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The Cochlear Potentials

II. The Nature of the Cochlear Endolymphatic Resting Potential

W. Kuijpers and S. L. Bonting

Departments of Otolaryngology and Biochemistry
University of Nijmegen, Nijmegen

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Summary. The effect on the endocochlear resting potential (ERP) of anoxemia, cyanide, pH changes and changes of the electrolyte composition of the perilymph was studied.

The ERP appears to be composed of two components: a negative potential E_- mainly determined by the K^+ gradient between endolymph and perilymph or plasma and a positive potential E_+ due to an ouabain- and anoxia-sensitive electrogenic K^+ pump represented by the Na^+–K^+-ATPase system of the stria vascularis.

Maintenance of the ionic concentrations of the endolymph appears to require in addition active transport of Na^+ and Cl^- out of the endolymph.

Key-Words: Endocochlear Resting Potential — Stria Vascularis — Electrogenic K^+-Pump — K^+-Diffusion Potential — Guinea Pig.

Schlüsselwörter: Endocochleares Potential — Stria vascularis — Elektrogene K^+-Pumpe — K^+-Diffusionspotential — Meerschweinchen.

Introduction

In a previous report we showed the dependence of the cochlear endolymphatic resting potential (ERP), a DC polarisation of the endolymph of about 80 mV positive with respect to perilymph or blood, on the functioning of the Na^+–K^+-ATPase system in the stria vascularis [24]. However, on the basis of this evidence we could not decide whether this potential is a diffusion potential due to the ionic gradients maintained by the cation pump, or whether it is an electrogenic potential arising directly from the cation pump activity.

The diffusion potential is given by the constant field equation formulated by Hodgkin and Katz [12]:

$$E = \frac{RT}{F} \ln \frac{P_K (K_i^+) + P_{Na} (Na_i^+) + P_{Cl} (Cl_o^-)}{P_K (K_o^+) + P_{Na} (Na_o^+) + P_{Cl} (Cl_i^-)} \text{ mV}.$$

In this equation R represents the gas constant, T the absolute temperature, F the Faraday constant and o and i refer to the concentrations of K^+, Na^+, and Cl^- outside and inside the cell, respectively while P_K, P_{Na} and P_{Cl} are the membrane permeabilities to these ions. In many

biological membranes the potential is mainly determined by the K^+ gradient, because the membrane is much more permeable to K^+ than to Na^+ and Cl^-. Since K_i is generally much larger than K_o, the inside of the cell is negative (about 80 mV) with respect to the outside.

Since the endolymphatic potential is positive with regard to plasma and perilymph and K^+ in the endolymph is high, it cannot be a K^+ diffusion potential. Neither could it be a Cl^- diffusion potential, since the Cl^- concentrations in endolymph, perilymph and plasma are nearly equal [16]. Johnstone [16] proposed that it could be a Na^+ diffusion potential. Neglecting the Cl^- contribution, he calculated from the observed Na^+ and K^+ concentrations a potential of + 82 mV, using a permeability ratio $P_{Na}/P_K = 50$ for the cochlear membranes, which is the ratio for the squid axon membrane during the rising phase of the action potential. He further proposed that the fast decrease of the endolymphatic potential in anoxemia, before a significant change in the Na^+/K^+ ratio has occurred [15], would be due to a change in the permeability ratio P_{Na}/P_K to 0.2, resulting in the observed negative potential of −30 mV. According to this hypothesis the endolymphatic resting potential would merely be the expression of the Na^+ gradient between endolymph and plasma or perilymph. The Na^+-K^+-ATPase system would act to maintain the intracellular-like ionic concentration of the endolymph. A difficulty of this hypothesis is that such drastic reversals of the permeability ratio have so far only been observed in excitable membranes.

An alternative possibility is that the ERP, rather than representing a diffusion potential between endolymph and perilymph or plasma, may be due to an electrogenic pump, which brings about an asymetrical distribution of ionic charges by pumping a single ion without or with only a partly coupled transport of a counterion. In that case, the sign of the ERP would indicate that the potential would be due to an electrogenic potassium pump.

The purpose of the present investigation was to elucidate the nature of the ERP. To that end the effects on the ERP of perfusing the perilymphatic space with solutions having various ionic contents were studied. The nature of the negative potential occurring after anoxemia or upon applying ouabain to the perilymph was studied in a similar way. In addition the pH dependence of the ERP was studied, because it had been suggested [31] that a slight difference in pH between peri- and endolymph could give an important contribution to the ERP. Finally we studied the effect on the ERP of acetazolamide, a strong inhibitor of the carbonic anhydrase system, which is present in very high activity in the stria vascularis [8] and which had been suggested to play a role in cochlear cation transport [9, 14].

Table. *Composition of perfusion fluids*[a]

	Normal Ringer	K^+ Ringer	Li^+ Ringer	SO_4^{2-} Ringer	Choline Ringer	Sucrose Ringer
Li^+	—	—	149	—	—	—
Na^+	149	—	—	149	—	—
K^+	4.5	153.5	4.5	4.5	4.5	4.5
Ca^{2+}	1.3	1.3	1.3	1.3	1.3	1.3
Mg^{2+}	0.8	0.8	0.8	0.8	0.8	0.8
Cl^-	131.2	131.2	153.2	2.6	166.2	4.2
HCO_3^-	25	25	3	3	3	3
HPO_4^{2-}	0.5	0.5	0.5	0.5	0.5	0.5
$H_2PO_4^-$	0.5	0.5	0.5	0.5	0.5	0.5
Choline	—	—	—	—	162	—
SO_4^{2-}	—	—	—	75.3	—	—
Sucrose	—	—	—	75	—	297

[a] All concentrations in mmole/l; pH adjusted to 7.4.

Methods

The experiments were performed on guinea pigs anaesthetised with nembutal The surgical procedure, the potential recording and the perfusion techniques have been described before [22, 24]. The composition of the perfusion fluids is given in the Table.

Results

Effects of Electrolyte Changes in the Perilymph

The effects on the ERP of perfusing the scala vestibuli with sucrose Ringer and Li^+ Ringer are shown in Fig. 1. There is a remarkable similarity between the effects of the two perfusion fluids, showing a decrease of the ERP of 18 and 15% respectively after continuous perfusion for 22 min.

An initial transient increase of the potential was observed with sucrose Ringer, perfused at the rate of 10 µl/min. At a perfusion rate of 15 µl/min the increase appeared to be higher, but it was absent at a rate of 6 µl/min. Since perfusion with normal Ringer at the same rates failed to show this effect, we ascribe this transient increase to an increase in hydrostatic pressure due to the viscosity of the sucrose solution. A similar increase in potential after application of positive pressure has been reported by Tasaki *et al.* [35]. In subsequent experiments with sucrose Ringer therefore, a perfusion rate of 6 µl/min was used.

The decrease of the ERP immediately ceased and was reversed upon perfusing with normal Ringer. The recovery process varied to a certain extent in different experiments. Sometimes the potential increased to nearly its normal value (Fig. 2), but often only a slow and partial recovery was obtained. This latter phenomenon was more frequently observed

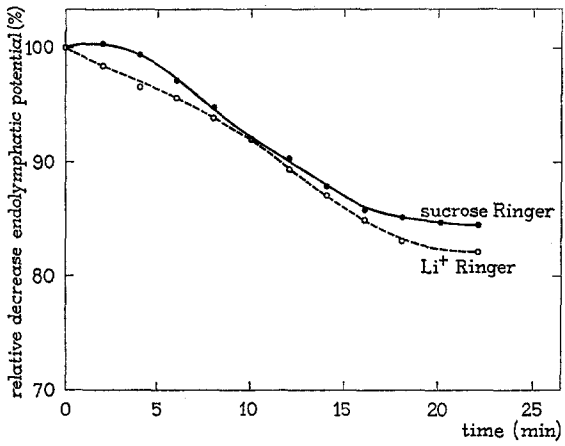

Fig. 1. Effect on ERP of continuous perfusion of the scala vestibuli with sucrose Ringer (•——•) and Li^+ Ringer (o----o). Each curve represents the mean value for six experiments

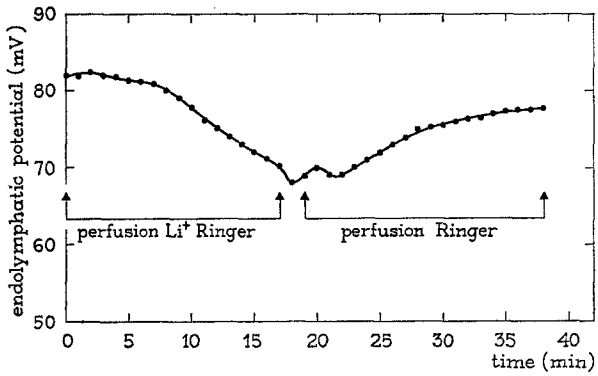

Fig. 2. Effect on ERP of continuous perfusion of the scala vestibuli with Li^+ Ringer and subsequent perfusion of normal Ringer

after extended perfusion. It seems unlikely that this effect could be due to damage of the cochlear membranes by the extended perfusion as such, because perfusion with normal Ringer for 45 min failed to show any effect on the ERP or CMP [24]. A more likely explanation would be that the absence of Na^+ affects cell metabolism or membrane permeability.

Additional evidence that the absence of Na^+ in the perfusate is responsible for the decrease of the ERP is presented in Fig. 3. The decrease of the potential, initiated by perfusion with sucrose Ringer, continued

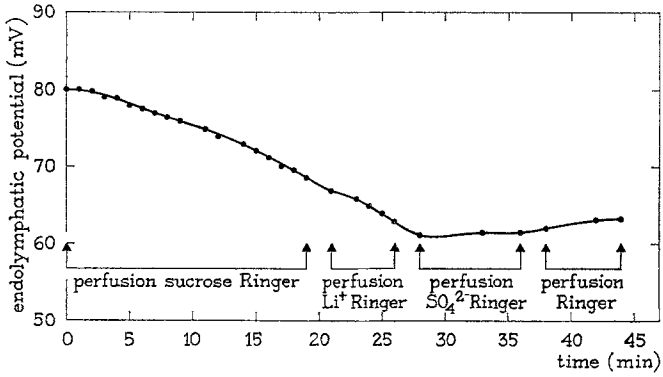

Fig. 3. Effect on ERP of continuous perfusion of the scala vestibuli with sucrose Ringer, followed by perfusion with Li^+ Ringer, SO_4^{2-} Ringer and normal Ringer

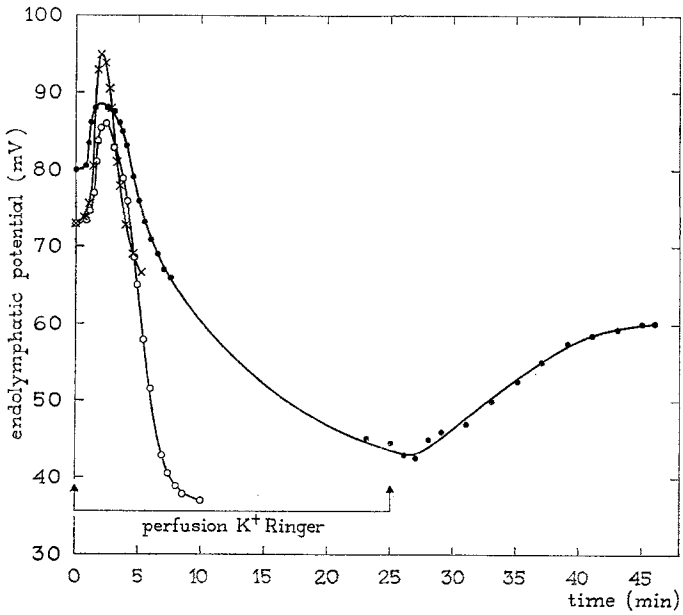

Fig. 4. Effect on ERP during and after continuous perfusion of the scala vestibuli with K^+ Ringer in three experiments

during subsequent perfusion with Li^+ Ringer, but ceased when Na_2SO_4 Ringer was perfused and a slight recovery was obtained with normal Ringer.

Fig. 4 shows the effect of perfusing the scala vestibuli with Ringer in which Na^+ was replaced by 150 mM K^+ in order to remove the K^+

gradient between the endolymph and the perilymph in the scala vestibuli. Within 1 min after starting perfusion the potential rose sharply, sometimes by more than 20 mV. Thereafter a fast decrease to about 50% of the original value was observed. When perfusion was stopped, the potential recovered slowly and only partially. This recovery, presumably due to exchange by Na^+ from the continuing flow of perilymph, could not be enhanced by perfusion with normal Ringer. The decrease of the ERP, also reported by Butler [5] after perfusing the scala tympani with 150 mM KCl, as well as the incomplete recovery upon perfusion with normal Ringer, may be due to an adverse effect of high extracellular K^+ concentration on cell metabolism [33, 37].

The contribution of Cl^- to the ERP was investigated by perfusing the scala vestibuli with Ringer in which Cl^- was replaced by SO_4^{2-} to which most membranes are much less permeable than to Cl^-. In the absence of Cl^- the potential was affected only very slightly, and this effect could be restored with normal Ringer. This is in agreement with the fact that sucrose Ringer, which has a very low Cl^- concentration, had the same effect on the ERP as Li^+ Ringer, which has a normal Cl^- content. The near equality of the Cl^- contents of the endolymph and perilymph [16] also indicates that the Cl^- contribution could not contribute significantly to the ERP.

In attempt to investigate the role of Reissner's membrane in the occurrence of the ERP, the bony wall of the exposed part of the basal turn was carefully removed and Reissner's membrane was ruptured thoroughly with a fine glass needle. The potential decreased rapidly to about +15 mV and thereafter diminished slowly, becoming zero within about 15 min. The rapid decrease of the ERP may be largely due to a leakage of ionic charges and the slow decrease to a deleterious effect of a fluid with a low K^+ and high Na^+ composition on the stria vascularis. The latter effect is confirmed by the degenerative changes in this tissue observed within a few hours after rupturing Reissner's membrane [7], and by the slow irreversible decrease of the ERP after 30 min perfusion of the scala media with high Na^+ Ringer [21]. However, when immediately after rupturing Reissner's membrane the cochlear fluid was removed by suction, the potential first increased by about 10 mV and upon ceasing suction slowly decreased to about +13 mV, coinciding with the rise of the fluid level in the cochlear space due to the perilymph production. This effect could be observed repeatedly even when the potential reached nearly zero about 15 min after the destruction of Reissner's membrane.

Effect of pH on the Potential

The dependence of the ERP on a pH difference between endolypmh and perilymph was investigated by perfusing the scala vestibuli with

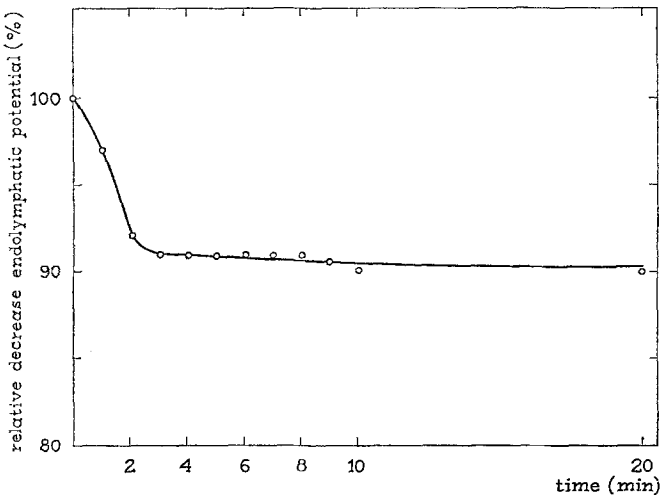

Fig. 5. Effect on ERP of continuous perfusion of the scala vestibuli with 10^{-3} M acetazolamide in Ringer's solution. Mean value for four experiments

Ringer solutions in which the pH was brought to 6.0 or 8.0 by addition of lactic acid or NaOH, respectively. Continuous perfusion with these solutions for 25 min failed to show any effect on the potential.

In another series of experiments a general respiratory acidosis was established by making the animal respire a gas mixture consisting of 80% O_2 and 20% CO_2. The pH of the blood was decreased from 7.3 to 6.8, while the pCO_2 rose to over 180 mm Hg. The ERP decreased by about 7% within 10 min, and could not be restored to normal by perfusing the scala vestibuli with normal Ringer of pH 7.4. A similar decrease in the pH of the endolymph has been reported by Misrahy *et al.* [27] in an identical experiment. When subsequently the animal was made to respire a gas mixture consisting of 95% O_2 and 5% CO_2, the potential returned within a few minutes to its original value.

The effect of acetazolamide, an inhibitor of carbonic anhydrase was studied by perfusing the scala vestibuli with Ringer containing 10^{-3} M acetazolamide. The results of four experiments are shown in Fig. 5. The potential decreased rapidly by 9(SE: 1.3)% within 3 min after starting perfusion. During the subsequent 17 min the potential remained virtually unchanged. The resemblance between the effects of CO_2 inhalation and of acetazolamide perfusion may be due to an increase of intracellular CO_2 resulting in cellular acidosis in both cases [34]. The resulting decrease in intracellular pH could inhibit the cation pump, since the Na^+–K^+-ATPase system in the stria vascularis has a pH optimum of 7.3 and its activity falls considerably at lower pH [23].

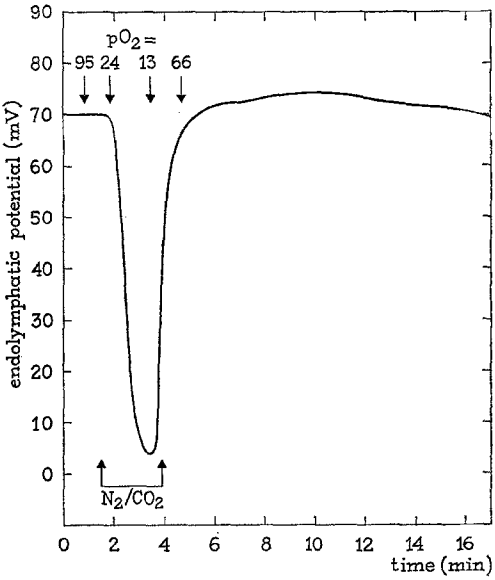

Fig. 6. The ERP and venous pO_2 (mm Hg) before, during and after temporary anoxemia from inhalation of a gas mixture consisting of 20% CO_2 and 80% N

Experiments on the Nature of the Negative Potential

The effects of anoxemia, induced by making the animal respire a mixture of 20% CO_2 and 80% N_2, is demonstrated in Fig. 6. The ERP started to decrease abruptly within 1 min, when the venous O_2 pressure fell to 24 mm Hg. The potential immediately returned after readmitting O_2. During prolonged anoxemia the potential decreased after 2 min to a negative value, which reached a maximal value after 4 min and thereafter slowly returned to zero in about 2 h. In our experiments the maximal negative value varied between 15 and 35 mV, which is in agreement with the data from other investigators [13,15,18].

It occurred to us that this negative potential might represent a K^+ diffusion potential. In order to test this, the scala vestibuli of the anoxemic animal was perfused with K^+ free Ringer. Surprisingly, this led to a rise of the potential to about +15 mV within 1 min after starting perfusion, while upon stopping perfusion the potential returned again to a negative value. This effect has previously been reported by Honrubia *et al.* [13], who suggested that it was due to removal of accumulated products of anaerobic metabolism. However, when we added 20 mM lactic acid to the perfusion fluid, the effect still occurred. This suggests that the potential reversal upon perfusion of the scala vestibuli is due to oxygen present in the perfusion fluid. To test this hypothesis, 10 mM

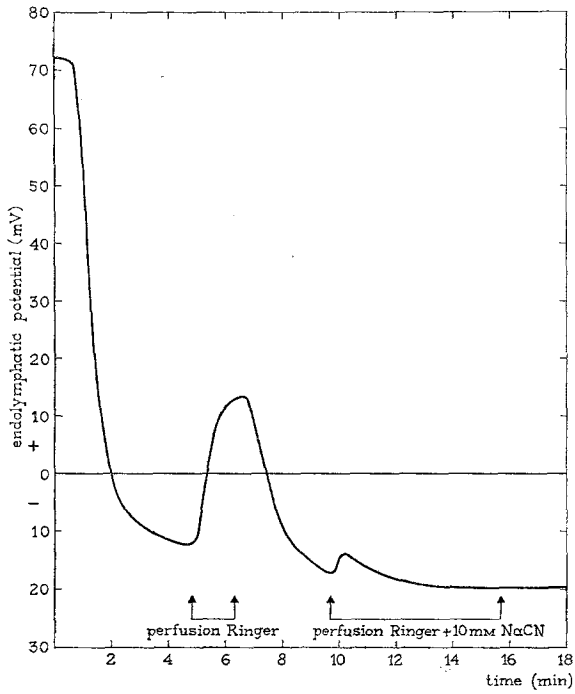

Fig. 7. Effect on ERP of prolonged anoxemia and of perfusion of the scala vestibuli of the anoxemic animal with normal Ringer and subsequently with Ringer containing 10 mM NaCN. Anoxemia was induced at $t = 0$

NaCN was added to the perfusion fluid. In this case, except for a slight transient increase, the potential remained negative (Fig. 7), which proves that the effect is indeed due to oxygen present in the perfusion fluid. In further experiments the negative potential was, therefore, obtained by the addition of 10^{-3} M ouabain to the perfusion fluid [24]. This has the additional advantage that the general metabolism of the animal remains unaffected, because the very small amount of ouabain administered to the perfusate leaves almost entirely with the outflowing mixture of perilymph and perfusate.

The scala vestibuli was perfused with Ringer containing 10^{-3} M ouabain until the potential had reached a stable negative value, usually in about 30 min. Thereafter the scala vestibuli was perfused with solutions of various ionic compositions, but always containing 10^{-3} M ouabain. When Ringer with choline replacing Na^+ was perfused, no effect on the negative potential was found (Fig. 8). The slight increase before starting choline Ringer perfusion may have been due to a movement of the electrode. When perfusion of K^+ Ringer was started, the potential rose

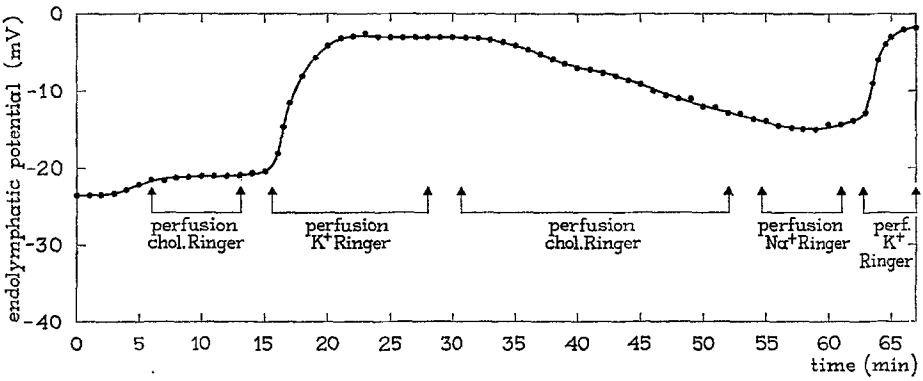

Fig. 8. Effect on the negative endocochlear potential during perfusion of the scala vestibuli with solutions of various ionic composition. The negative potential was obtained by perfusing the scala vestibuli for 30 min with Ringer containing 10^{-3} M ouabain

immediately to nearly zero in 5 min. A small fraction of the negative potential, about 2 mV, was repeatedly found to persist. Subsequent perfusion with normal or choline Ringer made the potential gradually decrease to negative values, due to removal of K^+. If K^+ Ringer was again perfused, the potential immediately increased to nearly zero (Fig. 8).

Discussion

The large increase of the ERP upon raising the K^+ concentration in the scala vestibuli and the small effect on this potential of lowering the Na^+ concentration in this compartment (Figs. 1—4) clearly show that the potential-determining cochlear membranes are much more permeable for K^+ than for Na^+. It is unlikely that the K^+ effect could be due to stimulation of the Na^+–K^+-ATPase system in the stria vascularis, because the enzyme system is nearly maximally activated at 5 mM K^+ [23], which is the normal K^+ concentration in blood plasma and interstitial fluid. Therefore, the 20 mV increase in the ERP must represent the disappearance of a K^+ diffusion potential, resulting from the K^+ gradient between perilymph and endolymph.

The permeability characteristics of the tympanal wall of the scala media cannot be very different from those of the other cochlear membranes in view of the fact that perfusion of the scala tympani with 150 mM K^+ caused an occasional increase of the ERP [5], while with Na^+ free solutions no significant effect on the ERP could be found [19]. In our experiments, the perfused fluids most likely reach through the spiral ligament the lateral and tympanal membranes changing there also

the ionic composition, because the amount of perfused fluid is high compared to the perilymph content of the perfused area and the blood volume of the whole membranous cochlea is only 0.2 μl [28].

An additional argument against a high P_{Na}/P_K ratio for the cochlear membranes might be derived from the failure to affect the ERP with tetrodotoxin [20], which blocks Na^+ channels in nerve membranes [25,29]. The suggestion of Johnstone [16] that the negative potential in anoxemia would be due to a drastic reversal of the permeability ratio is made unlikely by the occurrence of this negative potential after administration of ouabain, which is not known to affect passive cation permeabilities. These findings constitute convincing evidence against Johnstone's suggestion that the ERP would be a Na^+ diffusion potential. Johnstone (personal communication) has meanwhile obtained experimental evidence which also argues against this suggestion.

Our experiments also rule out a significant contribution of Cl^- ions and of H^+ ions to the ERP. Although prolonged inhibition of carbonic anhydrase may result in a change in the ionic composition of the endolymph [9], the slight effect of CO_2 inhalation and acetazolamide on the ERP in our experiments suggests that this enzyme is only secondarily involved in maintaining the ERP through affecting the intracellular pH.

The increase of about 20 mV, both in the ERP and the ouabain-induced negative potential, by K^+ Ringer in the scala vestibuli and the small effect of Na^+ on these two potentials, clearly show that the K^+ gradient between endolymph and perilymph does contribute to the ERP. This is, however, a negative contribution of about 20 mV. We must than conclude that the normal ERP of about + 80 mV is the sum of two potentials, a positive potential of about + 100 mV (designated E_+) and a negative potential of about − 20 mV (designated E_-) which is mainly a K^+ diffusion potential.

We still have to explain the E_+. We have shown that it cannot be an ion diffusion potential. This leaves the possibility that it is a potential, generated by an electrogenic cation pump. The cation pump must be the Na^+–K^+-ATPase system of the stria vascularis on the basis of our previously reported results [24]. The occurrence of such potentials has been observed in several tissues [6,11,17,30,36]. They all are highly sensitive to ouabain and to the action of the Na^+–K^+-ATPase system [1—3,38], while the potential is positive in the compartment to which Na^+ is being pumped. However, in the cochlea the potential is positive in the compartment from which Na^+ is extruded, which means that E_+ cannot be due to an electrogenic Na^+ transport from the endolymph. A logical conclusion would be that the E_+ is due to an electrogenic K^+ transport into the endolymph. Such a potential has so far only been observed in the midgut of the silkworm [10,39], where it was, however,

sensitive to anoxia but not to ouabain. Although there is as yet no precedent for an ouabain-sensitive electrogenic K^+ pump, there is some experimental evidence favouring this assumption for the cochlea. First, the ERP persists, though at a much lower value, after rupturing Reissner's membrane, which abolishes all ion gradients across stria vascularis and between scala media and scala vestibuli-tympani. Secondly in anoxemia the ERP disappears very rapidly, before the K/Na ratio undergoes a significant change [15].

The ERP would be high enough to maintain a large part but not all of the Na^+ gradient, because the Na^+ equilibrium potential for the observed Na^+ gradient (endolymph 0.41—1.44 mM, perilymph 115 to 180 mM [4]) lies between 129 and 150 mV. This would indicate the presence of active Na^+ transport. Furthermore Cl^- ions will tend to leak into the endolymph because of the high positive potential in this compartment. However, the actual Cl^- concentrations in the endolymph and perilymph are about equal. This suggests the existence of active Cl^- transport which extrudes Cl^- from the endolymph, as has previously been suggested by Johnstone [16], or an extrusion of Cl^- coupled to the active transport of Na^+.

The increase of the ERP during perfusion of K^+ Ringer and the initial decrease of E_- to zero under the same circumstances appeared to occur at about the same rate. However, the further decrease of E_- occurs at a much slower rate, and a potential of a few mV negative always persists. This may be due to an effect of Cl^- since at a negative cochlear potential Cl^- ions will move out of the endolymph and create a Cl^- concentration gradient between endolymph and blood-perilymph. This gradient could result in a small Cl^- diffusion potential, negative with respect to perilymph. On the other hand an incomplete change of the fluids at the tympanal wall of the scala media might also explain the slow phase in the K^+ effect on the E_-. The absence of this slow phase in the observations of the K^+ effect on the ERP may be due to the fast appearance of the deleterious effect of the high K^+ solution, resulting in a decrease of the potential.

Thus we feel that the ERP can be interpreted as composed of a larger positive potential E_+ due to an ouabain-sensitive, electrogenic K^+ pump located in the stria vascularis and a smaller negative K^+ diffusion potential E_-. A comparable situation has recently been shown to exist in the mollusk Anisodoris nobilis [26] where the resting potential of the neuron depends both on the cation gradients and on the electrogenic sodium transport. In addition we must assume an active transport of Na^+ and Cl^- out of the endolymph. Diffusion potentials for Na^+, Cl^- and H^+ do not seem to contribute to the ERP to a considerable extent. Confirmation of these conclusions could be obtained by measuring

the ionic content of the cells of the stria vascularis and by placing the stria vascularis with underlying spiral ligament in an Ussing chamber and measuring individual ion fluxes, potentials and short-circuit current. Our attempts to do this have so far failed, probably due to the smallness and fragility of the structure.

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Dr. W. Kuijpers
Department of Otolaryngology
University of Nijmegen
Geert Grooteplein Zuid 22
Nijmegen, The Netherlands