

rapid communication

KCNJ10 (Kir4.1) potassium channel knockout abolishes endocochlear potential

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Marcus, Daniel C., Tao Wu, Philine Wangemann, and Paulo Kofuji. KCNJ10 (Kir4.1) potassium channel knockout abolishes the endocochlear potential. *Am J Physiol Cell Physiol* 282: C403–C407, 2002; 10.1152/ajpcell.00312.2001.—Stria vascularis of the cochlea generates the endocochlear potential and secretes K⁺. K⁺ is the main charge carrier and the endocochlear potential the main driving force for the sensory transduction that leads to hearing. Stria vascularis consists of two barriers, marginal cells that secrete potassium and basal cells that are coupled via gap junctions to intermediate cells. Mice lacking the KCNJ10 (Kir4.1) K⁺ channel in strial intermediate cells did not generate an endocochlear potential. Endolymph volume and K⁺ concentration ([K⁺]) were reduced. These studies establish that the KCNJ10 K⁺ channel provides the molecular mechanism for generation of the endocochlear potential in concert with other transport pathways that establish the [K⁺] difference across the channel. KCNJ10 is also a limiting pathway for K⁺ secretion.

stria vascularis; null mutation; mouse; transepithelial potential; potassium secretion

STRIA VASCULARIS is a highly vascularized multilayered epithelium adjacent to the spiral ligament in the lateral wall of the cochlea and consists of two epithelial barriers, marginal cells and basal cells (Fig. 1; Ref. 9). Strial marginal cells secrete K⁺ into endolymph by an active mechanism, whereas strial basal cells are associated with intermediate cells inside stria vascularis and with fibrocytes outside in the spiral ligament. Stria vascularis has long been recognized to be the motor of K⁺ cycling (14, 29) in the cochlea and the origin of the endocochlear potential (EP) (26). The EP of about +80 mV in conjunction with the high K⁺ concentration ([K⁺]) of endolymph of about 150 mM drives the sensory transduction in the hair cells that leads to hearing (4). The molecular mechanisms of K⁺ secretion in strial marginal cells have been unambiguously determined

(3, 21, 29), whereas the molecular mechanisms that lead to the generation of the EP have remained elusive.

Several lines of evidence have led to the hypothesis that KCNJ10 (Kir4.1) K⁺ channels in intermediate cells generate the EP. First, strial marginal cells that had earlier been suspected to generate the EP were found to secrete K⁺ by a mechanism incompatible with EP generation (21, 29). In light of the similarity between strial marginal cells and vestibular dark cells (28), it became clear that strial marginal cells play an indirect role in the generation of the EP by establishing the low [K⁺] in the intrastrial spaces (24). Second, the EP was found to be generated across the basal cell barrier rather than across the marginal cell barrier (20). Third, intermediate cells were found to be connected to basal cells via a high density of gap junctions such that the membranes of intermediate cells could functionally be a part of the basal cell barrier (12). This finding opened the possibility that intermediate cells harbor the molecular mechanism that generates the EP. Fourth, intermediate cells were found to play a significant role because the EP was found to be absent in mutant mice lacking these cells (2). Finally, the EP was found to be sensitive to K⁺ channel blockers, suggesting that a K⁺ channel delivers K⁺ to strial marginal cells and contributes to EP generation (6, 16, 24).

Previous studies suggested that the KCNJ10 (Kir4.1) K⁺ channel could provide the requisite K⁺ selectivity at the basal/intermediate cell inner barrier. Transcripts for KCNJ10 were found in the stria vascularis, and immunostaining of KCNJ10 was found on cell membranes within the stria vascularis that developed in parallel with the EP during the second week after birth (6). Immunostaining of individually isolated cells from the stria vascularis (1) rigorously demonstrated that KCNJ10 was expressed in the membrane of intermediate cells and not in the basolateral mem-

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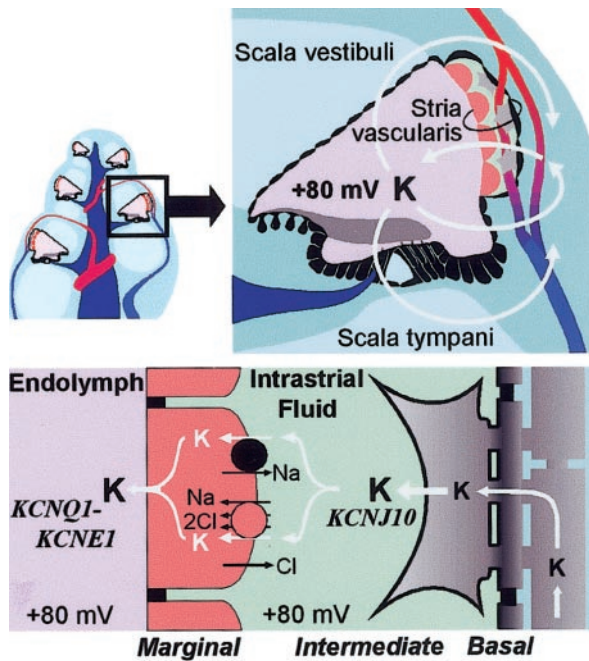


Fig. 1. Cross section of whole cochlea, single turn, and cell model of stria vascularis. The basal cells and intermediate cells are coupled via a high density of gap junctions; their intracellular potential with respect to perilymph is about -10 mV. The intrastrial space is at $+80$ mV or more because of the high density of KCNJ10 channels in the intermediate cell membrane and the large K^+ concentration ($[K^+]$) difference between intermediate cell cytosol and the intrastrial space. This voltage is the primary contribution to the endocochlear potential (EP). The marginal cell layer contributes to generating the EP by keeping the intrastrial $[K^+]$ low.

brane of marginal cells, as earlier reported (6). The localization of KCNJ10 to the intermediate cell membrane was recently confirmed (19, 25). A comparison of a drug sensitivity profile between the EP and the membrane voltage of isolated intermediate cells supported the hypothesis that KCNJ10 generates the EP (6, 24). The force of this conclusion, however, was reduced by the relatively low specificity of the drugs used and the presence of another K^+ channel type in intermediate cells (23).

We tested the hypothesis that the KCNJ10 K^+ channel generates the EP and delivers K^+ to the marginal cells by using mutant mice that lack this channel. If the KCNJ10 K^+ channel were essential for the EP and for K^+ delivery, it would be expected that mice lacking this channel would not generate an EP and could not secrete K^+ into endolymph, which should result in a collapsed endolymphatic space. Thus we measured the EP and the endolymphatic $[K^+]$ and prepared histological sections from homozygous ($-/-$) mutant mice with wild-type ($+/+$) and heterozygous ($+/-$) littermates serving as controls.

METHODS

The KCNJ10 mutant mice were produced and genotyped as described previously (13). The mutation consisted of replacement of most of the coding exon by a neomycin resistance gene. The absence of KCNJ10 gene expression in ho-

mozygous knockout mice was confirmed by immunolabeling of the inner ear (Neusch C, Rozengurt N, Kofuji P, and Lester H; personal communication).

Mice were used at an age of 17–21 days, when the auditory system is normally mature (18). Mice were anesthetized with inactin (thiobutobarbital sodium salt, 140 mg/kg ip; T-133, Sigma). The Institutional Animal Care and Use Committee of Kansas State University approved all experimental protocols. The EP and the endolymphatic $[K^+]$ were measured with double-barreled microelectrodes as described previously (16, 17). Measurements were made in the second turn after thinning the bone over the stria vascularis and picking a small hole (~ 30 μ m). K^+ -selective electrodes were calibrated in solutions of constant cation (K^+ and Na^+) concentration of 150 mM. The minor selectivity of the K^+ electrodes for Na^+ produced a nonlinearity in the calibration curve, which was closely fit by the Nicolski equation with nonlinear curve-fitting software (Originlab version 6.1): $V = V_i + S \times \log([K^+] + A \times [Na^+])$, where V_i is an offset term, S is slope, A is selectivity, and $[Na^+]$ is Na^+ concentration. Calibrations were made immediately after withdrawal of the electrodes from the cochlea.

The transepithelial utricular potential (UP) and $[K^+]$ were measured with techniques similar to those described above for the cochlea. The utricle was approached by removal of the stapes from the oval window.

For histological sections, temporal bones were removed after anesthesia, perfused with 10% formalin in phosphate-buffered saline through the oval and round windows, and postfixed in the same fixative at 4° C. The temporal bones were then decalcified in Cal-EX II (CS511-1D, Fisher Scientific), dehydrated in graded alcohol, embedded in paraffin, sectioned at 5- μ m thickness, and stained with hematoxylin and eosin. Images of sections were made with a laser scanning microscope (Pascal System 5; Zeiss).

RESULTS AND DISCUSSION

In homozygous mutant mice the EP was abolished, consistent with an essential role of the KCNJ10 K^+ channel (Fig. 2). The absence of an EP was associated with greatly compromised hearing as determined by the absence of a Preyer reflex, which is consistent with an auditory brain stem response threshold >81 dB (10). The endolymphatic $[K^+]$ was greatly reduced and

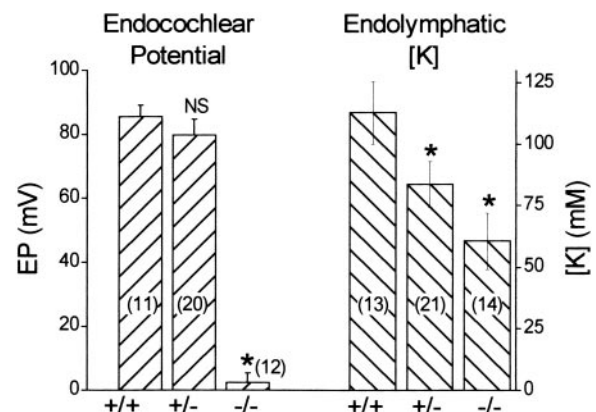


Fig. 2. Effects of mutation of KCNJ10 gene on EP and endolymphatic $[K^+]$. *Left*: EP of wild-type ($+/+$), heterozygous ($+/-$), and homozygous ($-/-$) mice. *Right*: cochlear endolymphatic $[K^+]$ of wild-type ($+/+$), heterozygous ($+/-$), and homozygous ($-/-$) mice. * $P < 0.05$; NS, not significant. No. of measurements in parentheses.

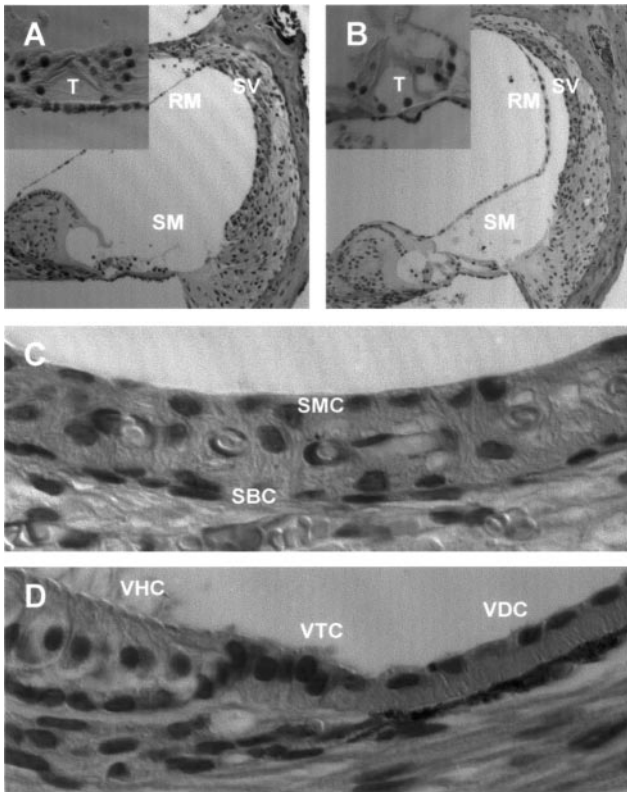


Fig. 3. Histological observations of inner ears from wild-type (+/+) and homozygous (-/-) mice. **A:** cross section of cochlear duct from wild-type mouse has normal position of Reissner's membrane (RM) giving a full cochlear lumen (scala media; SM) and normal thickness of stria vascularis (SV). *Inset:* tunnel (T) of Corti. **B:** cross section of cochlear duct from homozygous (-/-) KCNJ10 mutant mouse has partially collapsed position of Reissner's membrane leading to a reduced cochlear lumen. *Inset:* tunnel (T) of Corti. **C:** stria vascularis of homozygous mouse. Strial marginal cells (SMC) and strial basal cells (SBC) form the 2 barriers to diffusion between the intrastrial space and the external fluid spaces. The stria appears normal at the light microscopic level. **D:** cells of a vestibular ampulla from a homozygous mouse. The vestibular hair cells (VHC), vestibular transitional cells (VTC), and vestibular dark cells (VDC) appear normal at the light microscopic level.

the endolymphatic space was partially collapsed (Fig. 3), consistent with an important, but not exclusive, role of the KCNJ10 K^+ channel in delivery of K^+ to the marginal cells. In wild-type mice, both the EP and the endolymphatic $[K^+]$ were normal (85 mV, 113 mM). The EP was similar in heterozygous mutant mice, whereas the $[K^+]$ was intermediate between values found in wild-type and homozygous mice. The endolymphatic space in wild-type mice had its normal expanse. Partial collapse was measured as the maximum displacement of Reissner's membrane from a straight line between the points of attachment at the lateral wall and spiral limbus. This displacement in the first cochlear turn was not significant in wild-type and heterozygous mice but was large in homozygous mice: -1.4 ± 2.0 , 1.4 ± 1.5 , and $183 \pm 2.8 \mu\text{m}$ ($n = 3$, 3, and 2 ears, respectively; 3 or 4 sections per ear).

The conclusion that the KCNJ10 K^+ channel is important but not essential for K^+ delivery to the mar-

ginal cells suggests that other sources of K^+ may exist. The hypothesis that absence of K^+ delivery to marginal cells would lead to a collapse of the endolymphatic space was based on the finding that such a collapse occurred when K^+ uptake into marginal cells was abolished in mice lacking the SLC12A2 Na-K-2Cl cotransporter (5). A similar collapse was observed when K^+ secretion across the apical membrane of marginal cells was abolished in mice lacking either subunit of the apical KCNQ1-KCNE1 K^+ channel (15, 27).

The absence of an EP was not due merely to degeneration of the intermediate cells, because the thickness of the stria vascularis in the first cochlear turn was the same in wild-type, heterozygous, and homozygous mice (Fig. 3): 20.5 ± 1.1 , 20.1 ± 0.3 , and $19.9 \pm 1.1 \mu\text{m}$, respectively ($n = 3$ ears; 4 sections per ear). In contrast, mutant mice (Wv/Wv) in which the intermediate cells are missing and in which there is no EP were reported to have a significantly reduced strial thickness (2, 22).

KCNJ10 is predominantly expressed in glial cells and apparently acts to take up K^+ from the extracellular space during neuronal activity (7, 8). The question arises as to how an "inward rectifier" channel can contribute to an outward K^+ flux from the intermediate cells. Although the channels are moderately rectifying, they possess a significant conductivity in the outward direction (8) that could account for the efflux. On the other hand, the rectification may provide a check on the limit of efflux of K^+ from intermediate cells. If the efflux exceeded the putative supply from the gap junctions with the basal cells, it would be expected that the cytosolic $[K^+]$ in intermediate cells would decrease and the EP would be compromised by a decrease in the electromotive force produced across the KCNJ10 channels.

As shown in Figs. 2 and 3, mice lacking the KCNJ10 K^+ channel did not generate an EP and had a reduced endolymphatic $[K^+]$ and volume. It remained a question whether these effects on the cochlea were strictly due to the absence of the KCNJ10 K^+ channel from

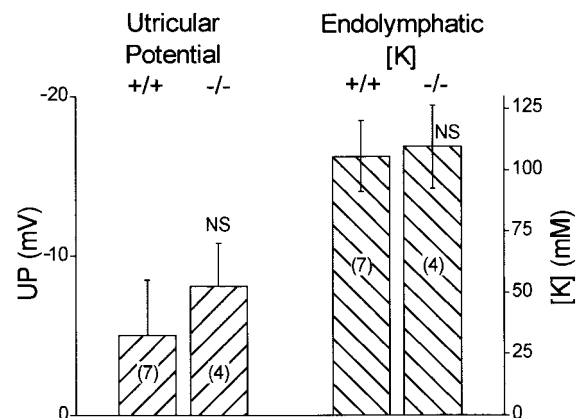


Fig. 4. Effects of mutation of KCNJ10 gene on utricular potential (UP) and endolymphatic $[K^+]$. **Left:** UP of wild-type (+/+) and homozygous (-/-) utricle. **Right:** endolymphatic $[K^+]$ of wild-type (+/+) and homozygous (-/-) utricle. No. of measurements in parentheses.

strial intermediate cells or whether systemic disturbances contributed to these observations. Such disturbances were expected to affect both the cochlea and the vestibular labyrinth. Vestibular endolymph has a high $[K^+]$ similar to that of cochlear endolymph and is produced by vestibular dark cells that transport K^+ by mechanisms similar to those in strial marginal cells (28). Unlike strial marginal cells in the cochlea, however, vestibular dark cells are not associated with an equivalence of strial intermediate and basal cells and the KCNJ10 K^+ channel is not expressed in the vestibular labyrinth (6). Consistent with this, the trans-epithelial potential in the vestibular labyrinth is close to zero.

If the observed cochlear defects were due solely to the lack of the KCNJ10 K^+ channel in intermediate cells and not to systemic disturbances, it would be expected that the $[K^+]$ in vestibular endolymph in mice lacking the KCNJ10 K^+ channel would be normal. Thus we measured the vestibular endolymphatic $[K^+]$ in the utricle and evaluated histological sections of the vestibular labyrinth. The endolymphatic $[K^+]$ in the utricle of mice lacking the KCNJ10 K^+ channel was 106 mM and was not different from that in wild-type mice (Fig. 4). No collapse of the vestibular labyrinth was observed (data not shown), and the principal cell types of the ampulla (hair cells, transitional cells, and dark cells) appeared normal (Fig. 3). Furthermore, the UP was -8 mV and was not different from that in wild-type mice (Fig. 4). The fact that endolymphatic $[K^+]$ and volume were not changed in the vestibular labyrinth by the mutation argues against the possibility that systemic complications caused the observed effects in the cochlea.

Another potential nonspecific basis of the absence of EP could be a slow-down in cochlear development associated with the genetic mutation. The EP normally does not begin development until after the first postnatal week (18, 30). If the mutation led in some way to a slowing of development such that the 18-day knockout animals were at a stage of inner ear development before the onset of EP, the observed absence of EP in these animals might not be caused by the absence of KCNJ10 but by a more general developmental defect.

We addressed this issue by noting that the tunnel of Corti also develops after the first postnatal week (11). Figure 3 shows that the tunnel in both normal and KCNJ10 mutant mice had a fully developed tunnel space at the time of the electrophysiological measurements. This finding, along with the normal vestibular endolymphatic $[K^+]$, gives confidence to our interpretation that the absence of EP and reduced $[K^+]$ are due specifically to the absence of KCNJ10 K^+ channels in strial intermediate cells.

In conclusion, these observations demonstrate that the KCNJ10 K^+ channel is the transport protein that provides the electromotive force for generation of the EP in concert with other transport pathways that establish the $[K^+]$ difference across the channel. In addition, KCNJ10 is an important mechanism for the delivery of K^+ to the K^+ -secretory strial marginal cells.

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