

Molecular and Physiological Bases of the K⁺ Circulation in the Mammalian Inner Ear

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Endolymph, the extracellular solution in cochlea, contains 150 mM K⁺ and exhibits a potential of approximately +80 mV relative to neighboring extracellular spaces. This unique situation, essential for hearing, is maintained by K⁺ circulation from perilymph to endolymph through the cochlear lateral wall. Recent studies have identified ion-transport molecules involved in the K⁺ circulation and their pathophysiological relevance.

It is well known to all physiologists that sounds from the outside world conduct through the external and middle ear and then stimulate the cochlea of the inner ear, a specialized peripheral organ in auditory system. It is also schoolbook knowledge that the cochlea transforms the mechanical energy of sounds to electrical signals via hair cells, the primary receptors for audition on the basilar membrane (FIGURE 1A), and transmits the signals to the brain. It is far less appreciated that the sensation of sounds relies on a complex system of ion-transport processes across multiple cell layers within the cochlea, arguably the most complex and intricate transcellular ion-transport process of the human body. It is those processes that are the subject of this short review.

The cochlea comprises three “tubular” structures, i.e., scala media, scala tympani, and scala vestibuli, and various types of cells such as epithelial cells and fibrocytes (FIGURE 1A). Scala tympani and scala vestibuli are filled with perilymph, whose ionic composition is identical to that of ordinary extracellular solution. On the other hand, the scala media stores another solution, endolymph. The endolymph contains 150 mM K⁺, 2 mM Na⁺, and 20 μM Ca²⁺, and possesses a highly positive potential of approximately +80 mV relative to blood plasma and perilymph, which is called “endocochlear potential” (102, 103). Such a high potential is not observed in any other organ of mammal. Cochlear hair cells bathe their cell bodies in perilymph and expose only their apical membrane, which harbors the hair bundles, to endolymph. The sound-driven vibration of the basilar membrane causes deflection of the bundles of hair cells and opening of mechano-sensitive channels at the top of their cilia. This process allows endolymphatic K⁺ to enter into hair cells, resulting in their excitation (26, 27). By forming a large driving force for K⁺ influx, the endocochlear potential directly contributes to the high sensitivity of hair cells to mechanical stimulation. This potential is therefore essential for hearing.

It is widely accepted that K⁺ is released from hair cells and then circulates from perilymph, rather than from blood, to endolymph through “cochlear lateral wall,” which is made up of two compartments, i.e., the spiral ligament, containing connective tissue and fibrocytes, and an epithelial tissue named stria vascularis

(FIGURE 1A). The “cochlear K⁺ circulation” is considered essential for establishment of high K⁺ concentration in endolymph and the endocochlear potential (36, 109). The concept of the K⁺ circulation was originally proposed from observations that 1) radioactive K⁺ applied to perilymph could be detected in endolymph much more efficiently than when perfused to blood plasma (40, 84) and 2) perilymphatic but not vascular perfusion of K⁺-free solution rapidly and prominently suppressed the endocochlear potential (59, 104).

Five components are considered to mediate cochlear K⁺ circulation (FIGURE 1A): 1) hair cells, which release K⁺ to perilymph via their basolateral K⁺ channels, 2) supporting cells beneath the hair cells, which take up K⁺ and three components in the cochlear lateral wall, i.e., 3) epithelial cells on basilar membrane, 4) spiral ligament, and 5) stria vascularis, through which K⁺ passes. K⁺ is finally excreted to the endolymphatic space, scala media, across the luminal side of stria vascularis. Recent studies have shown that each component expresses a unique set of ion-transport apparatuses and plays a specialized role in cochlear K⁺ circulation. In this review, we discuss the molecular bases, functional control, and pathological relevance of cochlear K⁺ circulation.

Cochlear Hair Cells and Supporting Cells in Organ of Corti

At the basolateral membrane of hair cells, there are two types of K⁺ channels; a voltage-gated K⁺ (K_v) channel KCNQ4, whose mutation in its pore region was found to cause a form of nonsyndromic dominant deafness DFNA2 (42), and a Ca²⁺-activated K⁺ channel (Maxi-K⁺ or BK) containing KCNMA1 subunit (2, 50, 51, 66, 73). Excitation of hair cells opens these channels for repolarization, which releases K⁺ to perilymph (FIGURE 1A). KCNQ4-knockout (KO) mice exhibit the degeneration of outer hair cells and progressive deafness (34).

The K⁺ must be immediately removed from the vicinity of hair cells, otherwise it would continuously depolarize the cells and interfere with normal excitability. In the organ of Corti, Deiters' cells, one type of supporting cells that cup the hair cells, seem to remove K⁺. Deiters' cells express K⁺,Cl⁻ cotransporters

Kcc3 and Kcc4 (5, 6), as well as an inwardly rectifying K⁺ (K_{ir}) channel K_{ir}4.1 (23), and thus would absorb excess extracellular K⁺. Until 2 wk after birth, Kcc4 is also expressed in the cells beside Deiters' cells in the organ of Corti and in stria vascularis. After that, the transporter remains expressed only in Deiters' cells. Kcc4-KO mice consistently start to show hearing impairment at ~3 wk after birth and become deaf at ~4 wk due to degeneration of hair cells (5). This transporter may therefore be crucial for the K⁺ removal by Deiters' cells. Targeted ablation of Kcc3 gene, whose mutation induces human Anderman syndrome exhibiting various neuronal defects (13, 25), causes degeneration of the organ of Corti and progressive deafness in mice (onset: several months to approximately 1 yr after birth) (6) (Table 2). Thus Kcc3 could also be involved in K⁺-uptake process by Deiters' cells. However, because Kcc3 distributes in spiral ligament as well, it is uncertain whether its dysfunction in Deiters' cells is responsible for the hearing disorder.

Epithelial Cells on Basilar Membrane and Spiral Ligament

The supporting cells, the epithelial cells on basilar membrane and the outer sulcus cells in spiral ligament are connected to each other through "gap junctions" at cell-cell contact (20) (FIGURE 1A). Because small molecules of <1 kDa, including ions and metabolites, can freely pass through gap junctions (46, 81), these cells constitute an electrical syncytium called "epithelial gap-junction network" (35) (FIGURE 1A). Cochlea has another syncytium known as "connective-tissue gap-

junction network" that is formed by gap junctions expressed in all the fibrocytes of spiral ligament and in some cells of stria vascularis (see below). It is proposed that these two networks mediate the K⁺ circulation from perilymph to endolymph (35) (FIGURE 1A).

Gap junction is an assembly of two hemi-channels, called "connexons," each of which locates at the lateral membrane of both cells. A connexon is composed of six connexin (Cx) subunits. Gap junctions of both networks express various subunits such as Cx26, Cx30, Cx31, Cx32, and Cx43 (36, 48, 55, 88). Dysfunction of different Cx subunits cause distinct types of hearing disturbances as shown in Table 1. Notably, abnormality of the gene encoding Cx26, a major constituent of cochlear gap-junction networks, is the most frequent genetic cause of deafness (85). Some of the Cx26 mutants impair the gap-junctional transport current in heterologous expression system (62, 111). Cx26-null mice and the transgenic mice overexpressing dominant-negative form of Cx26 had severe hearing loss (7, 43) (Table 2).

The spiral ligament of the cochlear lateral wall contains five distinct types (I-V) of fibrocytes. All of the fibrocytes are bathed in perilymph. Types II and IV fibrocytes, which are characterized by numerous extensions of their plasma membrane (64, 82), are neighbors to outer sulcus cells (Fig. 1A). Because there is no cellular connection between outer sulcus cells and either type II or IV fibrocytes, K⁺ passing through epithelial gap-junction network must be released to the perilymph by outer sulcus cells and then actively taken up by the fibrocytes. Two K⁺-uptake apparatuses, Na⁺-K⁺-ATPase composed of α₁ and β₁ subunits (77, 78) and Na⁺-K⁺-2Cl⁻ cotransporter NKCC1 (8, 75),

Table 1. Genes whose mutations cause hearing loss

Molecules	Distribution	Types of Hereditary Deafness and Other Phenotypes	References
Cx32 (GJB1)	Fibrocytes in LW and Lim; epithelia on BM	X-linked Charcot-Marie-Tooth and deafness	3
Cx26 (GJB2)	Fibrocytes in LW and Lim; epithelia on BM; intermediate cells; basal cells	DFNB1; DFNA3; hereditary palmoplantar keratoderma with deafness	10, 11, 15, 16, 21, 32, 56, 65, 71, 99, 114
Cx30 (GJB6)	Fibrocytes in LW and Lim; supporting cells in organ of Corti	DFNA3	19, 96
Cx31 (GJB3)	Fibrocytes in LW and Lim; supporting cells in organ of Corti; epithelia on BM	DFNA2; AR-nonsyndromic deafness	54, 112
Cx43 (GJA1)	Fibrocytes in LW and Lim; epithelia on BM; intermediate cells; basal cells	AR-nonsyndromic deafness	53
KCNQ4	Outer and inner hair cells	DFNA2	2, 42
Barttin (BSND)	Marginal cells (basolateral); dark cells (basolateral)	Type 4 Bartter's syndrome	4
Isk (min K or KCNE1)	Marginal cells (apical); dark cells (apical)	Jarvell and Lage-Nielsen syndromes	79
KvLQT1 (KCNQ1)	Marginal cells (apical); dark cells (apical)	Jarvell and Lage-Nielsen syndromes	67

BM, basilar membrane; AR, autosomal recessive; Lim, spiral limbus; SL, spiral ligament.

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are abundantly expressed in these particular fibrocytes (FIGURE 1B). Ouabain and furosemide, specific blockers for Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransporter, respectively, suppress the endocochlear potential dramatically when applied to perilymph (41, 44, 45, 47, 59). Therefore, both apparatuses may contribute to generation of the endocochlear potential by facilitating cochlear K⁺ circulation in the ligament (FIGURE 1B). K⁺ taken up by NKCC1 and Na⁺-K⁺-ATPase is then transported to stria vascularis via connective-tissue gap-junction network (FIGURE 1A).

Interestingly, administration of Ba²⁺ and Cs²⁺, blockers for K⁺ channels, to perilymph slightly increases the endocochlear potential (30, 57, 106). This effect is believed to be due to inhibition of K⁺ channels at the basolateral membrane of hair cells (57). However, since both Ba²⁺ and Cs⁺ inhibit K_{ir} channels more selectively and preferentially than K_v channels (70, 93), it is possible that some K_{ir} channels exist in fibrocytes and that the blockers may inhibit them to increase the endocochlear potential. We have recently found that K_{ir}5.1 is strongly expressed in types II, IV, and V fibrocytes of the ligament (22) (FIGURE 1B). Previous immunohistochemical studies suggest that K_{ir}5.1 occurs together with Na⁺-K⁺-ATPase and NKCC1 in these cells (8, 78, 113). Because the membrane potential (E_m) of the fibrocytes is approximately -5 mV (76, 101) and the perilymph surrounding the fibrocytes contains ~5 mM K⁺, the direction of K⁺ flow via K_{ir}5.1

should be outward (FIGURE 1B). K_{ir}5.1 would therefore stimulate Na⁺-K⁺-ATPase and NKCC1 by providing K⁺ for their K⁺ site in the narrow extracellular space surrounded by the infolded plasma membrane of the fibrocytes. The localized K⁺ recycling would attenuate the uptake of K⁺ released from outer sulcus cells by the K⁺-transport apparatuses. Thus the K⁺-recycling mechanism by K_{ir}5.1 could negatively regulate cochlear K⁺ circulation and prevent its overshoot. It is of note that K_{ir}5.1 is slightly expressed on the membrane surface but abundantly distributed in cytoplasm, presumably on the transport vesicles. K_{ir}5.1 may be therefore actively recycled between cytoplasm and membrane surface, and this recycling system could dynamically regulate the activity of K_{ir}5.1 in the fibrocytes, although its regulatory mechanism is still unknown.

In addition, several K⁺ channels such as ether-a-go-go (49, 68), K_v3.1 (80), and BK channels (52) distribute in fibrocytes of the ligaments. The physiological role of these molecules in cochlear K⁺ circulation is, however, still unclarified.

Stria Vascularis and the Possible Mechanism of Formation of the Endocochlear Potential

An early study demonstrated that a mutant guinea pig lacking stria vascularis lost the endocochlear potential (94). This tissue is therefore considered essential for

Table 2. Molecules whose genetic disruption causes hearing impairment in mice

Molecules	Distribution	Phenotypes of KO mice	References
Kcc3	Deiters' cells; types I and III fibrocytes in SL; epithelia on BM	Degeneration of organ of Corti; late onset progressive hearing loss	6
Kcc4	Deiters' cells	Degeneration of hair cells; early onset hearing loss with renal tubular acidosis with motor abnormality and seizures	5
Cx26 (GJB2)	See Table 1	Degeneration of hair cells, hearing loss	7
Cx30 (GJB6)	See Table 1	Loss of EP, hearing loss	96
Kir4.1 (KCNJ10)	Intermediate cells; Deiters' cells	Collapse of SM; deafness	61
NKCC1	Marginal cells; types II, IV, and V fibrocytes in SL; fibrocytes in Lim	Collapse of SM and VES; deafness; balance disorder	9, 12, 17
Claudin-11 (CLDN11)	Basal cells	Loss of EP; deafness	18, 37
KCNQ4	See Table 1	Degeneration of outer hair cells; progressive hearing loss	34
Isk (min K or KCNE1)	See Table 1	Collapse of SM and VES; deafness; balance disorder	100
KvLQT1 (KCNQ1)	See Table 1	Collapse of SM and VES; deafness; balance disorder	

EP, endocochlear potential; SM, scala media; VES, vestibular endolymphatic space.

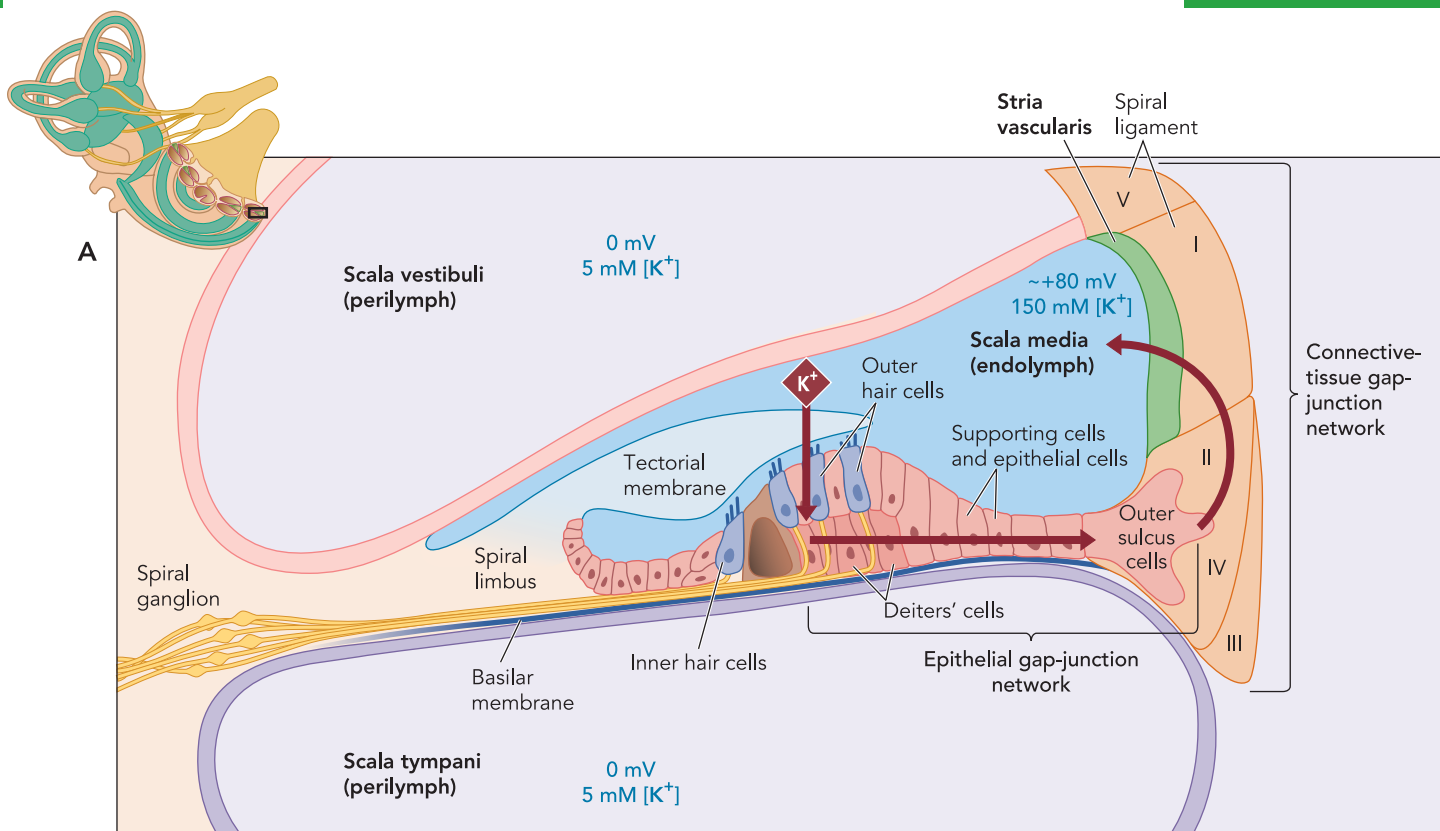


FIGURE 1. Schematic model of cochlear K⁺ circulation and formation of endocochlear potential in the lateral wall

A: K⁺ exiting from the hair cells is picked up by Deiters' cells. K⁺ is then circulated to the type II and IV fibrocytes in spiral ligament through the "epithelial gap-junction network," which is composed of the epithelial and supporting cells (purple) on the basilar membrane (dark gray) and the outer sulcus cells (purple) in the ligament. K⁺ is taken up by the types II and IV fibrocytes in spiral ligament and transported to the stria vascularis via the "connective-tissue gap-junction network" comprising the fibrocytes, basal cells, and intermediate cells (see detail in B). K⁺ is finally released to the endolymph in scala media across the luminal site of stria vascularis. I-V, type I-V fibrocytes. The concentration of K⁺ ([K⁺]) and the potential of each cochlear fluid are indicated. B: ion-transport apparatuses expressed in the stria vascularis and the spiral ligament that participate in cochlear K⁺ circulation. The potential and [K⁺] in each compartment are indicated. The involvement of various apparatuses in establishment of the endocochlear potential and cochlear K⁺ circulation is described in the text. NKCC1, Na⁺-K⁺-2Cl⁻ cotransporter. TJ, tight junctions.

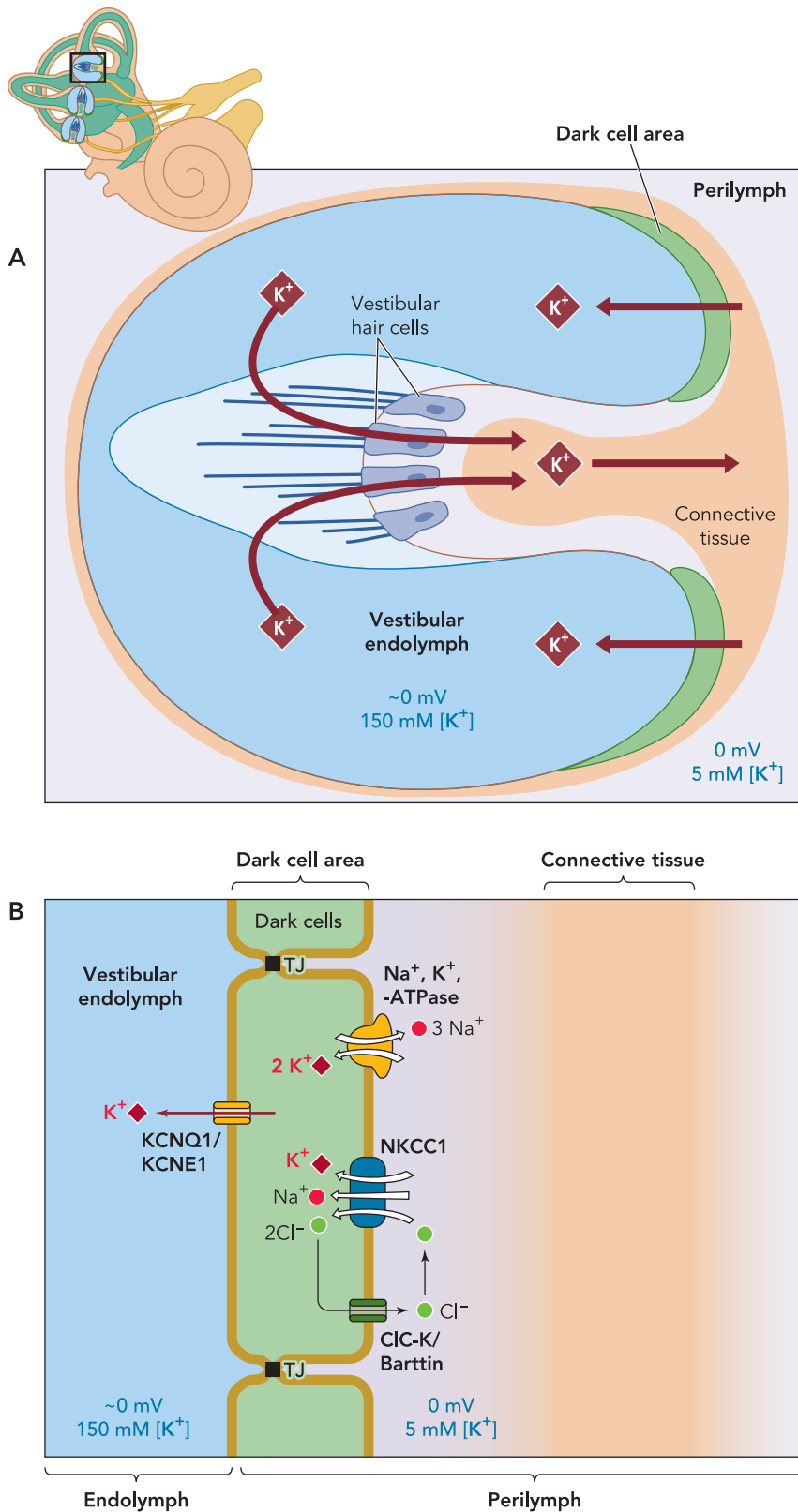


FIGURE 2. Schematic model of the K^+ -transport system in vestibule

A: vestibular dark cell area secretes K^+ from perilymph to endolymph. Vestibular stimuli enter K^+ into the vestibular hair cells (orange) from the endolymph. Note that vestibular endolymph contains 150 mM $[K^+]$ but that its potential is ~ 0 mV. **B:** cells composed of dark cell area and ion-transport apparatuses mediating vestibular K^+ transport. Note that the molecules expressed in dark cells are similar to those in stria vascularis. However, there is neither intermediate cells nor basal cell layer in the vestibule.

formation of the endocochlear potential (109). Stria vascularis contains three types of epithelial cells, i.e., marginal, intermediate, and basal cells. Marginal cells are connected to each other by tight junctions and forms a monolayer epithelium (FIGURE 1B). Basal cells also constitute another layer by tight-junctional connection. Claudin is a major functional constituent of tight junctions, and its ~ 20 isoforms have been identified (98). Marginal cells express claudin-1, -2, -3, -8, -9, -10, -12, and -14, whereas basal cells harbor only claudin-11 (38). Intermediate cells, which are melanocytes, are scattered between these two layers. Abundant capillaries composed of endothelial cells and pericytes penetrate the tissue, the feature from which the name “stria vascularis” originates. Intermediate and basal cells are electrically connected by gap junctions not only to each other but also with the endothelial cells and pericytes. Because basal cells are also connected to fibrocytes via gap junctions, all of these cells are incorporated into connective-tissue gap-junction network. This network transports K^+ from types II and IV fibrocytes to intermediate and basal cells (FIGURE 1B).

Anoxia and application of either ouabain or furosemide to the basilar artery, from which strial capillaries diverge, dramatically reduced the endocochlear potential (39, 47, 105). Importantly, these drugs affect the endocochlear potential much more rapidly when perfused via vascular route than via perilymphatic route (47, 105). Moreover, vascular perfusion of Ba^{2+} largely suppressed the endocochlear potential (23, 60, 91). These data suggest that Na^+ - K^+ -ATPase, NKCC1, and K^+ channels (probably K_{ir} channels) expressed in stria vascularis are more critically involved in generation of the endocochlear potential than those in spiral ligament. It also seems probable that the strial K^+ homeostasis plays the key role in establishment of the endocochlear potential.

Salt et al. (76) measured simultaneously the potential and K^+ concentration in stria vascularis by gradually inserting a double-barreled electrode and found a unique extracellular space sandwiched between the basolateral membrane of marginal cells and the apical membrane of basal cells. This so-called “intrastrial space” is filled with the extracellular fluid whose K^+ concentration is 1–2 mM. The potential of the fluid in intrastrial space is ~ 100 mV more positive than the perilymph (termed “intrastrial potential”) (28, 76) (FIGURE 1B). Notably, intrastrial space is isolated from endolymph in scala media and perilymph in the ligament by marginal and basal cell layers, respectively. Compared with the intrastrial potential, the E_m of marginal cells relative to perilymph and the endocochlear potential are reported to be lower, i.e., +90 to 100 mV and +80 mV, respectively (69, 76). Thus the intrastrial potential must be a source of the endocochlear potential. The E_m of basal cells relative to the perilymph is approximately -5 mV (28, 63, 76). The

potential difference between the E_m of basal cells and the intrastrial potential is close to the equilibrium potential of K^+ (E_K) toward intrastrial space (76). It was therefore hypothesized that 1) the apical membranes of basal cells are highly K^+ permeable so that passive K^+ flow would generate a highly positive intrastrial potential and 2) the basal cell layer acts as the main electrical barrier (76). Later studies identified the gap-junctional connection between basal and intermediate cells (36, 89), and an isolated intermediate cell expressed a large K^+ conductance (90). Accordingly, K^+ diffusion was defined to occur across the apical membrane of intermediate cells. The barrier function of basal cells was confirmed by findings that 1) the potential of the endolymph in vestibular system, which lacks basal cells, is ~ 0 mV (see *Comparison of Ion-Transport Mechanisms Between Cochlear and Vestibular Systems*) and 2) targeted ablation of claudin-11, which destroys tight junctions of basal cells, caused loss of the endocochlear potential in mice (18, 37). One further important factor for formation of the intrastrial potential is the maintenance of low K^+ concentration in intrastrial space, which is essential to promote K^+ flow across the apical membrane of intermediate cells. Indeed, vascular perfusion of high K^+ -containing solution, which might increase K^+ concentration in intrastrial space, suppresses the endocochlear potential (60, 91). The basolateral membrane of marginal cells abundantly expresses Na^+K^+ -ATPase (α_1 plus β_1 or β_2 subunits) and NKCC1 (8, 29, 33, 77, 110) (FIGURE 1B). Notably, this membrane is highly infolded and contains an extremely large number of mitochondria whose metabolic energy may be required to drive the pump. These two apparatuses appear to maintain low K^+ concentration in intrastrial space by taking up K^+ from this space, as required for a large K^+ -dependent potential difference between intrastrial space and intermediate cells. Inhibition of Na^+K^+ -ATPase and NKCC1 by their specific blockers and anoxia-induced inhibition of the pump are all expected to increase K^+ concentration in intrastrial space and thereby reduce the K^+ -diffusion potential with resulting suppression of the intrastrial and endocochlear potentials. Only a small alternation of K^+ concentration in intrastrial space may be enough to decrease the potential, because the space is very narrow (only 15–20 nm in width) (24, 83). In this light, it is not surprising that disruption and mutation of the NKCC1 gene in mice causes collapse of the scala media and deafness (9, 12, 17).

The identity of the strial K^+ channel, essential for generation of the intrastrial potential, has only recently been established by work from our laboratory, showing the K_{ir} channel $K_{ir}4.1$ is abundantly expressed in stria vascularis (23). Because $K_{ir}4.1$ was the only K_{ir} channel found in stria vascularis, we concluded that this channel must be the target of Ba^{2+} inhibition of the endocochlear potential (23). Later, another group determined that $K_{ir}4.1$ is expressed specifically at the apical

membrane of intermediate cells (1) (FIGURE 1B). Further studies have indicated the pivotal role of $K_{ir}4.1$ in formation of the endocochlear potential. Takeuchi et al. (91) revealed by patch-clamp analysis that the isolated intermediate cell expressed abundant K_{ir} current and that its resting E_m was almost identical to E_K . These authors studied the effects of Ba^{2+} in bath solution containing low K^+ concentration, similar to that in intrastrial space, on the E_m of the intermediate cell. The dependence of E_m changes on different concentrations of Ba^{2+} are quite similar to the dependence of decline of the endocochlear potential on the concentration of Ba^{2+}

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perfused into the artery (91). Thus the potential difference across the apical membrane of intermediate cells, which is generated due to expression of the K_{ir} channel, may account for formation of the intrastrial potential that is highly positive relative to the body fluid.

If $K_{ir}4.1$ is the origin of the potential difference across the apical membrane of intermediate cell, then the low K^+ concentration in intrastrial space (1–2 mM) is essential to form the potential difference. The low K^+ concentration in intrastrial space will be dynamically maintained by the balance between the K^+ inflow to intrastrial space via $K_{ir}4.1$ and the K^+ outflow from intrastrial space mediated by the K^+ transport apparatuses, namely Na^+K^+ -ATPase and NKCC1 (FIGURE 1B). Inhibition of these apparatuses may increase K^+ concentration in intrastrial space, which would then reduce the potential difference across the apical membrane of intermediate cells. On the other hand, Ba^{2+} applied to the artery should reach the intrastrial space, inhibit $K_{ir}4.1$ -channel activity, and dramatically reduce the potential difference. Consistently, $K_{ir}4.1$ -KO mice completely lost EP (61) (Table 2). Accordingly, the intrastrial potential can be prominently suppressed by application of either Ba^{2+} or inhibitors for the K^+ -transport apparatuses to the artery, albeit by distinct mechanisms, i.e., inhibition of $K_{ir}4.1$ or augmentation of K^+ concentration in intrastrial space (23).

Transport of ion(s) other than K^+ also seems important for strial K^+ homeostasis. In marginal cells, because NKCC1 unidirectionally transports Na^+ and Cl^- together with K^+ , the former two ions must be supplied to intrastrial space to keep the transporter active. Both Na^+ and Cl^- are enriched in the intrastrial space solution whose ionic composition is the same as that of the ordinary extracellular fluid. Additionally, Na^+ is supplied by Na^+K^+ -ATPase at the basolateral membrane. As for the Cl^- pathway to intrastrial space, marginal cells are found to express a functional Cl^- chan-

nel comprising a pore-forming CLC-K and an accessory barttin subunit (14, 92, 108) (FIGURE 1B). Mutations of the barttin gene causes hereditary hearing disorder accompanied with renal failure, which is known as type 4 Bartter's syndrome (4) (Table 1). These mutants impaired the Cl⁻ current when expressed with CLC-K in *Xenopus* oocytes (14). Therefore, it is likely that, at the basolateral membrane of marginal cells, this Cl⁻ channel dominates in the Cl⁻-recycling pathway to maintain NKCC1 activity.

K⁺ traveling to strial marginal cells is finally excreted to endolymph across their apical membrane (86, 108). A K_v channel containing KCNQ1 (K_vLQT1: α subunit) and KCNE1 (I_{SK} or minK: β subunit) plays this role (14, 74) (FIGURE 1B). Loss of function mutations of either subunit cause Jarvell and Lange-Nielsen syndromes, which exhibit sensorineural deafness as well as QT prolongation in electrocardiogram (31, 67, 79, 97, 107) (Table 1). Both KCNE1- and KCNQ1-null mice show a collapse of scala media and a severe hearing impairment (72, 100) (Table 2).

Comparison of Ion-Transport Mechanisms Between Cochlear and Vestibular Systems

The vestibular labyrinth also stores endolymph. Although vestibular endolymph contains ~150 mM K⁺, its potential relative to body fluid is ~0 mV (58, 108) (FIGURE 2A). Vestibular endolymph is thought to be maintained by "dark cells," the epithelia in ampulla (FIGURE 2A). Thus "vestibular dark cell area" corresponds to "cochlear stria vascularis." Like strial marginal cells, dark cells form a monolayer by tight junctional connection at the boundary between endolymph and perilymph.

Histochemical and electrophysiological studies have identified many similarities between dark cells and marginal cells (FIGURE 2B). Dark cells harbor a large number of mitochondria, Na⁺-K⁺-ATPase, NKCC1, and CLC-K/barttin at their infolded basolateral membrane (9, 14, 17, 58, 95, 110). They also express KCNQ1/KCNE1 at the apical membrane (14). KCNE1-, KCNQ1-, and NKCC1-KO mice exhibit a collapse of vestibular endolymphatic space and balance defect in addition to cochlear dysfunctions (9, 12, 17, 72, 100) (Table 2). Accordingly, like strial marginal cells, vestibular dark cells unidirectionally transport K⁺ from basolateral to apical side.

On the other hand, several prominent differences have been recognized between the two tissues (FIGURE 2B). First, dark cell area has no basal cell layer between the epithelial cells and the vestibular perilymph. Second, the dark cell area lacks intermediate cells and thus the K⁺ channel comparable to K_{ir}4.1. Therefore, the vestibule possesses none of the compartment corresponding to intrastrial space, the K⁺ conductance generating the positive potential, and an electrical barrier.

This highlights essential roles of both the basal cell layer and the intermediate cells expressing K_{ir}4.1 in formation of the endocochlear potential.

Notably, K_{ir}4.1-KO mice retain normal level of the potential and K⁺ concentration in vestibular endolymph (~0 mV and ~140 mM, respectively). The mice, in turn, had ~0 mV of the endocochlear potential and lost ~50% of K⁺ concentration from the cochlear endolymph (61), although both types of endolymph are directly connected. This implies that a mechanism for maintenance of vestibular endolymph is separate from that of cochlear endolymph.

Further Questions and Perspectives

As outlined up to now, highly unique properties of cochlea are clearly implicated for the generation of the endocochlear potential, and many important details of formation of the endocochlear potential and cochlear K⁺ circulation and their pathophysiological relevance remain to be elucidated and/or proved. It remains to be proved, for example, whether K⁺ transport via the epithelial gap-junction network is necessary to maintain the endocochlear potential and high K⁺ concentration in endolymph. Kcc3-KO mice become deaf due to degeneration of hair cells. Their cochlea, therefore, should be unable to transport sufficient K⁺ from hair cells to epithelial gap-junction network, but its endocochlear potential and K⁺ concentration in endolymph are almost normal (6). Dysfunction or defect of Cx26 protein in mice did not affect the endocochlear potential (7, 43). Perilymphatic perfusion of blockers for gap junctions, n-heptanol and hexanol, reduced the endocochlear potential by only ~8 mV (87). The role of the K⁺ transport via the epithelial gap-junction network in the K⁺ circulation should therefore be reevaluated.

It is enigmatic that inhibition of only the Na⁺-K⁺-ATPase expressed in the lateral wall by ouabain completely suppresses the endocochlear potential, because, in turn, the potential can also be totally suppressed by inhibition of only NKCC1 (40, 44, 47, 59, 105). The K⁺ circulation may be achieved by functional coupling between these two apparatuses. To prove this hypothesis, we should evaluate the role of each apparatus in the cochlear system in a quantitative manner by a new technical approach.

Endothelial cells constituting strial capillaries must be extremely specialized, since they expose one membrane side to approximately +100 mV in intrastrial space and the other to 0 mV in blood. This suggests that these cells form a tight barrier and provide a high electrical resistance. However, Ba²⁺ applied to the artery appears to easily penetrate capillaries and directly reach the intrastrial space, because it rapidly and strongly suppresses the endocochlear potential. Vascular perfusion of high K⁺-containing solution decreases the endocochlear potential to some extent,

suggesting that a certain amount of K^+ enters into the intrastrial space from blood. It must be addressed how stria capillaries, while forming high resistance, can allow the flow of ions such as Ba^{2+} and K^+ into intrastrial space that affects the endocochlear potential.

It may not be understandable why cochlear endolymph possesses approximately +80 mV, whereas the vestibular endolymph is at ~0 mV, although both endolymphatic spaces are directly connected by a narrow tunnel. It is speculated that this tunnel acts as a high-resistance passageway between the two spaces, but this hypothesis has not yet been proven. It is clearly necessary to analyze the electrical resistance between the two systems to clarify this point. Furthermore, the mechanisms that maintain high K^+ concentration in endolymph remain enigmatic, although cochlear K^+ circulation seems to be critically involved. Resolution of these enigmas is prerequisite to adequately understanding the role of the K^+ circulation in perception of sounds in cochlea.

Finally, it is firmly established that dysfunction of ion transporters mediating the cochlear K^+ circulation causes hearing impairments. However, we do not know yet the genetic background of many other defects of cochlear and vestibular labyrinth, including Ménière's disease. Further studies of a challenging nature are needed to elucidate details and pathophysiological relevance of cochlear K^+ circulation. ■

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