

How is the highly positive endocochlear potential formed? The specific architecture of the stria vascularis and the roles of the ion-transport apparatus

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Abstract Cochlear endolymph, an extracellular solution containing 150 mM K^+ , exhibits a positive potential of +80 mV. This is called the endocochlear potential (EP) and is essential for audition. The mechanism responsible for formation of the EP has been an enigma for the half century since its first measurement. A key element is the stria vascularis, which displays a characteristic tissue structure and expresses multiple ion-transport apparatus. The stria comprises two epithelial layers: a layer of marginal cells and one composed of intermediate and basal cells. Between the two layers lies an extracellular space termed the intrastrial space (IS), which is thus surrounded by the apical membranes of intermediate cells and the basolateral membranes of marginal cells. The fluid in the IS exhibits a low concentration of K^+ and a positive potential similar to the EP. We have demonstrated that the IS is electrically isolated from the neighboring extracellular fluids, perilymph, and endolymph, which allows the IS to sustain its positive potential. This IS potential is generated by K^+ diffusion across the apical membranes of intermediate cells, where inwardly rectifying Kir4.1 channels are localized. The low K^+ concentration in the IS, which is mandatory for the large K^+ -diffusion potential, is maintained by Na^+, K^+ -

ATPases and $Na^+, K^+, 2Cl^-$ -cotransporters expressed at the basolateral membranes of marginal cells. An additional K^+ -diffusion potential formed by KCNQ1/KCNE1- K^+ channels at the apical membranes of marginal cells also contributes to the EP. Therefore, the EP depends on an electrically isolated space and two K^+ -diffusion potentials in the stria vascularis.

Keywords Epithelium · Potential · Cochlea · Electrophysiology · Potassium transport

Introduction

Sounds from the outside world propagate through the external and middle ears and then stimulate the cochlea of the inner ear, a specialized peripheral organ of hearing. Hair cells, receptors on the basilar membrane that vibrates when acoustic stimuli reach the inner ear, transform the mechanical energy of sounds into electrical signals (Fig. 1a). The mammalian cochlea is a highly sensitive sensor of hearing; it can detect basilar-membrane motion near ± 0.1 nm that is elicited by a threshold stimulus [31]. To achieve such exquisite sensitivity, the cochlea is equipped with multiple systems enhancing its mechanical inputs. The endocochlear potential, the topic of this article, is one of the key systems. Model mice lacking the endocochlear potential (EP) are deaf [11, 22, 42], indicating that this potential is mandatory for proper hearing.

The snail-shaped mammalian cochlea comprises three tubular structures—the scala media, scala tympani, and scala vestibuli—and various cell types such as epithelial cells and fibrocytes (Fig. 1a). The scala tympani and scala vestibuli are filled with perilymph whose ionic composition resembles that of ordinary extracellular solutions. On the

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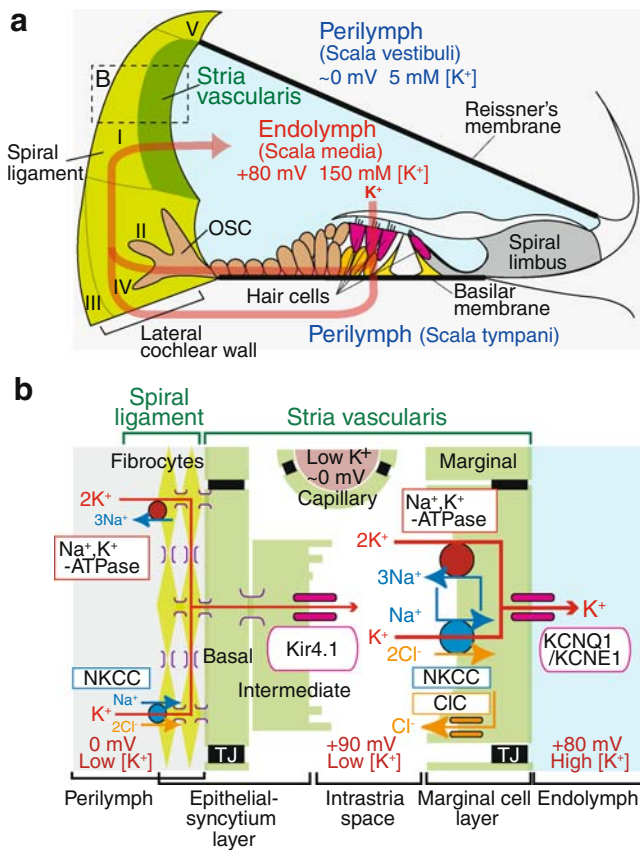


Fig. 1 Structure of the cochlea and its lateral wall. **a** The ionic composition and potential of the endolymph are maintained by K^+ circulation through the lateral cochlear wall (*left*). There are two possible routes of K^+ transport from hair cells to the lateral wall: one through the gap-junctional network of epithelial cells on the basilar membrane and the other through perilymph. The locations of five types of fibrocyte are indicated by *Roman numerals*. **b** A schematic enlargement of the boxed region in (**a**) depicts the ion-transport apparatus involved in the formation of the EP (endocochlear potential). *NKCC* $Na^+, K^+, 2Cl^-$ -cotransporter, *CIC* Cl^- -channel, *TJ* tight junction. Reproduced and modified from [69]

other hand, the scala media holds a distinct solution termed endolymph. The endolymph contains 150 mM K^+ , 2 mM Na^+ , and 20 μM Ca^{2+} , and exhibits an EP of about +80 mV relative to blood plasma and perilymph [107, 108]. Such a specialized electrochemical milieu is not observed in the extracellular fluids of any other organ in mammals. Each cochlear hair cell bathes its cell body in perilymph and exposes its apical membrane, which is surmounted by a mechanosensitive hair bundle comprising dozens of stereocilia, to endolymph. The basilar-membrane motion elicited by acoustic stimuli deflects the bundle and opens cation-permeable mechano-electrical transduction channels at the top of the stereocilia. This process permits endolymphatic K^+ to enter hair cells, resulting in their electrical excitation [29, 30, 32]. By forming a large driving force, the EP

accelerates not only the influx of K^+ but also the entry of Ca^{2+} that enhances the motility of the hair bundle [10]. The EP therefore serves as a biological battery to increase the sensitivity of hair cells.

Since Georg von Békésy, who was awarded with Nobel Prize for his research on traveling waves in the cochlea, first measured the EP in 1952 [107, 108], the mechanism underlying its formation has remained an enigma. Our recent electrophysiological assays have clarified key elements in the mechanism, which will be described in this article. We will also discuss the physiological merit of the EP as an amplifier for sound signaling.

K^+ circulation in the cochlea

After entering hair cells, K^+ exits across their basolateral membranes through K^+ channels [3, 18, 39, 47, 48, 52, 71, 75, 87] and reaches the lateral cochlear wall either by a perilymphatic route or through the gap-junctional network comprising Deiters' cells and epithelial cells on the basilar membrane [6, 7, 24, 40, 56] (Fig. 1a). K^+ is subsequently transported across the lateral cochlear wall and finally returned back to endolymph (Fig. 1a).

This cochlear K^+ circulation from endolymph to perilymph and then to endolymph, which is also referred to as cochlear K^+ recycling, is thought to be involved in the establishment of the EP and possibly the high K^+ concentration in endolymph [27, 40, 41, 113, 114]. The concept of the K^+ circulation was originally proposed on the basis of the following observations. First, radioactive K^+ applied in perilymph can be detected in endolymph much more efficiently than when perfused in blood plasma [45, 90]. Second, perilymphatic but not vascular perfusion of K^+ -free solution rapidly and prominently suppresses the EP [59, 109]. Zidanic and Brownell [118] electrophysiologically measured the current flow from endolymph to perilymph and estimated it as 0.8–1.6 μA per 1-mm wedge of cochlea. This may represent a fraction of the total K^+ circulation.

Architecture of the lateral cochlear wall

The lateral cochlear wall is composed of two components, the spiral ligament containing five distinct types (I–V) of fibrocytes and connective tissue, and an epithelial tissue, the stria vascularis, comprising marginal, intermediate, and basal cells (Fig. 1a, b). In the ligament, types II and IV fibrocytes are characterized by numerous extensions of their plasma membranes [88]. All of the fibrocytes, which are bathed in perilymph, and the basal and intermediate cells in the stria vascularis are connected together through

gap junctions and are therefore considered to share the same electrochemical properties [11, 93]. These three types of cells appear to form a functional syncytium; its basolateral and apical surfaces correspond to the membranes of fibrocytes and to the apical membranes of intermediate and basal cells, respectively (Fig. 1b).

Marginal cells, whose apical surfaces directly face endolymph, constitute a monolayer of epithelial cells (Fig. 1b). Thus, the lateral wall beside scala media can be considered to be made up of the epithelial syncytium and the marginal-cell layer. Between the two layers, there are numerous capillaries and an extracellular space called the intrastrial space (IS) [28, 89] (Fig. 1b). The basolateral membranes of marginal cells and the apical membranes of intermediate cells are highly infolded and twisted together, and the IS is very narrow, with a width of only 15 nm. The interior of the stria is separated from perilymph, endolymph, and blood by the tight-junctional networks of basal cells in the epithelial-syncytium layer, of marginal cells, and of vascular endothelial cells [35] (Fig. 1b).

The tissues and ion-transport apparatus critically involved in formation of the EP

Contribution of the spiral ligament to the EP

Types II and IV fibrocytes in the spiral ligament express on their membranes two K^+ -uptake transporters: Na^+,K^+ -ATPase composed of α_1 and β_1 subunits [82, 83] and $Na^+,K^+,2Cl^-$ -cotransporter (NKCC) made up of NKCC1 subunit [12, 78] (Fig. 1b). Ouabain or furosemide, a specific blocker for Na^+,K^+ -ATPase or NKCC, respectively, dramatically suppresses the EP when applied to perilymph [46, 49–51, 59]. It is therefore likely that both transporters contribute to the EP by carrying K^+ from perilymph into the fibrocytes across their membranes that correspond to the basolateral surface of the epithelial-syncytium layer. K^+ taken up by the fibrocytes then moves through the gap junctions of basal and intermediate cells to the apical surface in the epithelial-syncytium layer (Fig. 1b).

The stria vascularis is essential for the EP

Since the first measurement of the EP, audiologists have wondered which cochlear tissue generates the potential. Tasaki and Spyropoulos found by electrophysiological techniques that a deaf guinea pig called *waltzing* exhibited an EP of normal amplitude although it had no hair cells [100]. This observation ruled out an involvement of hair cells in the establishment of the EP. They additionally conducted an experiment with a wild-type guinea pig to precisely identify the tissue responsible for formation of the EP. After

removing Reissner's membrane separating endolymph from perilymph (Fig. 1a), they sucked most of the endolymph in the scala media into a glass pipette. In this condition, a thin film of endolymph might have remained over the stria vascularis and the organ of Corti. They then measured the potential along the surface of these tissues with the electrode, and recorded positive potential of +30 to +50 mV near the stria vascularis, whereas the potential was near 0 mV in the proximity of the organ of Corti [100]. These two observations strongly suggest that the stria vascularis but not the organ of Corti is essential for generation of the EP.

Strial ion-transport apparatus crucial for the EP

Anoxia or vascular perfusion of either ouabain or furosemide dramatically reduces the EP [44, 51, 110]. Of importance, these drugs affect the EP much more rapidly when perfused by a vascular route than by a perilymphatic one [51, 110]. The Na^+,K^+ -ATPase and NKCC expressed in the stria vascularis are therefore more critically and directly involved in the generation of the EP than the corresponding transporters in the spiral ligament. NKCC and Na^+,K^+ -ATPase, which consist respectively of NKCC1 subunits and of a complex of α_1 plus β_1 or β_2 subunits, occur at the basolateral membrane of marginal cells [12, 34, 38, 82, 116]. Moreover, vascular perfusion of Ba^{2+} , a blocker of K^+ channels, sharply decreases the EP [26, 57, 60, 96], so the channels expressed in the stria vascularis are suggested to play a pivotal role in the formation of the EP. It is now known that a target of Ba^{2+} is the inward rectifier Kir4.1 [26], and that this K^+ channel is localized at the apical membranes of intermediate cells [25, 94, 96]. Slc26a4-knockout mice, which lack the strial Kir4.1 for unknown reasons, and Kir4.1-null mice completely lose the EP [61, 115]. In addition, several human mutations of the gene encoding Kir4.1, which are expected to impair the channel's function, cause sensorineural deafness accompanied by neuronal disorders and electrolyte imbalance [5, 81].

Previous model for the mechanism of formation of the EP

As for the mechanism for formation of the EP, the following two-cell model [112, 113], also called the five-compartment model [96], has been widely accepted. Salt et al. [79] measured the electrochemical properties of the stria vascularis by inserting a K^+ -selective electrode. They found that the fluid of the IS contained a low K^+ concentration like that of perilymph and exhibited a highly positive potential of +90 mV [79] (Fig. 1b). This positive IS potential was presumed to represent the

majority of the EP [33, 79]. Some groups reported that the membrane potential of basal cells in the epithelial syncytium relative to the perilymph was -5 mV and their intracellular K^+ concentration was high [33, 63, 79] (but see [69] reporting 0 to $+4$ mV). In general, if the cell membranes were selectively K^+ permeable and there was a concentration gradient across them, the K^+ -diffusion potential, which is the K^+ -equilibrium potential (E_K) represented by Nernst's equation, would occur across the membranes. The potential difference across the apical surface of the epithelial syncytium is close to the E_K with respect to the IS [79]. Because vascular perfusion of Ba^{2+} prominently suppresses the EP [26, 60], it was hypothesized that the K^+ -diffusion potential formed by Kir4.1 produces the IS potential and is therefore critical for the EP [79, 113, 114, 116]. In this model, Na^+,K^+ -ATPase and NKCC at the basolateral membranes of marginal cells sustain a low K^+ concentration in the fluid of the IS to facilitate K^+ diffusion through Kir4.1 channels. The IS potential of $+90$ mV decreases across the apical membrane of marginal cells by 10 mV [63, 70], resulting in a potential of $+80$ mV in endolymph.

The current model for generation of the EP based on our findings

Questions to be addressed in the previous hypothesis

Although the two-cell or five-compartment model seems reasonable, no *in vivo* experiments have been performed to verify it because of technical difficulties. In addition, Melichar et al. reported that strong inhibition of Na^+,K^+ -ATPase reduces the EP to a value of -10 mV, which is called the negative EP, but leaves the stria vascularis at a positive potential of $+14$ mV [63]. This potential difference cannot be explained by the two-cell model, which proposes that the IS potential is nearly equivalent to the EP. Therefore, some elements that are essential for formation of the EP may be missing in the two-cell model. Furthermore, it is not clear how the stria vascularis maintains the positive IS potential. It is reported that, in two lines of knockout mice, the tight-junctional networks of the stria vascularis are disrupted and the EP is eliminated: mice lacking claudin-11, a major constituent of tight junctions of basal cells [22, 42, 43], and mice missing the gene encoding connexin30 (Cx30) [11, 102]. Although Cx30 is a gap-junctional protein of fibrocytes [41, 53, 54], all cell types in the stria vascularis of the mutant mice display an elevation of homocysteine, which damages endothelial cells and disrupts their tight junctions [11]. Because the IS potential is important for the EP, the results from the two knockout mice suggest the involvement of a

unique electrical property of the stria vascularis in sustaining the IS potential. However, neither this property nor the process inducing the phenotype of the mutant animals has been clearly identified [11, 22, 42, 102].

Measurement of the electrochemical properties in the lateral cochlear wall

To clarify the precise mechanism underlying the generation of the EP, we measured the potential, K^+ activity, and input resistance by inserting a double-barreled K^+ -selective electrode into the lateral cochlear wall under various conditions [69]. The experimental results are summarized in Fig. 5.

An ionic activity is an effective concentration of an ion, or in other words, a measure of the fraction of the ions that are free and active and actually elicit a variety of chemical and biological phenomena including the diffusion potential monitored with the ion-selective electrode. An ionic activity is the product of the ion's concentration and its activity coefficient. Because the coefficient is variable and unmeasurable in intact cells and tissues, the K^+ activity was measured in our assays. In some experiments, the EP was simultaneously monitored with a separate electrode.

First, in normal conditions, we found four compartments in the lateral wall, each of which shows distinct electrochemical property as follows (see Fig. 5): (1) epithelial syncytium—slightly positive potential (0 to $+4$ mV) and 60 mM K^+ activity; (2) IS—high potential similar to the EP and low K^+ activity like perilymph (5 mM); (3) marginal cells—high potential similar to the EP and 80 mM K^+ activity; and (4) endolymph—high potential of $+80$ mV and high K^+ activity exceeding 100 mM.

The stria vascularis may have a barrier function to maintain the positive potential in the IS. To test this idea, we measured the input resistance in the IS with the K^+ -selective electrode (Fig. 2). The electrode was driven through the lateral wall and, when it encountered the IS, the potential changes of this region in response to current pulses became much larger than those of perilymph and endolymph (Fig. 2). This finding indicates that the resistance inside the IS is much higher than those of the perilymph and endolymph. This space is therefore electrically isolated from neighboring extracellular fluids to sustain the positive potential.

Next, we examined the electrochemical property of the IS that would be crucial for formation of the EP (Fig. 3). During inhibition of Na^+,K^+ -ATPase by anoxia, the potential in the IS fluid falls and then rebounds slightly, whereas the K^+ activity shows a reverse pattern (Fig. 3a). Under the same conditions, the electrochemical milieu inside the epithelial syncytium barely changes. Because the apical membranes of intermediate cells of the epithelial-

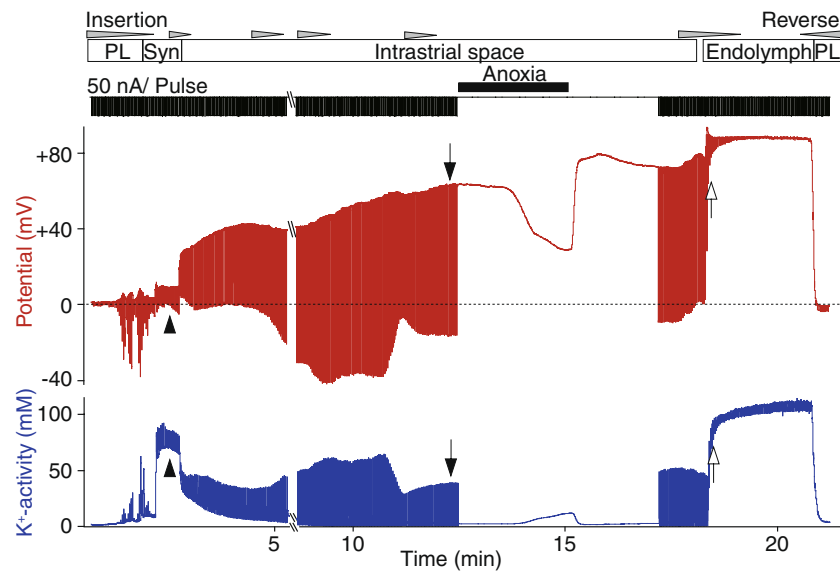


Fig. 2 Input-resistance measurement of lateral-wall compartments. A double-barreled, K^+ -selective electrode was driven through the lateral wall of the cochlea to record the potential (red) and K^+ activity (blue). The input resistance was measured simultaneously as downward deflections in the potential record in response to 50-nA current pulses 200 ms in duration. The upward deflections in the recording of K^+ activity are artifacts resulting from the current pulses applied to the

neighboring electrode. The electrode encountered successively the perilymph (PL), the epithelial syncytium (Syn, filled arrowheads), the intrastrial space (IS, filled arrows), and finally the endolymph (EL, open arrows). Note the decline in the IS potential during a period of anoxia (see Fig. 3a). In this and subsequent recordings, the gray wedge above the trace indicates the period during which the electrode was advanced. Reproduced and modified from [69]

syncytium layer express Kir4.1 channels [1, 25] (see Fig. 1b), these two observations suggest that the IS potential is mainly generated by K^+ diffusion. Indeed, the E_K calculated by Nernst's equation with measured K^+ -activity values in the epithelial syncytium and in the IS corresponds well to the IS potential recorded during anoxia (Fig. 3b). Similar results are obtained when ouabain or bumetanide is arterially applied to the stria vascularis to specifically inhibit Na^+, K^+ -ATPase and NKCC. Furthermore, perfusion of Ba^{2+} to the stria vascularis decreases the potential of the IS with little change of its K^+ activity (Fig. 3a). Taken together, the IS potential is likely to be formed mainly by K^+ diffusion through Ba^{2+} -sensitive Kir4.1 channels in the apical membranes of intermediate cells. The low K^+ concentration in the IS seems to be maintained by Na^+, K^+ -ATPase and NKCC expressed basolaterally in marginal cells. During anoxia and perfusion of Ba^{2+} , the EP changes roughly in parallel with the IS potential (Fig. 3a). Thus, the IS potential represents a significant fraction of the EP.

During anoxia, the IS potential remains positive but the EP decreases and finally reaches -15 mV (Fig. 3a; see also [63]). This potential difference may occur in the marginal-cell layer, for that is the sole tissue component between the IS and endolymph (see Fig. 1b). We thereby examined the K^+ activity and potential of marginal cells (Fig. 4). After passing the epithelial syncytium and the IS, the K^+ -selective electrode encounters the inside of

marginal cells (Fig. 4a). This compartment is distinguishable from the endolymph, because the K^+ activity of the former (80 mM) is lower than that of the latter (>100 mM; Fig. 4a). In marginal cells, the K^+ activity is barely changed by vascular perfusion of Ba^{2+} but prominently decreased in a sigmoidal fashion by application of anoxia, although the potential is sharply reduced in both cases (Fig. 4a). Of note, neither anoxia nor perfusion of Ba^{2+} alters the K^+ activity in endolymph (data not shown). In the anoxic condition, when the K^+ -selective electrode is advanced from marginal cells to endolymph, a large potential difference is detected (Fig. 4a). It indicates that the potential difference occurs across the apical membranes of marginal cells. For two reasons, most of the difference is attributable to the K^+ -diffusion potential. First, during a period of anoxia, E_K calculated by Nernst's equation with the recorded K^+ activity of marginal cells and that of endolymph [62, 86, 112] matches well the measured potential difference between these two compartments (Fig. 4b). Second, in the apical membranes of marginal cells, the K^+ permeability considerably exceeds those of Na^+ and Cl^- [98]. The K^+ -diffusion potential is likely to be formed by the KCNQ1/KCNE1 channels that are strongly expressed at the apical membranes of marginal cells [9, 68, 77, 104]. Because the basolateral membranes of marginal cells express little K^+ conductance [95], the K^+ -concentration gradient across this membrane barely produces a potential difference.

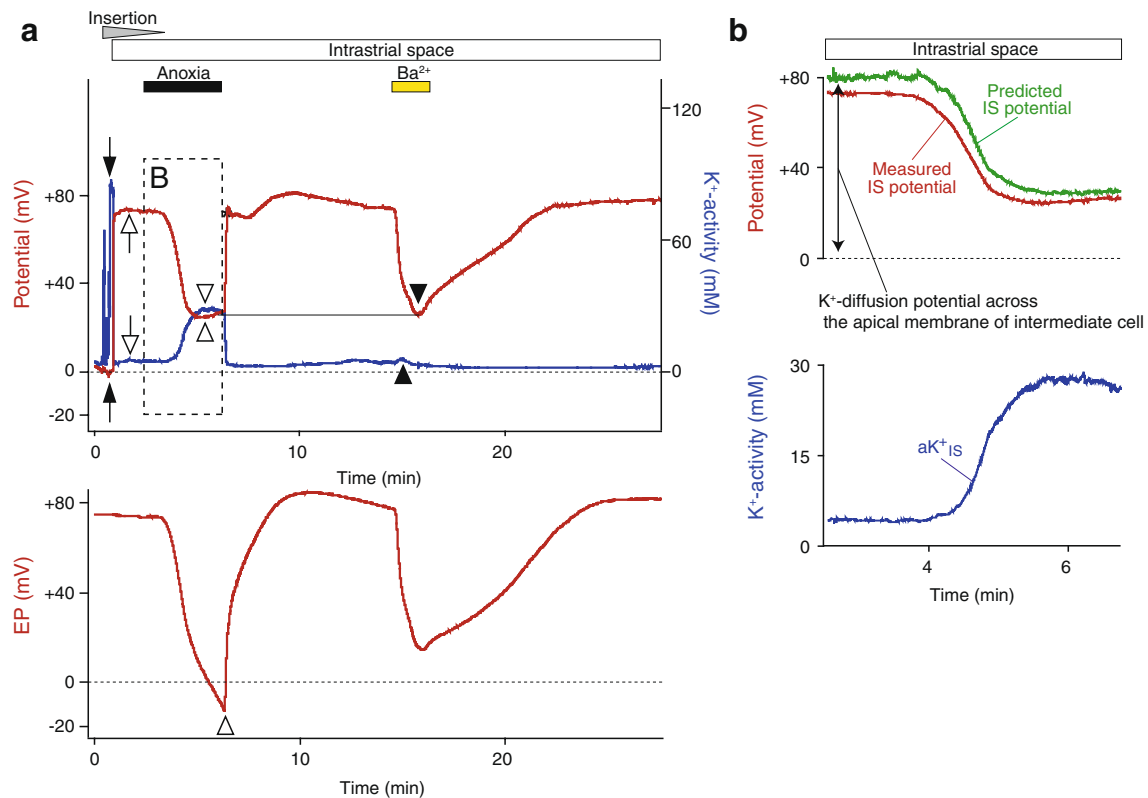


Fig. 3 Effects of blocking Na⁺,K⁺-ATPase and K⁺ channels on the property of the IS. **a** After passing through the epithelial syncytium (filled arrows), a K⁺-selective electrode (upper panel) was held in the intrastrial space (IS, open arrows). The endocochlear potential (EP) in a different portion of the cochlear turn was monitored simultaneously with a single-barreled electrode (lower panel). Open and filled arrowheads show the peak changes in the potentials (red) and K⁺ activity (blue) during anoxia and vascular perfusion of 1 mM Ba²⁺.

Anoxia increased K⁺ activity in the IS (aK_{IS}⁺) much more than did the application of Ba²⁺, but both treatments reduced the IS potential to a similar value (thin horizontal line). **b** The IS potential during anoxia was predicted (green) with the equation IS potential = V_{Syn} + (RT/F) ln(aK_{i(Syn)}⁺/aK_{IS}⁺). The potential and K⁺ activity in the syncytium (V_{Syn} and aK_{i(Syn)}⁺) and in the IS (aK_{IS}⁺) (blue) were obtained from (a) (upper panel, filled arrows, and box). The IS potential recorded in (a) (boxed) is also shown (red). Reproduced and modified from [69].

According to all of the experimental data and calculations, the EP can be represented by the simple relation:

$$EP = V_{Syn} + \frac{RT}{F} \ln\left(\frac{aK_{i(Syn)}^+}{aK_{IS}^+}\right) + \frac{RT}{F} \ln\left(\frac{aK_{i(MC)}^+}{aK_{EL}^+}\right)$$

where V_{Syn} is the potential of the epithelial syncytium (constant); aK_{i(Syn)}⁺, aK_{IS}⁺, aK_{i(MC)}⁺, and aK_{EL}⁺ are the K⁺ activities of the epithelial syncytium (constant), the IS fluid, marginal cells, and endolymph (constant), respectively; and R, T, and F have their usual meanings. The first two terms on the right represent the IS potential, and the final term represents the potential difference across the apical membranes of marginal cells. The EP thus depends on the electrical isolation of the IS (Fig. 2) and two K⁺-diffusion potentials (Figs. 3 and 4) in the stria vascularis. See Fig. 5 to review the electrochemical properties of the lateral wall in different conditions.

The key elements in formation of the EP

Our findings not only have experimentally confirmed the two-cell (five-compartment) model but also have demonstrated three additional factors crucial for formation of the EP. First, the IS is electrically isolated from the perilymph, endolymph, and blood (Fig. 2). This feature is required for maintenance of the high potential and seems to depend on the separation of this space by tight-junctional networks of basal cells in the epithelial syncytium, marginal cells, and endothelial cells. The loss of the EP observed in claudin-11- and Cx30-null mice [11, 22, 42, 102] must result from disruption of the electrical barrier constituted by basal and endothelial cells, respectively. In spite of the electrical isolation of the stria, arterially perfused Ba²⁺ appears to penetrate the endothelial cells and reach the IS. The endothelial barrier may therefore be loose enough to permit the permeation of Ba²⁺ but tight enough to guarantee the high input resistance of the IS. The abundantly infolded membranes of intermediate and margin-

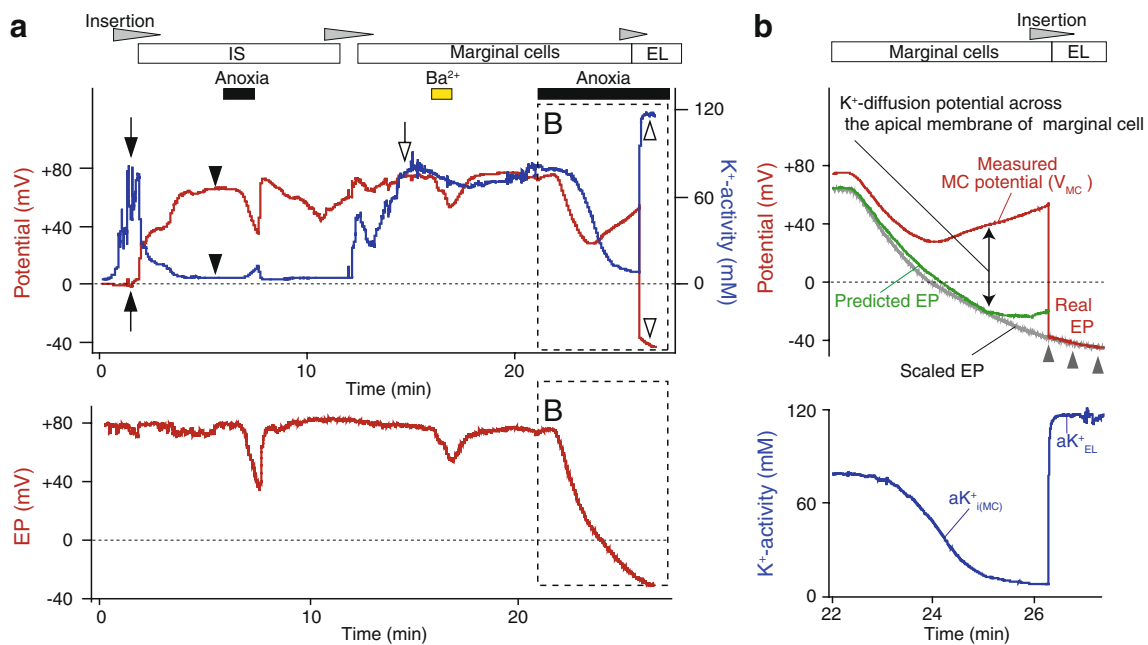


Fig. 4 Analysis of the properties of marginal cells. **a** A K⁺-selective electrode (*upper panel*) advanced from the perilymph towards the endolymph first encountered the epithelial syncytium (*filled arrows*) and the intrastrial space (*IS, filled arrowheads*). Anoxia was imposed briefly when the electrode was in the IS. Entry into the marginal cells was signaled by a highly positive potential and elevated K⁺ activity (*open arrow*). Anoxia and 1 mM Ba²⁺ were tested while the electrode was held at that location. The electrode was ultimately inserted into the endolymph during a period of anoxia (*open arrowheads*). Throughout the experiment, the endocochlear potential (*EP*) was

recorded with a second electrode (*lower panel*). **b** The EP during anoxia was predicted (*green*) with the equation $EP = V_{MC} + (RT/F) \ln(aK_{i(MC)}^+ / aK_{EL}^+)$. The potential (V_{MC} , *red*) and intracellular K⁺ activity ($aK_{i(MC)}^+$, *blue*) of marginal cells and the endolymphatic K⁺ activity (aK_{EL}^+) were obtained from (**a**) (*upper panel, box*, and *upper panel, open arrowhead*). The vertical scale of the EP in (**a**) (*lower panel, box*) was adjusted (scaled EP, *gray line*) to fit the curve of the potential that was recorded by the K⁺-selective electrode when it was in endolymph (*red line* highlighted by *gray arrowheads*). *IS* intrastrial space, *EL* endolymph. Reproduced and modified from [69]

al cells tightly wrap the capillaries. The IS between the membranes is very narrow, only 15 nm [28]. These anatomical features could provide an additional mechanism to increase the resistance between the IS and blood while permitting the effective diffusion of substances across this border. However, the precise properties of the barrier in the stria and the mechanism underlying its establishment are still largely unknown, and thus further experimental studies are needed.

A second important point is that the epithelial syncytium is virtually clamped at 0 to +4 mV. Because of this property, the K⁺-diffusion potential at the apical membranes of intermediate cells represents a significant fraction of the positive IS potential. Gap junctions interconnect many fibrocytes and constitute an extremely large functional cell volume that provides a large membrane capacitance for the basolateral surface of the epithelial-syncytium layer. Therefore, even if the amplitude of K⁺ transport across the lateral wall should alter, the potential of the syncytium would barely change. The mechanism that sets the potential of the epithelial syncytium at 0 to +4 mV has not yet been identified. Maintenance of a high K⁺ concentration in the epithelial syncytium is also important to set the large K⁺-diffusion

potential across the apical surface. Perilymphatic perfusion of K⁺-free solution [59], ouabain, or furosemide [46, 49–51, 59] reduces the EP because it may attenuate K⁺ influx across the membranes of the fibrocytes by blocking Na⁺,K⁺-ATPase and NKCC, decreasing K⁺ concentration in the epithelial syncytium and thus decreasing the IS potential. Brn-4-null mice, which show reduction of the EP by 45–50 mV, lose membrane infoldings in their type II fibrocytes [64, 72] and are therefore expected to display a reduced density of K⁺-uptake transporters. This abnormality would decrease the K⁺ concentration in the epithelial syncytium, which could result in considerable reduction of the IS potential.

Third, the K⁺-diffusion potential at the apical membranes of marginal cells also contributes to the generation of the EP (Fig. 3). In the normoxic condition, the K⁺ activity of endolymph, which exceeds 100 mM, is significantly higher than the intracellular K⁺ activity of marginal cells, about 80 mM. The potential difference of 10 mV measured at the apical membranes of marginal cells [63, 70] is therefore likely to represent mainly the K⁺-diffusion potential. The negative EP observed during strong block of the K⁺-uptake transporters of marginal cells reflects the combination of a decrease in the IS potential

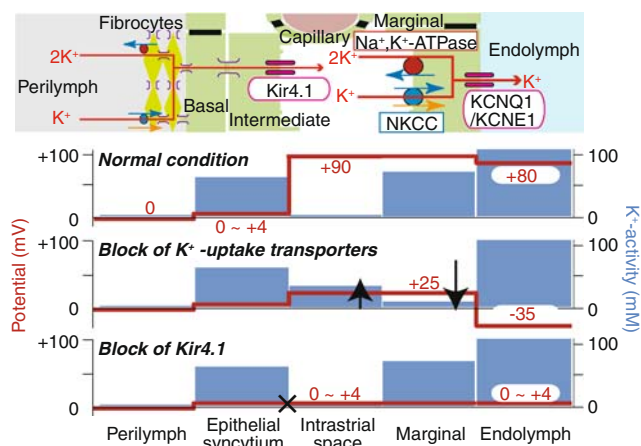


Fig. 5 Summary profile of electrochemical properties of the lateral cochlear wall. The *top panel* depicts the structure of the lateral wall and the K^+ -transport apparatus involved in the generation of the EP. The predicted potential and K^+ activity in each compartment under normal conditions and during inhibition of the stria K^+ -uptake transporters and Kir4.1 are respectively shown in *other panels*. NKCC $Na^+,K^+,2Cl^-$ -cotransporter. Reproduced and modified from [69]

and an increase in the diffusion potential across the apical membranes of marginal cells (Figs. 3, 4, and 5).

Unidirectional K^+ transport through the lateral cochlear wall and its involvement in generation of the EP

As mentioned earlier, K^+ is transported from perilymph to endolymph across the lateral cochlear wall: in each of the two epithelial layers, K^+ is taken up across the basolateral membranes by Na^+,K^+ -ATPase and NKCC and then secreted across the apical membranes by K^+ channels (Fig. 1b). This unidirectional K^+ transport is achieved by Na^+ recycling between the two K^+ -uptake transporters as discussed below and drives K^+ circulation throughout the cochlea. Disruption of K^+ transport by blocking K^+ -uptake transporters prominently affects K^+ concentration in the IS and in marginal cells and consequently changes the amplitude of the two K^+ -diffusion potentials that form the EP (Figs. 3, 4, and 5). Therefore, unidirectional K^+ transport is critically involved in generation of the EP.

In general, if the potential of the membranes expressing K^+ channels is identical to E_K , then K^+ is not moved across them. In the stria vascularis, the measured potential differences across the apical membranes of intermediate cells and those of marginal cells always modestly exceed E_K of these membranes (Figs. 3 and 4). Thus, K^+ should be secreted from these cells into the IS and the endolymph through Kir4.1 and KCNQ1/KCNE1 channels, respectively. This machinery supports unidirectional K^+ transport across the lateral cochlear wall and thus K^+ circulation. In addition, local Na^+ homeostasis may be involved in unidirectional

K^+ transport. Because Na^+,K^+ -ATPase and NKCC occur together in the basolateral membranes of marginal cells and in the fibrocyte membranes of the epithelial syncytium, Na^+ is expected to cycle between the two K^+ -uptake transporters (Fig. 1b). This local cycling may couple the Na^+ efflux through Na^+,K^+ -ATPase and the influx through NKCC, which would be required for K^+ uptake across the basolateral membranes. Then K^+ would exit from intermediate cells of the epithelial syncytium and marginal cells to the IS and the endolymph through the K^+ channels localized in the apical membranes, respectively. This mechanism could be called Na^+ -recycling, unidirectional transport of K^+ .

K^+ secretion from the stria vascularis to endolymph

K^+ transported to stria marginal cells may finally be excreted into endolymph through KCNQ1/KCNE1 K^+ channels in their apical membrane [9, 77, 92, 104, 112]. In support of this idea, both KCNE1- and KCNQ1-null mice show a collapse of scala media, probably due to abrogation of K^+ secretion, and a severe hearing impairment [74, 104]. Human loss-of-function mutations of either subunit cause Jervell and Lange-Nielsen syndromes, which involve sensorineural deafness as well as prolongation of the QT interval in the electrocardiogram [36, 67, 84, 103, 111].

Other ion-transport mechanisms in the lateral cochlear wall

Type 4 Bartter's syndrome, which is caused by loss-of-function mutations in the pore-forming CIC-K subunits or barttin accessory subunits of Cl^- channels, is accompanied by hearing disturbance [4, 80]. Barttin-knockout mice show deafness with loss of the EP [73]. These observations imply that Cl^- transport in the stria vascularis is involved in formation of the EP. The basolateral membranes of marginal cells are Cl^- permeable and express the subunits listed above [2, 19, 76, 97] (Fig. 1b). The contribution of Cl^- channels to the EP may be the following. NKCC, when actively working, would cause local depletion of the Cl^- concentration in the IS fluid surrounding the basolateral membranes of marginal cells, which would result in dysfunction of the transporter. The Cl^- channels may provide intracellular Cl^- for the extracellular K^+ site of NKCC to sustain its activity. In barttin-knockout mice [73], dysfunction of the NKCC induced by loss of the Cl^- conductance would reduce the K^+ concentration in marginal cells. This reduction could further increase the K^+ -diffusion potential at the apical membranes of marginal cells and suppress the EP (see Fig. 5).

Patch-clamp analyses of isolated marginal cells demonstrated that their basolateral and apical membranes express

non-selective cation channels [91, 97, 98]. TRPML3, whose mRNA is abundantly detected in marginal cells [66], could be a constituent of these cation channels, although its distribution pattern inside the stria vascularis is controversial (see [13, 117]). A gain-of-function mutation of TRPML3 observed in deaf mice called *varitint-waddler* disturbs the structure of the stria vascularis and results in reduction of the EP [8, 16, 23, 66, 117]. In spite of this evidence, the physiological significance of TRPML3 in the formation of the EP is unknown. Other molecular candidates of the non-selective cation channels in marginal cells are TRPV4 [13, 55, 99] and TRPC-family channels [65].

Comparison of ion-transport mechanisms between the cochlea and vestibule

The vestibular labyrinth, which includes the sacculus, utriculus, and semicircular canals, is also filled with endolymph. Although vestibular endolymph contains 150 mM K^+ , its potential is 0 mV [58, 112]. A highly positive potential in endolymph may not be necessary in the vestibule, for the low-frequency response of the vestibular organs implies their transduction currents can be far smaller. Indeed, in the whole-cell configuration of in vitro condition, the transduction-current conductance of a mouse vestibular hair cell (2.6 nS) is about half of that of a cochlear hair cell (5.5 nS) [21]. Except in the sacculus, the endolymph of the vestibular system seems to be maintained by dark cells located in specialized epithelia of the ampullae [112] (Fig. 6a). Dark cells in the utriculus could be involved in the maintenance as well. The function of the dark-cell areas in ampullae may resemble that of the cochlear stria vascularis. Like marginal cells, dark cells form a monolayer by tight-junctional connection at the boundary between endolymph and perilymph (Fig. 6b).

Many similarities are observed between dark cells and marginal cells. Dark cells express Na^+, K^+ -ATPase, NKCC1, and CIC-K/barttin in their infolded basolateral membranes [15, 19, 20, 58, 101, 116]. They also harbor KCNQ1/KCNE1 in the apical membranes [19, 68] (Fig. 6b). KCNE1-, KCNQ1-, and NKCC1-knockout mice exhibit a collapse of the vestibular endolymphatic space and balance disorders in addition to cochlear dysfunction [15, 17, 20, 74, 104]. Accordingly, like strial marginal cells, vestibular dark cells unidirectionally transport K^+ from their basolateral to their apical sides.

On the other hand, several prominent differences are detected between the two organs (Figs. 1b and 6b). The dark-cell area has neither basal cells nor intermediate cells, so the vestibule possesses none of the characteristics corresponding to the IS, the K^+ conductance generating the IS potential, and the electrical barrier. It is therefore

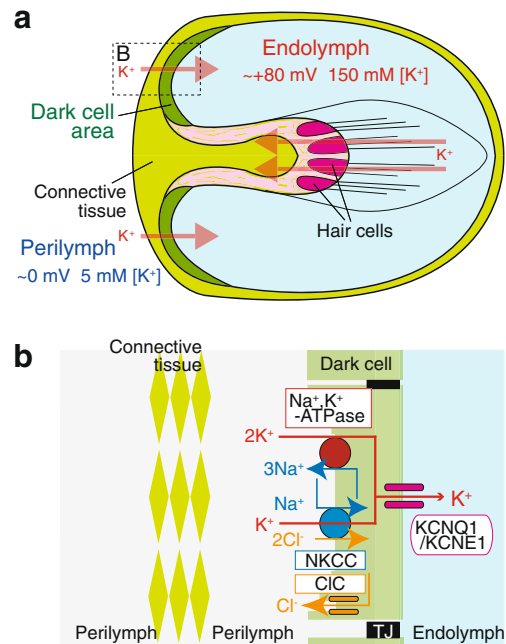


Fig. 6 Structure of the vestibule. **a** Vestibular endolymph is likely to be maintained by K^+ transport from perilymph to endolymph in the dark cell area. **b** Ion-transport mechanisms in the dark cell area of ampullae (boxed region in (a)). NKCC $Na^+, K^+, 2Cl^-$ -cotransporter, CIC Cl^- -K/barttin-type Cl^- channel, TJ tight junction

reasonable that the potential of vestibular endolymph is 0 mV. Comparison of the epithelial architecture and function of the cochlea and vestibule clearly indicates that the two-layer system equipped with two K^+ -diffusion potentials plays an essential role in the formation of the positive potential in the endolymph while maintaining the K^+ circulation and high endolymphatic K^+ concentration. If the cochlea possessed only one epithelial layer that faced the endolymph and harbored only one K^+ -diffusion potential, then this organ, like the vestibule, would maintain a negligible EP because of the small K^+ -diffusion potential across the apical membrane. To achieve a highly positive potential in the endolymph with one epithelial layer and one K^+ -diffusion potential, the cochlea might be able to substitute high Na^+ for high K^+ in the endolymph, but this condition would not be suitable for the hair cell's function.

The scala media of the cochlea is connected to the endolymphatic space of the sacculus by ductus reuniens, a narrow tunnel. In spite of this direct connection, cochlear endolymph exhibits a potential of +80 mV, whereas the endolymph in the vestibular system is at 0 mV. The mechanism underlying the maintenance of such a prominent difference is unknown. It is speculated that ductus reuniens provides an electrical barrier between the two endolymphatic spaces. Because the sacculus does not harbor any epithelial cells like the stria vascularis and the dark-cell area that maintains endolymph and its properties, Sellick and Johnstone

proposed that the potential of saccular endolymph derives from the EP [85]. In support of this idea, the potential of saccular endolymph during a period of anoxia altered and became negative in parallel with the EP. They further assumed that current would flow through ductus reuniens and calculated that the resistance of the tunnel was 180 k Ω . However, a direct measurement of the current and the resistance has not yet been performed, and the nature of the electrical barrier remains uncertain. Further studies will be necessary to clarify these points.

Physiological merit of the EP and high endolymphatic K⁺ concentration

To amplify the mechanical inputs of sounds, the mammalian cochlea bears two mechanisms in hair cells: active hair-bundle motility and membrane-based electromotility. In the former system, the movement of hair bundles is enhanced by a myosin-based process in the stereocilia [31]. In the latter, depolarization shortens the outer hair cells, which amplify the motion of the basilar membrane [14]. Why have mammals acquired the EP in the course of evolution to further amplify sound signals in the cochlea? Even without the EP, if cochlear endolymph contained a high Na⁺ concentration and hair cells were excited by Na⁺ influx, the cochlea would be sufficiently sensitive to weak acoustic stimulation. Indeed, mechano-electrical transduction channels are Na⁺ permeable [29, 32, 37]. The combination of the potential difference and the chemical gradient of Na⁺ between Na⁺-enriched endolymph and hair cells would establish the large driving force for Na⁺ influx. Nevertheless, why has the endolymph accumulated K⁺ instead of Na⁺ while maintaining the highly positive potential? Because mechano-electrical transduction channels are open 15% of the time even at the resting position of stereocilia [29, 106], Na⁺ would continue to enter the hair cells from Na⁺-enriched endolymph. The hair cells would have to continuously spend large amounts of ATP to extrude Na⁺ into the perilymph against the concentration gradient, which would exhaust or even destroy the cells. To supply sufficient energy, an enormous number of capillaries should lie in the vicinity of the hair cells. In this situation, however, vibrations produced by blood flow would significantly interfere with the proper motion of the extremely sensitive basilar membrane and hair bundle. To avoid these disadvantages but simultaneously sensitize hearing, it is reasonable that the cochlea has acquired a high K⁺ concentration and a high endolymphatic potential. In this environment, K⁺, after exciting hair cells, can diffuse into the perilymph across their basolateral membrane without wasting their energy while the large driving force for K⁺ influx from endolymph to the cells is maintained.

The physiological significance of the large endolymphatic space remains uncertain. However, because the stria vascularis is positioned far from the hair cells and basilar membrane, any vibration elicited by blood flow in the stria capillaries can be dissipated before reaching them. The damping effect may be further strengthened by the viscosity of endolymph [105], which would act as a cushion, like the synovial fluid in joints, to absorb mechanical vibration.

Several properties of endolymph contribute to the proper operation of the basilar membrane and hair cells in response to subtle acoustic stimuli. It will be important for inner-ear physiologists to confirm the ideas mentioned above.

Conclusion

The key elements underlying formation of the EP have been now demonstrated: this potential depends on an electrical barrier function in the stria vascularis and two K⁺-diffusion potentials at the apical membranes of intermediate cells and of marginal cells [69]. These factors are established by a specific spatial arrangement of ion-transport apparatus and fine cellular and tissue architecture in the lateral cochlear wall including the stria vascularis. However, several enigmas remain. Moreover, although evidence has been accumulated to prove that dysfunction of the ion-transport mechanisms maintaining the cochlear K⁺ circulation and the EP causes hearing impairments, we do not know the genetic background and pathophysiological sources of many other disorders of the cochlear labyrinth including Ménière's disease and sudden deafness. Further studies are necessary to completely understand the mechanisms underlying the formation of the EP and their physiological relevance to hearing function.

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