

## **Endolymph composition: paradigm or inevitability?**

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## **Summary**

This review is focused on the unusual composition of the endolymph of the inner ear and its function in mechano-electrical transduction. The role of  $K^+$  and  $Ca^{2+}$  in excitatory influx, the very low  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  concentrations of endolymph, stereocilia structure of hair cells and some proteins involved in mechanosensory signal transduction with emphasis on auditory receptors are presented and analyzed in more details. An alternative hypothetical model of ciliary structure and endolymph with a 'normal' composition is discussed. It is concluded that the unique endolymph cation content is more than an energy saving mechanism that avoids disturbing circulatory vibrations to achieve a much better mechanosensory resolution. It is the only possible way to fulfil the requirements for a precise ciliary mechano-electrical transduction in conditions where pressure events with quite diverse amplitudes and duration are transformed into adequate hair cell membrane depolarizations, which are regulated by a sensitive  $Ca^{2+}$ -dependent feedback tuning.

## **Key words**

Hair cells •  $K^+$  influx • Mechanosensing • Mitochondria

## **Composition of endolymph of the inner ear**

Mechanical sensor cells of the cochlear and vestibular receptor organ are called hair cells (HC) because of the presence of stereocilia. These stereocilia are bathed in high  $K^+$ , low  $Na^+$ ,  $Mg^{2+}$ ,  $Ca^{2+}$  and a protein-containing solution called endolymph. The cochlea of the inner ear has two types of receptor cells – outer and inner auditory HC depending on their position in the organ of Corti. The intimate mechanosensing mechanism of the auditory signal transduction in HC has been intensively studied and exhaustively reviewed (Fettiplace and Hackney 2006, Nin *et al.* 2016, Wangemann 2006, Zdebik *et al.* 2009). To a great extent the transduction of mechanical stimuli into membrane depolarization, mediator secretion and action potential generation in auditory or vestibular afferent sensory neurons are clear (Fettiplace and Hackney 2006, Wangemann 2006). However, one important detail of the transduction mechanism, the unique endolymph ion content, is not completely explained. Concentrations of about 157 mmol/l  $K^+$ , 1 mmol/l  $Na^+$ , 0.02 mmol/l free  $Ca^{2+}$  and 0.01 mmol/l  $Mg^{2+}$  (Bosher and Warren 1978, Scheibe *et al.* 1999, Wangemann 2006) are really exceptional for extracellular body fluid. The differences of mammalian endolymph cation content versus perilymph, blood plasma and other common extracellular solutions varies between about 30 times higher for  $K^+$ , >60 times lower for free  $Ca^{2+}$  and  $Mg^{2+}$ , and >100 times lower for  $Na^+$ .

## **Excitatory $K^+$ and $Ca^{2+}$ influx: mechanical, spatial and metabolic considerations**

The excitation of neurons, muscle and receptor cells depends mainly on  $Na^+$  influx, in primarily through voltage-dependent  $Na^+$  channels (Moczydlowski 2012a), ionotropic receptors (Moczydlowski 2012b) and cAMP or cGMP gated channels (Connors 2012). The influx of  $Na^+$  transiently depolarizes cell membranes and thus generates nonregenerative and graded receptor potentials in the sensory part of sensory neurons and in receptor cells, or

elicits an all-or-nothing response - action potential in axons and skeletal muscle cells (Moczydlowski 2012a, b). This mechanism seems to be suitable also for HC but surprisingly  $K^+$  influx is the way for depolarization of their ciliary membranes (Fettiplace and Hackney 2006) that is followed by the secretion of glutamate for exciting afferent auditory sensory neurons (Ottersen *et al.* 1998). An indication of the reason of this phenomenon is the fact that the only part of the HC in contact with the endolymph are stereocilia (Fig. 1). Endolymph is produced in the stria vascularis of the lateral cochlear wall (for details see Uetsuka *et al.* 2015; Zdebik *et al.* 2009), i.e. outside the mechanical signal transduction area of the inner ear. It was stated that the reason for its high  $K^+$  and low  $Na^+$  content is to decrease the energy requirements of inner and outer hair cells, and thus of the organ of Corti as a whole (Zdebik *et al.* 2009). Using  $K^+$  and  $Ca^{2+}$  influx instead of  $Na^+$  for ciliary membrane depolarization avoids the need for many high ATP-consuming  $Na^+/K^+$  pumps there. Cell membranes possess millions of these ion transporters that utilize around one third of the body energy (Simmers 2012) and up to 66-70% of the neuronal ATP (Howarth *et al.* 2012; Simmers 2012). On the other side  $K^+$  influx only slightly increased high  $K^+$  concentration of ciliary cytoplasm.  $K^+$  homeostasis of HC is sparingly achieved by opening of  $K^+$  channels outside the ciliary region (Connors 2012). As a consequence, vascularization of the mechanosensory area is more distant (Shi 2011) and in this way the mechanical influence of closely situated microcirculatory vessels is prevented (Zdebik *et al.* 2009). However, there are additional undervalued considerations for the formation of such exceptional body fluid. In the first place this is the low diffusion rate of ions between ciliary space and cell bulk. A clear sign for restricted diffusion between ciliary and HC body cytoplasm is the very low free  $Ca^{2+}$  concentration in endolymph (Bosher and Warren 1978, Wangemann 2006), as well as the many plasma membrane  $Ca^{2+}$ -ATPases 2 isoform (PMCA2), present in the ciliary membrane with density of about  $2200/\mu m^2$  (Chen *et al.* 2012). The limited ability of PMCA2 to extrude

ciliary  $\text{Ca}^{2+}$  'may constitute a major cause of outer HC vulnerability and high-frequency hearing loss' (Chen *et al.* 2012). Significant  $\text{Ca}^{2+}$  influx through the mechanoelectrical transduction channel complex containing transmembrane channel-like 1 and 2 as its essential components (Kurima *et al.* 2015) is sufficient to saturate ciliary cytoplasm and to induce  $\text{Ca}^{2+}$ -dependent sensitization of these channels despite PMCA2 and the abundant presence of  $\text{Ca}^{2+}$  buffer proteins there (Hackney *et al.* 2005). The diffusion between cilia and HC body is troubled not only by its tiny ankle base but also by the presence of a rootlet inside it. This rootlet represents a protein dense bundle comprised mainly of  $\beta$ - and  $\gamma$ -actin, but also of spectrin, tropomyosin and TRIOBP, a cytoskeleton-associated protein, which extends with similar length in cilia and cell body, and fulfils the significant part of the section of their connection (Fettiplace and Hackney 2006, Furness *et al.* 2008, Kitajiri *et al.* 2010). As a result, the diffusion between these well distinguishable HC parts is reduced. In this way the ATP movement towards cilia and ADP backwards to HC body is restricted. Therefore, mitochondria in HC body will not be able to provide sufficient ATP to support energy consumption of many  $\text{Na}^+/\text{K}^+$  pumps, together with a significant number of PMCA and other ciliary ATPases. Another reason for  $\text{K}^+$  instead of  $\text{Na}^+$  influx is the fact that mechanoelectrical transduction in the restricted ciliary space would not be troubled by the moderate increase of intraciliar  $\text{K}^+$  as it would only barely influence the reversal potential of  $\text{K}^+$  due to the high potassium concentration in the cytoplasm.

< Figure 1 >

An alternative hypothetical way to ensure ciliary HC metabolic requirements if  $\text{Na}^+$  depolarization occurs is to place mitochondria directly into stereocilia. This way, problems with ATP and ion diffusion limitation would be solved, but several others would arise. First, the need of intense vascularization that will generate continuous micro mechanical vibrations and thus a much lower signal/noise ratio of the sensor (Zdebik *et al.* 2009). Second, the

average mitochondrial size – about 0.25-0.5  $\mu\text{m}$  wide and 1-2  $\mu\text{m}$  or more in length, and a volume ranging from a few thousandths of  $\mu\text{m}^3$  to a several  $\mu\text{m}^3$  (Kayar *et al.* 1988) are comparable with the dimensions of the entire stereocilium as the diameter of the tallest auditory stereocilium is about 0.25  $\mu\text{m}$  and the height varies from 1  $\mu\text{m}$  (calculated volume  $\sim 0.05 \mu\text{m}^3$ ) to 6  $\mu\text{m}$  (volume  $\sim 0.3 \mu\text{m}^3$ ) depending on the location (Lim 1980). The presence of mitochondria will harm the mechanical sensitivity due to an improper ciliary architecture caused by the reduction of their abundant core of filaments (mainly actin, Slepecky and Chamberlain 1985), which should be replaced by mitochondria. Additionally, the absence of mitochondria in the ciliary area (Furness *et al.* 2008; Sipe *et al.* 2013; Bullen *et al.* 2015), decreases the probability of oxidative stress damage of HC because they are a main cellular source of reactive oxygen species (Murphy 2009). This influence is vulnerable even under ambient noise conditions while recurrent oxidative stress significantly enhances HC death (Baker and Staecker 2012, Nuttall *et al.* 2016). An impaired  $\text{Ca}^{2+}$  buffering is also expected due to the mitochondrial  $\text{Ca}^{2+}$ -buffer capacity (Williams *et al.* 2013) that ultimately will spoil the precise  $\text{Ca}^{2+}$ -dependent regulation of the mechanosensory channel complex (Kurima *et al.* 2015) by abundant ciliary  $\text{Ca}^{2+}$ -binding proteins (Hackney *et al.* 2005). Therefore, the unusual endolymph content is the only possible way to avoid the mitochondrial presence in the HC mechanosensing area, which ensures adequate ciliary elasticity, a proper and sustained working mode of mechano-electrical transduction and its precise  $\text{Ca}^{2+}$ -dependent regulation.

The risk of  $\text{Ca}^{2+}$  overload in the ciliary area exists due to a basic characteristic of the mechanotransduction channel – a very large single channel conductance in the range of 100-320 mS, depending on the species, type of sensory HC (outer, inner), as well as their location on the top of stereocilia and high  $\text{Ca}^{2+}$  selectivity (Beurg *et al.* 2006). The last specificity is overcome by another special feature of mammalian endolymph – very low concentration of free  $\text{Ca}^{2+}$  - 0.02 mmol/l vs about 1.2 mmol/l in the other extracellular body fluids

(Wangemann 2006). The combination of low extracellular free  $\text{Ca}^{2+}$  and abundant  $\text{Ca}^{2+}$  binding proteins in ciliary cytoplasm forms the optimal  $\text{Ca}^{2+}$  influx/ciliary free  $\text{Ca}^{2+}$  ratio for a precise regulation of the mechanotransducer channel complex conductivity during continuous auditory stimulation by a variety of pressure amplitudes.

$\text{Mg}^{2+}$  concentration of mammalian endolymph is also very low (about 0.01 mM, Bosher and Warren 1978). This is needed to avoid  $\text{Mg}^{2+}$ -dependent reduction of the ciliary inward current and the prolonging of its time constant of adaptation (Ricci and Fettiplace 1998), i.e. the low amount of this bivalent cation is necessary for optimal gating and kinetic properties of ciliary mechanosensory channels.

In conclusion the unique endolymph  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  content is more than an energy saving mechanism that avoids disturbing circulatory vibrations to achieve a much better mechanosensory resolution. It is the only possible way to fulfil the requirements for a precise ciliary mechano-electrical transduction in conditions where the pressure events with quite diverse amplitudes and duration are transformed into adequate HC membrane depolarizations, which are regulated by a sensitive  $\text{Ca}^{2+}$ -dependent feedback tuning. No doubt, the vestibulo-cochlear mechanosensing is a wonderful phenomenon and the endolymph is one of its major and unavoidable components.

### **Conflict of interest**

No conflict of interest to declare.

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Figure caption

**Figure 1.** Compartments of cochlea with organ of Corti and stereochilia basic structure. Endolymph fills scala media and perilymph – scala vestibule and scala tympani. The corresponding concentrations of inorganic cations in mammals are presented. MET (mechanoelectrical transduction). Data for the cations content are from Scheibe *et al.* (1999) and Wangemann (2006).

