

Ionic and potential changes of the endolymphatic sac induced by endolymph volume changes

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Abstract

The endolymphatic sac (ES) is believed to be the locus for endolymph volume regulation in the inner ear. It has recently been shown that induced endolymph volume changes in the cochlea result in anatomical changes in the ES, suggesting that function of the sac varies according to endolymph volume status. In the present study we have recorded luminal concentrations of K^+ and Na^+ from the ES and the endolymphatic sac potential (ESP) during cochlear endolymph volume changes. ES recordings were made by an extradural approach, thereby preserving normal cerebrospinal fluid resting pressure. Cochlear endolymph volume changes were generated by performing injections or withdrawals through a pipette inserted into endolymph by a round window approach. The pre-treatment concentrations of K^+ and Na^+ in the ES were found to be 8.4 mM (S.D. 3.3, $n=8$) and 128.6 mM (S.D. 18.4, $n=10$) respectively, and the mean ESP was 14.4 mV (S.D. 5.2, $n=18$). Endolymphatic injections were found to produce a sustained increase in the K^+ content of the ES by an average of 19.9 mM and to decrease Na^+ by 30.7 mM measured 50 min after the start of injection. The time for K^+ increase to occur was found to correlate with the injected volume, with larger injected volumes producing a more rapid increase. Endolymphatic withdrawals were found to induce a slow decline in endolymphatic K^+ by an average of 3.4 mM measured at 50 min after withdrawal, although no significant change of Na^+ was detected. Volume-induced ESP changes were highly variable. Injections produced a small increase in the mean ESP and withdrawals produced a small decrease but neither change was statistically significant and some animals showed potential changes in the opposite direction. These data show that a change in cochlear endolymph volume status results in a physiologic response of the ES which is sustained for a considerable period. If the ES plays a part in the restoration of normal endolymph volume, this process appears to proceed slowly, based on the prolonged time courses of ionic changes observed. © 2000 Elsevier Science B.V. All rights reserved.

Key words: Cochlea; Endolymphatic sac; Endolymph volume; Endolymphatic hydrops

1. Introduction

Although the endolymphatic sac (ES) is believed to be involved in endolymph volume regulation, many details of the mechanisms underlying this process have not been established. It remains unknown how endolymph volume change is detected, exactly how the ES responds to the volume change, and how endolymph volume is ultimately corrected. The importance of the ES in volume regulation was demonstrated by observation of endolymphatic hydrops in many rodent species following surgical ablation of the endolymphatic duct

and sac (Kimura and Schuknecht, 1965; Kimura, 1967; Sziklai et al., 1992). The morphology of the ES is also sensitive to systemically applied procedures which are believed to disturb endolymph volume. Systemic glycerol administration was reported to increase the density of intraluminal contents and change the morphological appearance of cells bounding the endolymphatic space (Erwall, 1988; Erwall et al., 1988; Takumida et al., 1989; Jansson et al., 1992; Jansson and Rask-Andersen, 1993). The glycerol-induced changes were altered by pre-treatment with colchicine, which is known to inhibit synthesis and secretion of proteoglycans (Takumida et al., 1989). Meyer zum Gottesberge and Mai (1996) reported glycerol-induced alteration of CD15 expression in cells comprising the intraosseous and extraosseous portions of the endolymphatic sac. CD-15 is a

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glycoconjugate, which may be involved in water transport of cell recognition and adhesion processes. More recently, morphological changes of the ES have been shown to result from direct manipulations of endolymph volume (Rask-Andersen et al., 1999). An endolymph volume increase, generated by injection of artificial endolymph into the cochlea, decreased the density of homogeneous substance (HS) present in the sac lumen. It also produced an apparent deactivation of light cells and an activation of dark cells, in which some expanded to veil light cells. The veiling phenomenon, in which dark cells cover the light cells, has been previously described as a potential mechanism by which the light cells are regulated (Erwall, 1988). Conversely, an endolymph volume decrease generated by endolymph withdrawal from the cochlea resulted in an increased density of HS, an activation of light cells, and a deactivation and shrinking of the dark cells.

The above findings indicate that the endolymphatic sac responds to induced endolymph volume changes. To further understand the physiologic nature of this response, we have recorded luminal concentrations of Na^+ and K^+ and the endolymphatic sac potential (ESP) during cochlear endolymph volume manipulations. The luminal ion composition of the ES has been shown to be quite different from that of the rest of the inner ear, where K^+ is the predominant cation. The ionic composition of the ES contents have been measured to be approximately 12–18 mM for K^+ and 80–130 mM for Na^+ (Mori et al., 1987; Ikeda and Morizono, 1991; Mori et al., 1998; Couloigner et al., 1999). There are therefore substantial gradients for K^+ and Na^+ between the saccule and the endolymphatic sac, which are separated by the endolymphatic duct which is characterized by an extremely narrow lumen, termed the isthmus (Lundquist, 1965). It was therefore anticipated that longitudinal displacement of endolymph would result in ionic changes in the endolymphatic sac and contribute to our understanding of homeostatic processes active in the endolymphatic duct and sac.

2. Methods

The experiments required an ion-selective microelectrode to be placed into the lumen of the endolymphatic sac to record luminal composition, followed by an injection or withdrawal from a second pipette located in the endolymphatic space of the cochlea. The experimental approach is shown schematically in Fig. 1. This study reports the findings from 18 NIH strain pigmented guinea pigs in which stable ionic and ESP recordings, followed by successful endolymph volume manipulation, were achieved. Animals weighing 300–500 g were anesthetized with Inactin (sodium thiobuta-

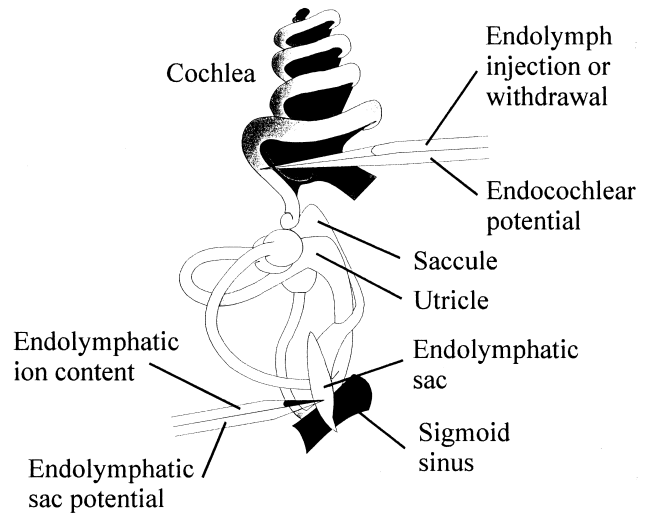


Fig. 1. Schematic of the guinea pig inner ear showing the experimental procedure. Luminal concentrations of K^+ and Na^+ and ESP were recorded by an extradural, dorsal approach to the endolymphatic sac. In addition, manipulation of cochlear endolymph volume was performed by injections or withdrawals of endolymph. This was performed with a double-barreled pipette which was inserted into endolymph through the round window and the basilar membranes, that had been accessed by a lateral approach.

barbital, RBI Chemicals, MA) at a dose of 100 mg/kg given intraperitoneally. Body temperature was maintained at 38°C with a thermistor-controlled heating pad. A cannula was placed in the left jugular vein for the administration of anesthetic supplements and for muscle relaxant (pancuronium bromide) given to effect. The trachea was cannulated and animals were artificially ventilated, maintaining an end-tidal CO_2 level close to 5%.

2.1. Recordings from the endolymphatic sac

Access to the endolymphatic sac was gained by an extradural approach. The posterior fossa and temporal bone were drilled from the dorsal side and the dura was exposed. The dura and sigmoid sinus were retracted slightly to visualize the dorsal margin of the bony niche in which the ES lies. An ion-selective electrode was inserted into the sac lumen from the dorsal side, as indicated by registration of a stable positive potential. The double-barreled ion selective microelectrodes were fabricated by techniques similar to those described in prior publications (Salt and DeMott, 1992, 1997). One side of a double-barreled pipette was silanized by exposure to dimethyldichlorosilane vapor and the tip was beveled to 2–4 μm with a Narishige EG-40 pipette grinder. For K^+ -selective electrodes, the non-silanized reference barrel was filled with 500 mM NaCl and the ion barrel was filled with 500 mM KCl, after which a small column of Fluka 60403 ion exchanger (Fluka

Chemicals, Ronkonkoma, NY) was drawn into the barrel by suction. Each electrode barrel was connected via an Ag/AgCl wire to an electrometer amplifier. A differential amplifier was incorporated to subtract the potential detected by the reference barrel from that detected by the ion barrel. Electrodes were calibrated before and after use in standards containing 2, 10 and 20 mM K^+ in a background of 150 mM NaCl. The mean sensitivity of electrodes to K^+ change was 55.8 mV/decade (S.D. 4.1, $n=9$). For Na^+ -selective electrodes the reference barrel was filled with 500 mM KCl, the ion barrel filled with 500 mM NaCl and the Fluka 71176 ion exchanger was used. Calibration standards contained 50, 100 and 150 mM NaCl. The mean electrode sensitivity to Na^+ was 54.4 mV/decade (S.D. 4.53, $n=9$). Potentials from reference and ion-selective electrodes were sampled and stored at 10 s intervals under computer control, and were transformed to ionic concentration values off-line using the experiment-specific calibration data. Although time courses are presented with 10 s resolution, error bars are only shown at 1 min intervals for clarity.

2.2. Manipulations of endolymph volume

Direct manipulations of endolymph volume of the cochlea were performed by injections or withdrawals into the endolymphatic space of the basal cochlear turn. The auditory bulla was first exposed by a post-auricular approach and was opened sufficiently to visualize the round window membrane. A double barreled pipette was inserted into endolymph of the basal turn by advancing it through the round window and basilar membrane until the endocochlear potential (EP) was registered. The tip of the pipettes was beveled to approximately 5 μ m for injections and 12 μ m for withdrawals. The barrel used to record EP contained 150 mM KCl. The side used for injections and withdrawals in the tip contained an artificial endolymph, comprised of 140 mM KCl and 25 mM $KHCO_3$ (Salt and DeMott, 1997), behind which was mineral oil. The distance moved by the oil–water meniscus was used to calculate the volume of fluid injected or withdrawn. Injections were performed mechanically, using a WPI A1400 nanopump for injection rates up to 80 nl/min and a WPI Ultrapump for higher rates. Consistent withdrawals through small-tipped pipettes could not be achieved with these pumps, so endolymph withdrawals were performed by hand in a manner identical to endolymph sampling. A 5 ml syringe was attached to the withdrawal pipette and suction applied while movement of the oil meniscus was observed. In the eight animals in which withdrawals were performed, an average of 643 nl (S.D. 217) were aspirated during a period which averaged 5.6 min (S.D. 2.3). As movements of the experimenter temporarily disturbed ion-selective

electrode recordings during endolymph withdrawals, ionic composition could not be reliably measured during the period when withdrawal was actually taking place.

Significance of induced changes were assessed by *t*-tests or Pearson correlations performed using Sigstat software (SPSS Inc.). Experimental protocols for the study were reviewed and approved by the Animal Studies Committee of Washington University, approval number 19990029.

3. Results

The baseline composition and potential of the fluid surrounding the sac and in the sac lumen, as measured in this study, are summarized in Table 1. In the ES lumen, K^+ is higher and Na^+ is lower than the respective concentrations in the fluid bathing the sac.

3.1. Endolymph volume increase

When endolymph volume was increased by injection of artificial endolymph into the cochlea, changes of K^+ concentration were observed in the ES, as shown in Fig. 2. Fig. 2 shows the result of injecting volumes varying from 237 to 1400 nl into the endolymphatic space of the cochlea. Injections occurred over a 15 min period, except for the highest rate in which 1400 nl was injected during a 7 min period. For all injections, the endolymph K^+ in the sac increased and reached a plateau which was maintained or even continued to rise slowly over the 50 min observation period. There was no indication of recovery toward the pre-injection concentration during the entire observation period. The amount of K^+ increase, summarized in the lower panel for the 15 min injections averaged 19.9 mM (S.D. 8.1, $n=5$), which was statistically significant ($P=0.0006$).

In separate experiments, Na^+ concentration was recorded in the ES during endolymph volume increases. The individual Na^+ concentration time courses and the

Table 1
Composition and potential of endolymphatic sac and surrounding fluid

	Fluid bathing endolymphatic sac	Endolymphatic sac lumen
ESP (mV)	–	14.4 S.D. 5.2 $n=18$
K^+ (mM)	4.3 S.D. 1.2 $n=8$	8.4 S.D. 3.3 $n=8$
Na^+ (mM)	157.4 S.D. 16.3 $n=10$	128.6 S.D. 18.4 $n=10$

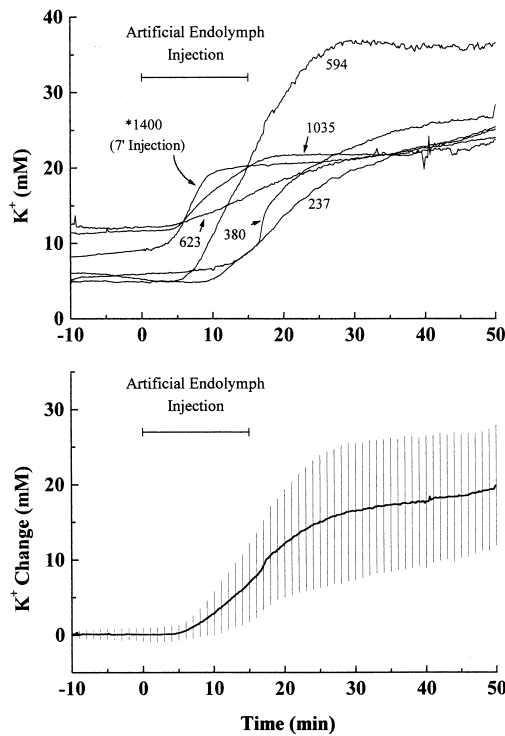


Fig. 2. Upper panel: Luminal K⁺ concentration in the endolymphatic sac in six experiments during injection of artificial endolymph into the cochlea. The total volume of artificial endolymph injected is indicated for each trace. All injections occurred over a 15 min period, except for one experiment (indicated *) where a high-rate injection was performed for only 7 min. Lower panel: Average K⁺ increase for the five experiments in which 15 min injections were performed. S.D. bars were calculated at 1 min intervals.

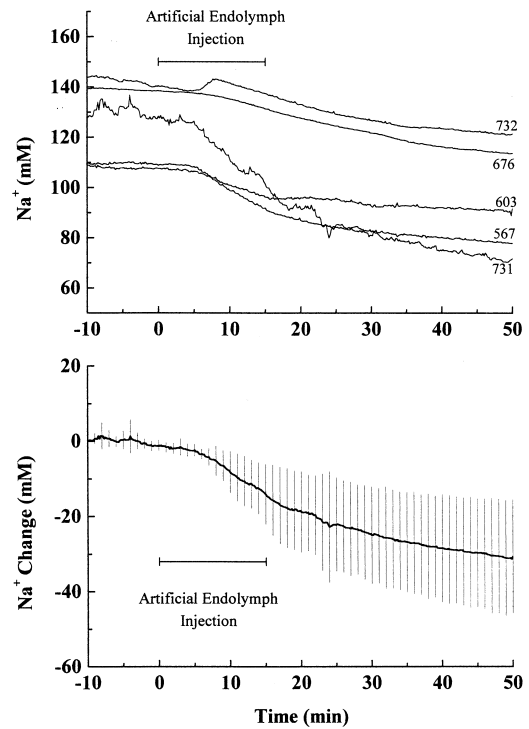


Fig. 3. Upper panel: Individual traces of Na⁺ concentration in the endolymphatic sac as a function of time during endolymph volume increase. The total volume of endolymph injected during the 15 min period is indicated for each trace. Lower panel: Average Na⁺ change with S.D. bars calculated at 1 min intervals.

average change in concentration are shown in Fig. 3. Na⁺ decreased with a time course which approximately mirrored the K⁺ increase. The mean decrease was 30.7 mM (S.D. 15.1, $n=5$) which was statistically significant compared to the pre-injection control period ($P=0.0019$). The mean Na⁺ decrease was somewhat larger than the K⁺ increase, suggesting the sac contents may become hypoosmotic during the response to endolymph volume increase. However, the absolute magnitudes of the Na⁺ and K⁺ changes were not significantly different ($P=0.20$), so it also remains possible that the ionic changes in the sac occur isoosmotically.

The magnitudes of measured K⁺ increases were remarkably consistent as the volume of artificial endolymph injected was varied, as shown in the left side of Fig. 4. The Pearson correlation coefficient between the amount of K⁺ increase and the injected volume was -0.386 , which was not significant ($P=0.45$). No attempt was made to correlate Na⁺ changes with injected volume as volume injections during Na⁺ recordings were not varied to the same degree as those for K⁺. Individual Na⁺ changes are shown only for comparison. In contrast to the consistent amount of K⁺ change,

the rate at which the K⁺ increase initially occurred did correlate with the injected volume. With larger injected volumes, the change in endolymph K⁺ occurred faster, which was also apparent in Fig. 2. The rate of response

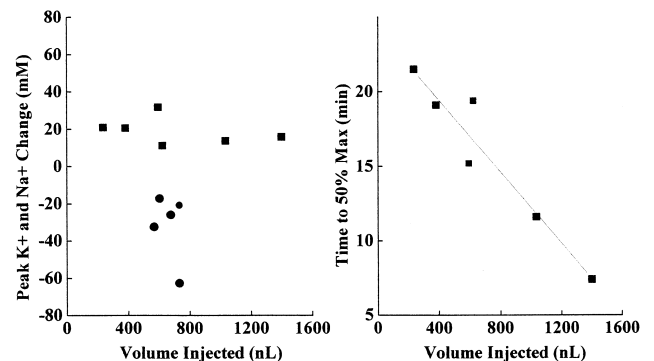


Fig. 4. Relationship between the amount of K⁺ change (left: squares) and the rate of the K⁺ response (t_{50}) (right: squares) as a function of the volume of artificial endolymph injected. The rate of the sac response (t_{50}) was calculated as the time taken to reach 50% of the maximum K⁺ change observed in the 50 min period. There was no significant correlation of the amount of K⁺ increase with injection rate, but the rate of sac response did correlate with the injection rate ($R=-0.961$). Larger injection volumes induced a faster response of the sac. Sodium decreases with endolymphatic injections are shown (left: circles) for comparison.

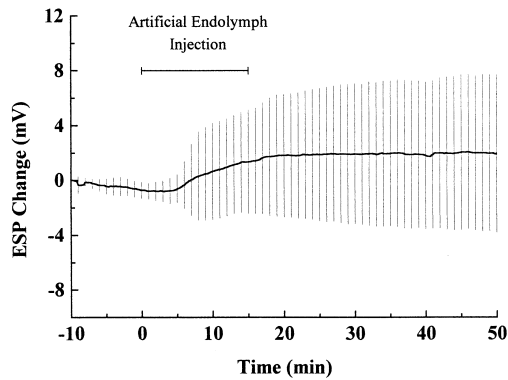


Fig. 5. Changes of ESP produced by injections of artificial endolymph into the cochlea. Bars indicate the S.D. calculated at 1 min intervals. Although the ESP increased in some animals, there was considerable variation in response and the amount of change was not statistically significant.

was quantified in terms of the time taken to reach 50% of the maximum K^+ elevation (t_{50}) seen during the 50 min observation period. The variation of t_{50} with injection volume is summarized in the right side of Fig. 4, in which a significant correlation (Pearson coefficient = -0.961 , $P=0.0022$) is apparent. Larger injected volumes thus resulted in a faster, but not larger, increase in K^+ compared to smaller volumes.

Changes of ESP measured during endolymph volume increase are summarized in Fig. 5. Although the mean response consisted of an ESP increase which averaged $+2.4$ mV (S.D. 5.6, $n=10$), there was tremendous variation in both the amount and the direction of the response, as evidenced by the standard deviation bars shown in Fig. 5. The ESP change was not statistically significant. Furthermore, the magnitude and direction of ESP change did not correlate with either the rate of injection or with the magnitude of measured ionic changes. During endolymphatic injections, the EP measured at the injection site showed a decline from the

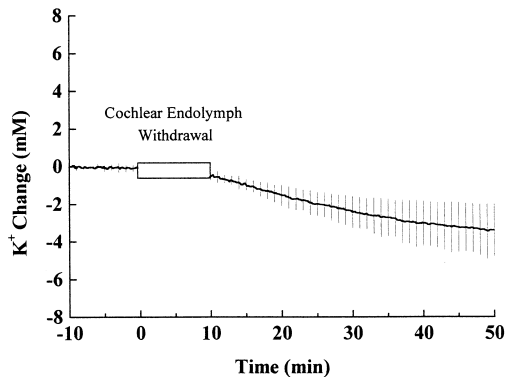


Fig. 6. Mean changes of endolymphatic K^+ induced by withdrawal of an average endolymph volume of 492 nl (S.D. 76) from the cochlea of four animals. Bars indicate S.D. which was calculated at 1 min intervals.

pre-treatment values of 93.6 mV (S.D. 2.6, $n=10$) by an amount which averaged 5.6 mV (S.D. 2.4, $n=10$) at the end of the injection period. After injection, EP recovered to reach an average value of 89.6 mV (S.D. 3.4, $n=10$) at 50 min after the onset of injection.

3.2. Endolymph volume decreases

When endolymph volume was decreased by aspirating endolymph from the cochlea, the K^+ concentration of the ES decreased, as shown in Fig. 6. The decrease occurred more slowly than with volume injection and did not appear to reach an asymptote or show any recovery towards the pre-injection value during the observation period. The decrease averaged 3.4 mM (S.D. 1.4, $n=4$) which was significant relative to the pre-injection period. In contrast, no significant changes of Na^+ concentration were observed. In four experiments where Na^+ concentration in the sac was measured following the withdrawal of an average volume of 794 nl (S.D. 208) from the cochlea, the mean Na^+ increase at 50 min was 0.9 mM (S.D. 5.8) which was not statistically significant. However, the absolute magnitudes of the K^+ and Na^+ changes were also not significantly different so it remains possible that ionic changes occur isototically.

The ESP changes during endolymphatic withdrawals are summarized in Fig. 7. Although the average response declined by 0.95 mV (S.D. 3.6, $n=8$), this change was not statistically significant relative to the pre-injection value ($P=0.47$). During withdrawals, the EP measured at the withdrawal site on average declined by 14.2 mV (S.D. 13.9, $n=8$) from the pre-withdrawal value of 88.1 mV (S.D. 7.7, $n=8$). It recovered to reach 78.7 mV (S.D. 23.1, $n=8$) at 50 min after the start of withdrawal.

In some experiments, endolymph withdrawals and injections were combined. Fig. 8 shows an experiment in which endolymph was first withdrawn from the co-

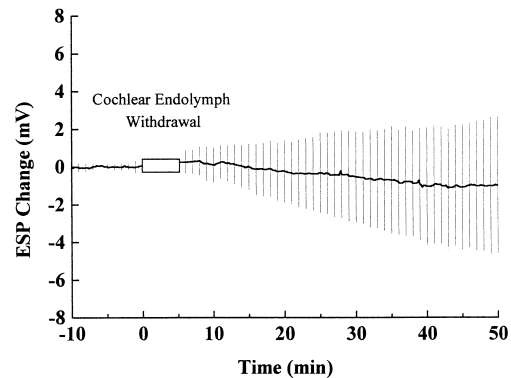


Fig. 7. Mean changes of ESP induced by withdrawal of an average of 643 nl (S.D. 218) from the cochlea of eight animals. Bars indicate S.D. which was calculated at 1 min intervals.

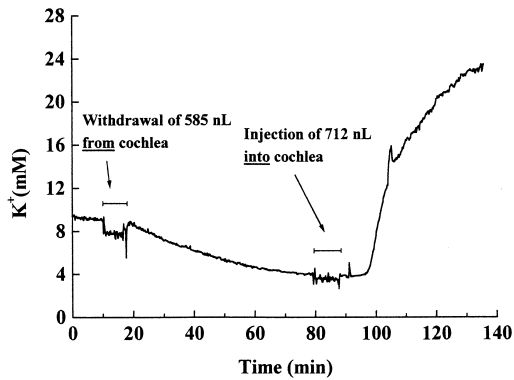


Fig. 8. Example experiment showing a slow decrease in K^+ of the ES induced by withdrawal of 585 nl of cochlear endolymph. Re-injection of 712 nl of native/artificial endolymph mixture resulted in a substantial K^+ increase after a delay of some minutes.

chlea, followed 70 min later by the injection of a slightly greater volume back into the endolymphatic space. The withdrawal resulted in a slow decline of K^+ in the ES below the pre-treatment level. Re-injection resulted in no K^+ change for approximately 8 min, following which there was a rapid increase with K^+ rising substantially above the pre-treatment level. This experiment demonstrates the bi-directional nature of the ES response within a single preparation.

4. Discussion

The present data confirm that the ES is highly sensitive to endolymph volume changes, with the composition of endolymph in the lumen undergoing substantial changes following small volume manipulations. Following endolymph volume increase, K^+ increased by 19.9 mM and Na^+ decreased by 30.7 mM. The magnitude of K change was similar as the injection rate and total volume injected was varied, but the rate at which K change occurred was faster with higher injection rates. The sac response was sustained, with no indication of recovery towards pre-manipulation levels within the observation period. This suggests that if the ES is involved in the restoration of normal endolymph volume, it must act very slowly to correct the volume disturbance.

Since little is known about the basic physiology of the ES, there are numerous explanations for the ion changes we have observed. The first possibility is that the changes arise mechanically, by the movement of high- K^+ , low Na^+ saccular endolymph into the ES lumen as a result of cochlear endolymph volume increase. If ionic turnover in the sac can be neglected, then the volume of saccular endolymph entering to induce a 19.9 mM K^+ increase can be calculated. Assuming a luminal volume of the guinea pig endolymphatic sac

of 123 nl (Shinomori et al., 1999) and a saccular K^+ concentration of 150 mM, the calculated volume which must enter is 20.3 nl, which represents an increase in the luminal volume by 16%. This is comparable to the 12% volume increase calculated to occur when an average of 574 nl is injected into the endolymphatic compartment, assuming a total endolymph volume of 4.93 μ l (Shinomori et al., 1999). However, the assumption of a low or absent rate of ion turnover of the endolymph in the ES is thought to be unlikely in view of the evidence for Na/K ATPase and Na/K/2Cl cotransport occurring in the tissues (Ichimaya et al., 1994, Teixeira et al., 1999). In this case, the observed steady-state increase of K^+ in the sac as a result of endolymph volume increase could represent an increased, and sustained, endolymph influx rate, balanced by an ongoing rate of K^+ clearance from the sac. In order to evaluate what flow and clearance rates could give the observed findings we made use of our inner ear fluids simulation model (a public-domain program available on the internet at <http://oto.wustl.edu/model.htm>) with dimensions of the endolymphatic duct and sac derived from magnetic resonance images (Ghiz et al., 2000). The ES and duct was estimated to be 2 mm long with the area varying from 0.001 mm² in the duct to an area of 0.133 mm² at the widest part of the sac and with a total luminal ES volume of 94 nl. By

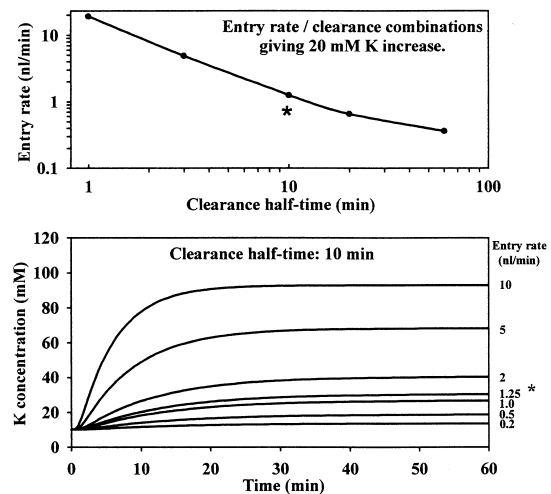


Fig. 9. Results of simulations incorporating different rates of high- K^+ (150 mM) endolymph influx into the ES, balanced by different rates of K^+ -clearance. Upper Panel: Shows calculated combinations of volume influx rate and clearance which would result in a plateau of 20 mM K^+ increase. For lower half-times (which correspond to faster rates of clearance) higher rates of endolymph influx would be required to give 20 mM increase. Lower Panel: calculated K^+ -concentration time courses for a clearance half time of 10 min, on which the point in the upper panel (marked*) is based. Each curve shows the time course for the endolymph entry rate shown at the right. With higher inflow rates, higher plateau concentrations would be achieved. An inflow rate of 1.25 nl/min resulted in a 20 mM K^+ increase, which is the value plotted in the upper panel for a clearance half-time of 10 min.

varying the simulated rates of entry of 150 mM K^+ and rates of K^+ clearance, combinations were derived which resulted in a 20 mM K^+ concentration increase as plotted in the upper panel of Fig. 9. The lower panel of Fig. 9 shows example K^+ concentration time courses for a clearance rate of 10 min half-time with varying rates of volume influx. For a K^+ clearance half-time of 10 min, a volume influx rate of 1.25 nl/min results in a 20 mM K^+ increase. Entry rates of greater than 10 nl/min are thought unlikely as this would correspond to the entry of a total volume of 500 nl over the observation period which would not be consistent with the absence of K^+ recovery for injections of a little as 237 nl. Alternatively, if K^+ turnover in the ES occurred at a rate comparable to that of cochlear endolymph, with a half-time of 55 min (Konishi et al., 1978), then a much slower rate of 0.4 nl/min (total entry of 20 nl over a 50 min period) would have to be considered. It is thought unlikely that the sac has the capacity for rapid ion clearance as lower amounts of Na/K ATPase in the ES relative to other parts of the inner ear were reported by Ichiyama et al. (1994). Combined, these data suggest that volume entry rates must be low. However, since neither the rate of K^+ clearance nor the rate of volume influx are yet reliably known, it is not possible on the basis of the present data to quantify either of these parameters.

Another possibility is that the observed ion changes may not represent saccular endolymph influx, but instead may correspond to some change in local homeostasis induced by the volume change. Prior studies (Erwall, 1988; Rask-Andersen et al., 1999) have shown changes in the luminal HS and in cellular morphology as a result of volume manipulations. It remains possible that the ionic composition of the lumen of the sac is entirely dominated by local homeostatic mechanisms. If saccular endolymph moves towards the sac, ion transport processes in the endolymphatic duct may be adequate to normalize the composition before it reaches the sac. Although this may not be in keeping with immunochemical studies that have shown Na/K ATPase is low in the endolymphatic duct (Ichiyama et al., 1994), it is possible that such normalization could involve passive ion movements and not require active processes. Thus, the ionic changes we observe could be locally generated as an integral part of the luminal changes, or could be a secondary consequence of some change in ion transport by the epithelium.

The endolymphatic boundary is unlikely to be damaged by the volumes injected in the present study as the average injected volume of 643 nl was well below that required to rupture the boundary. Takeuchi et al. (1991) injected up to 2 μ into endolymph and did not observe any membranous ruptures. Pressure measurements made during endolymphatic injections suggest

that approximately 4 μ l of solution can be injected before rupture occurs (Wit et al., 2000). It is also unlikely that volume leakages at the electrode penetration sites have any major effect on the measured ion changes. The magnitude and stability of the EP and ESP potentials measured in the study demonstrate that electrodes are placed with minimal damage. High potentials cannot be recorded when significant damage to the endolymphatic boundary occurs. In the cochlea, volume leakage around inserted electrodes was demonstrated to be minimal by the fact that leakage of an injected marker was almost nonexistent (Salt and DeMott, 1994). There is no evidence that the ES is unduly damaged by electrode penetration as our findings do not indicate a large or variable amount of endolymph outflow from this site. A more detailed characterization of leaks and clearances from the ES will require future studies with marker techniques.

The time courses of the ionic changes we measured were initially unexpected, based on our prior measurements showing the rate of basally directed endolymph flow to decline quickly after injection ceases (Salt and DeMott, 1997). However, pressure measurements made during endolymphatic injections demonstrate that the endolymphatic system behaves as two compliant compartments, corresponding to the cochlear and vestibular portions, separated by a flow resistance provided by the ductus reuniens (Wit et al., 2000). Although the calculated pressure differential between the two compartments rapidly dissipates following injections, the pressure in the vestibular endolymph slowly rises to a low value which is sustained for a long period. These findings are consistent with the rapid decline of longitudinal flow in the cochlea following endolymphatic injection, as the pressure differential declines, and also with the slower, sustained pressure increase in the vestibular endolymph representing the stimulus to the ES.

Another important finding of the study was the fact the ES shows a bi-directional response, with K^+ increases associated with endolymph volume increases and K^+ decreases associated with endolymph volume decreases. These findings are consistent with our previous morphological observations, in which the normal state was a balance between two extreme states. One extreme consisted of dense luminal HS, activated light cells and deactivated dark cells seen after endolymph volume decreases. The other extreme consisted of reduced or absent HS and activated dark cells, in some cases veiling the light cells, seen after endolymph volume increases (Rask-Andersen et al., 1999). The suggestion that the ES performs two functions under different conditions, presumably secretion and resorption, and in the normal state is balanced between these two extremes is also in agreement with our previous measurements of endolymph volume flow. In the normal co-

chlea we found longitudinal flow to be extremely slow to the extent that the rate was not significantly different from zero and could not make a significant contribution to the ionic homeostasis of cochlear endolymph. In experiments where flow markers were introduced into endolymph by volume injections, flow directed towards the endolymphatic sac was induced (Salt and DeMott, 1997; Lundquist et al., 1964; Guild, 1927) which has been shown to be the direct result of the endolymph volume disturbance induced by the injection (Salt and DeMott, 1997). Other studies have shown that if cochlear endolymph volume is reduced by osmotic dehydration, volume flow directed towards the cochlear apex is generated which may correspond to endolymph generation by the ES (Salt and DeMott, 1995). On the basis of these observations it appears likely that volume regulation is a bidirectional process in which endolymph volume is generated or resorbed according to the needs of the endolymphatic system. Other morphological studies also support the concept that under some conditions the endolymphatic sac performs a secretory role (Friberg et al., 1986; Takumida et al., 1991). Part of the variation in measured electrolyte composition in the lumen of the sac (e.g. Couloigner et al., 1999) may also be accounted for by variations in endolymph volume status at the time of testing.

The ESP showed small, inconsistent changes associated with endolymph volume manipulations and ionic changes in many cases occurred with negligible change in ESP. This contrasts markedly with the situation for cochlear endolymph, where the K^+ content is remarkably stable and cannot usually be changed without substantial changes of the EP. These observations demonstrate that the ESP cannot be used as an index of ES function with regard to volume status. Other studies have found the ESP to be sensitive to direct mechanical manipulations of the ES (Teixeira et al., 1999), interruption of the endolymphatic duct (Tsuji-kawa et al., 1992), anoxia (Mori et al., 1987), acetazolamide (Mori et al., 1998, Couloigner et al., 1998), amiloride (Couloigner et al., 1998), catecholamines (Mori et al., 1990a) and to the aldosterone antagonist, canrenoate (Mori et al., 1991). In contrast to the EP of the cochlea, the ESP is insensitive to systemically applied furosemide (Mori et al., 1990b) or perisacculary bumetanide (Couloigner et al., 1998), although it is sensitive to lumenally applied bumetanide (Teixeira et al., 1999). It is therefore apparent that the homeostatic mechanisms for endolymph in the ES differ considerably from the rest of the endolymphatic system and it remains unknown which transport systems regulate the luminal K^+ and Na^+ concentrations and the ESP.

As already mentioned, a complete interpretation of these findings is limited by the lack of supporting data concerning how the ES responds to volume changes. In

reality, we do not know whether endolymph enters the sac in volume. Both the present study and prior histological studies are not conclusive in this regard as marker substances present in saccular endolymph could quickly reach the ES lumen simply by diffusion. We do not know whether the sac can generate volume or exactly how the morphological and physiological changes of the ES relate to endolymph homeostasis. This study does, however, add to our understanding of ES physiology by demonstrating the time course and magnitude of induced physiological changes. The data are consistent with the ES performing a more active and complex function than that of resorbing endolymph volume at a uniform rate, as has been widely believed for many years.

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