accepts pH equivalents raised the hypothesis that pendrin controls endolymphatic pH. pH was measured with double-barreled ion-selective electrodes in endolymph and perilymph of \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice. Measurements were made before and after the onset of hearing. At all ages, endolymph of \( \text{Slc26a4}^{+-} \) mice was more alkaline than perilymph. In contrast, endolymph of \( \text{Slc26a4}^{-/-} \) mice was more acidic than perilymph (Fig. 6). No difference was found in the pH of perilymph or blood between \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice (Fig. 6, Table 1).

Cochlear epithelial cells express \( \text{Ca}^{2+} \) channels \( \text{Trpv5} \) and \( \text{Trpv6} \). Epithelial \( \text{Ca}^{2+} \) channels \( \text{Trpv5} \) and \( \text{Trpv6} \) have recently been found to contribute to \( \text{Ca}^{2+} \) reabsorption from vestibular endolymph (24, 46). This finding raised the question of whether these channels are also expressed in cochlear epithelial cells and contribute to \( \text{Ca}^{2+} \) absorption in the cochlea. Expression of \( \text{Trpv5} \) was mainly found in marginal cells of stria vascula, and expression of \( \text{Trpv6} \) was found mainly in inner and outer sulcus epithelial cells (Fig. 7). Staining for \( \text{Trpv5} \) and \( \text{Trpv6} \) was similar in \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice.

Lack of pendrin causes elevation of endolymphatic \( \text{Ca}^{2+} \) concentration. \( \text{Ca}^{2+} \) concentration was measured with double-barreled ion-selective electrodes in endolymph and perilymph of \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice before and after the onset of hearing. At \( \text{P10} \), \( \text{Ca}^{2+} \) concentration in endolymph of \( \text{Slc26a4}^{-/-} \) mice was lower than \( \text{Ca}^{2+} \) concentration in perilymph. During further development, endolymphatic \( \text{Ca}^{2+} \) concentration progressively decreased to adult levels (Fig. 6). In contrast, endolymphatic \( \text{Ca}^{2+} \) concentration in \( \text{Slc26a4}^{-/-} \) mice at \( \text{P10} \) was similar to \( \text{Ca}^{2+} \) concentration in perilymph. During further development, endolymphatic \( \text{Ca}^{2+} \) concentration progressively increased. No difference was found in \( \text{Ca}^{2+} \) concentration of perilymph or blood between \( \text{Slc26a4}^{+-} \) or \( \text{Slc26a4}^{-/-} \) mice (Fig. 6, Table 1).

**DISCUSSION**

The most salient findings of the present study are as follows. 1) Pendrin in spiral prominence epithelial cells of the cochlea is a functional anion exchanger. 2) At \( \text{P10} \), before the onset of hearing, \( \text{Slc26a4}^{-/-} \) mice express \( \text{Kcnj10} \) in the stria vascula and generate a small endocochlear potential. During further development, \( \text{Kcnj10} \) expression and endocochlear po-

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**Fig. 3.** Evaluation of hearing in \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice. Hearing thresholds were determined by auditory brain stem recordings using click and tone-burst stimuli at 8, 16, and 32 kHz. A: example of auditory brain stem recordings made in a postnatal day 15 (P15) \( \text{Slc26a4}^{-/-} \) mouse using 16-kHz tone-burst stimuli at different amplitudes. B: data summary. The onset of hearing occurred in \( \text{Slc26a4}^{-/-} \) mice at \( \text{P12} \). Hearing in \( \text{Slc26a4}^{-/-} \) mice improved daily, reaching nearly adult levels by \( \text{P15} \). \( \text{Slc26a4}^{-/-} \) mice did not develop hearing.

**Fig. 4.** Measurements of endocochlear potential (EP) in \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice before and after the onset of hearing. At \( \text{P10} \), \( \text{Slc26a4}^{+-} \) and \( \text{Slc26a4}^{-/-} \) mice generated an endocochlear potential. This potential grew with further development in \( \text{Slc26a4}^{-/-} \) mice but was lost in \( \text{Slc26a4}^{-/-} \) mice. *\( P < 0.05 \).
tential are lost and Slc26a4<sup>−/−</sup> mice fail to develop hearing. 3) Lack of pendrin leads to an acidification and an increase in the Ca<sup>2+</sup> concentration of endolymph. 4) Epithelial cells enclosing endolymph express the acid-sensitive Ca<sup>2+</sup> channels Trpv5 and Trpv6 in the apical membrane.

Pendrin mediates HCO<sub>3</sub><sup>−</sup> secretion into endolymph. The observation that pendrin is a functional anion exchanger and that loss of pendrin leads to an acidification of endolymph suggests that the main anions transported are alkaline equivalents such as HCO<sub>3</sub><sup>−</sup>. HCO<sub>3</sub><sup>−</sup> is a likely substrate for pendrin since the stria vascularis generates CO<sub>2</sub> and the spiral prominence is heavily expressing carbonic anhydrase, which converts CO<sub>2</sub> to HCO<sub>3</sub><sup>−</sup> (14, 26). The stria vascularis is a source of CO<sub>2</sub> because it has a higher metabolic rate than neighboring tissues and because it has a respiratory quotient of 1.2, which means that it generates 1.2 CO<sub>2</sub> for every O<sub>2</sub> molecule consumed (22). In addition, spiral ligament and spiral prominence fibrocytes express Slc4a7, which is a Na<sup>+</sup>/HCO<sub>3</sub><sup>−</sup> cotransporter likely involved in the uptake of HCO<sub>3</sub><sup>−</sup> into cells (3). Consistent with pendrin-mediated HCO<sub>3</sub><sup>−</sup> secretion is the observation...
that the HCO$_3^-$ concentration in endolymph is higher than in perilymph and that inhibition of carbonic anhydrase leads to an acidification of endolymph and a reduction in HCO$_3^-$ (16, 38). Furthermore, acoustic stimulation, which increases metabolism and CO$_2$ production, has been shown to cause an alkalization of endolymph (15).

Coincidentally, pendrin-mediated HCO$_3^-$ secretion has recently been demonstrated in the cortical collecting duct of the kidney (33). A systemic effect as origin of the acidified endolymphic pH, however, is unlikely since no difference in plasma or perilymphatic pH was observed.

In the absence of pendrin, endolymph was more acidic than perilymph. Acid secretion into endolymph, which was uncovered in the absence of pendrin, may occur in several endolymph-facing epithelial cells, including cochlear interdental cells, known to express the subunits E and B1 of the NH+-ATPases in the apical membrane and the Cl-/HCO$_3^-$ exchanger Slc4a2 (AE2) in the basolateral membrane (19, 37). Additional acid secretion sites may include strial marginal cells, which express the E subunit of the NH+ -ATPases in their apical membrane (37).

The possibility that the endocochlear potential would provide a driving force for pH equivalents and that loss of the endocochlear potential in Slc26a4$^{-/-}$ mice would be the sole cause for endolymphatic acidification is unlikely since a similar acidification of endolymph was found in the vestibular labyrinth, although there is no difference in the transepithelial voltage between Slc26a4$^{-/-}$ and Slc26a4$^{-/-}$ mice (24). HCO$_3^-$ secretion into vestibular endolymph is likely mediated by pendrin expressed in vestibular transitional cells (44).

Early loss of Kcnj10 prevents the development of hearing. The endocochlear potential, which is generated by the K$^+$ channel Kcnj10, is crucial for hearing since it drives sensory transduction (23, 43). Adult Slc26a4$^{-/-}$ mice lack Kcnj10 protein expression and fail to generate an endocochlear potential, which is consistent with the fact that adult Slc26a4$^{-/-}$ mice are deaf (9, 44).

In general, mice are born blind and deaf. Eyes and ear canals open at P11, and hearing begins at P12. Expression of the K$^+$ channel Kcnj10 in the stria vasularis begins in mice around P8, and the onset of expression is paralleled by the onset of an endocochlear potential (13). At P10, Slc26a4$^{-/-}$ mice expressed Kcnj10 in the stria vasularis and generated a small endocochlear potential. This finding demonstrates that the stria vasularis of Slc26a4$^{-/-}$ mice can express Kcnj10 protein and can generate an endocochlear potential.

A window of opportunity may exist to prevent loss of Kcnj10 expression and to enable Slc26a4$^{-/-}$ mice to develop a normal endocochlear potential and normal hearing. This narrow window of opportunity appears to close with the degeneration of the organ of Corti that begins between P7 and P15 (9). Pendrin and Kcnj10 are expressed in different cells. The link between loss of pendrin function and loss of Kcnj10 expression remains unclear.

Endolymphatic acidification inhibits Ca$^{2+}$ absorption. Under physiological conditions, endolymphatic Ca$^{2+}$ concentration is 20–30 μM, which is very low for an extracellular compartment (4). Higher or lower concentrations have been shown to suppress transduction currents and microphonic potentials (25, 40). The endolymphatic Ca$^{2+}$ concentration is likely controlled by secretory and absorptive processes. Ca$^{2+}$ secretion may involve basolaterally expressed Ca$^{2+}$ influx mechanisms in conjunction with apically expressed Ca$^{2+}$-ATPases such as PMCA2 (45). Support for active Ca$^{2+}$ secretion into endolymph comes from the finding that pharmacological inhibition of Ca$^{2+}$-ATPases leads to a fall in endolymphatic Ca$^{2+}$ concentration (17) and that deaf-waddler mice, which bear a loss-of-function mutation in the apically expressed Ca$^{2+}$-ATPase PMCA2, have a very low endolymphatic Ca$^{2+}$ concentration (45).
Reabsorption of Ca\(^{2+}\) may entail apically expressed Ca\(^{2+}\) channels Trpv5 and Trpv6 in conjunction with basolaterally expressed Ca\(^{2+}\) -ATPases and Na\(^+\)/Ca\(^{2+}\) exchangers (27, 45, 46). The finding that Trpv5 is expressed in the apical membrane of stria marginal cells in conjunction with the finding of the Ca\(^{2+}\) -ATPase PMCA1 in their basolateral membrane (45) suggests that the stria vascularis is involved in Ca\(^{2+}\) reabsorption. Expression of Trpv5 and Trpv6 is of great interest in view that these channels are inhibited by extracellular acidification (29, 42). Endolymphatic acidification may inhibit Ca\(^{2+}\) absorption and lead to the observed elevation in endolymphatic Ca\(^{2+}\) concentration. The majority of Ca\(^{2+}\) that enters the hair cell bundle through the transduction channel under physiological conditions is extruded by Ca\(^{2+}\) -ATPases that are expressed in the hair cell bundle (47). Although Ca\(^{2+}\) is necessary to maintain the sensitivity of the bundle through adaptation and motility (1, 5, 8), an entry of excess Ca\(^{2+}\) into the sensory cells leads to Ca\(^{2+}\) overloading and cell death. The capacity of cochlear hair cells to extrude Ca\(^{2+}\) may be limited, especially in outer hair cells that appear to not express plasma membrane Ca\(^{2+}\) -ATPases in their basolateral membrane (7). Ca\(^{2+}\) over-load may be the cause of cellular degeneration that was observed to begin with outer hair cells between P7 and P15 (9).

In conclusion, our data demonstrate that pendrin is a functional formate-permeable and DIDS-sensitive anion exchanger that likely mediates HCO\(_3\) secretion into endolymph. Hence endolymph is alkaline, and loss of the pendrin leads to acidification. Endolymphatic acidification may be responsible for inhibition of Ca\(^{2+}\) reabsorption via the acid-sensitive Ca\(^{2+}\) channels Trpv5 and Trpv6. Failure to lower endolymphic Ca\(^{2+}\) may inhibit sensory transduction necessary for hearing and promote the degeneration of the sensory cells. Degeneration of the sensory cells closes a window of opportunity to restore the normal development of hearing in Slec26a4\(^{-/-}\) mice and possibly human patients suffering from Pendred syndrome.

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