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Research Article

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The Mutual Independence of the Endolymphatic Potential and the Concentrations of Sodium and Potassium in Endolymph

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ABSTRACT The relationship between the endolymphatic potential (EP) and the sodium and potassium concentration gradients between endolymph and interstitial fluid was studied both by measuring the EP at varying concentrations of sodium and potassium in endolymph and by measuring the effect of a depressed EP on the concentrations of these cations. Ethacrynic acid was used in dogs to change the concentration of sodium and potassium (meq/liter) in endolymph from 5.8 and 148 to 134 and 24.3, respectively. No change in the EP accompanied these alterations. In a second series of experiments the EP was reduced from + 72 mV to + 31 mV for a mean duration of 20 min. No change in the concentration of sodium and potassium in endolymph was found during the period of reduced EP. These data suggest that there is little relationship between the EP and the sodium and potassium concentrations in endolymph.

INTRODUCTION

Von Bekesy (1) discovered that the endolymphatic potential (EP)[1] is + 75 mV and Smith, Lowry, and Wu (2) showed that the potassium concentration in endolymph is approximately 150 meq/liter and the sodium concentration is less than 20 meq/liter. It has also been reported that the chloride concentration (3) and pH (4) (and presumably the bicarbonate concentration) of endolymph are not very different from those of plasma. Because the only known ionic concentration gradients between endolymph and interstitial fluid involve sodium and potassium, attempts have been made to implicate these ions in the genesis of the EP. Two possibilities for this relationship include a sodium diffusion potential or an inwardly directed electrogenic potassium pump (5). A sodium diffusion potential between endolymph and interstitial fluid would account for the polarity of the EP although some reservations exist concerning the precise relationship between the sodium concentration gradient and the magnitude of a possible diffusion potential (3), because of the varying reported values of the concentration of sodium in endolymph (5).

Recently Kuijpers and Bonting (6, 7) have suggested that the EP is the sum of a small negative potassium diffusion potential and a much larger positive potential due to an inwardly directed electrogenic potassium pump. Their evidence for a potassium diffusion potential lay in the finding of an 8–20 mV increase in the EP when potassium concentration gradient was decreased by perfusing the scala vestibuli with a 0.15 M potassium solution. The short duration (3 min) of this increase, followed by a rapid fall of the EP to 30% of the control value, was unexplained. An electrogenic potassium pump was deduced from their finding of progressive diminution of the EP when the scala vestibuli was perfused with solutions containing 10^{-8} M to 10^{-3} M ouabain. Kuijpers and Bonting, however, did not measure the composition of cochlear fluids after observing the ouabain effect on the potential. Konishi and Mendelsohn (8), while performing similar experiments, measured the concentrations of sodium and potassium in endolymph after the EP was reduced in response to a perfusion of the scala tympani with 10^{-5} M and 10^{-4} M ouabain. They found the concentration of potassium of endolymph was unaffected by ouabain, although the concentration of sodium rose slightly when 10^{-4} M ouabain was used.

Although attention has been placed on a relationship between the EP and sodium and potassium concentra-

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[1] *Abbreviation used in this paper:* EP, endolymphatic potential.

tion gradients, there is evidence suggesting that no such relationship exists. Utricle endolymph has a cation composition similar to that of scala media endolymph, but the EP in the utricle is + 4 mV (9). Konishi, Kelsey, and Singleton (10) showed that perfusion of the scala media with perilymph was unassociated with an immediate fall in the EP. Of particular significance is the study of Bosher and Warren (11), where it was established that in maturing infant rats the cochlear fluids developed the adult pattern of sodium and potassium concentration gradients well before the appearance of the EP.

The present experiments were designed to study the effect on the EP of altering the concentration of sodium and potassium in endolymph by ethacrynic acid administration to the dog (12), and the effect of altering the EP on the composition of endolymph.

METHODS

The surgical approach to the inner ear in dogs and guinea pigs included a ventromedial incision in the neck followed by sharp and blunt dissection to expose the auditory bulla. The bulla was removed to expose the round window. After approximately 2 μl of perilymph was obtained, the round window was removed to expose the basilar membrane, through which approximately 1.5 μl of endolymph was obtained and potential measurements made. In early experiments single-barrelled pipettes and electrodes were used with a tip diameter of 20 μm for puncture of the round window and 10 μm for puncture of the basilar membrane. In later experiments double-barrelled pipettes (13) were used with a tip diameter of 10-15 μm. One barrel was filled with mineral oil and served as a pipette and the other barrel was filled with 0.15 M KCl and served as an electrode. Measurement of the tip resistance in random electrodes revealed it to be 5×10^6 to 10×10^6 Ω.

The 0.15 M KCl electrodes were linked to an electrometer input coupler of a Beckman-Offner Model R Dynograph (Beckman Instruments Inc., Fullerton, Calif.) through silver-silver chloride electrodes. A reference 0.15 M KCl electrode was placed in a neck muscle adjacent to the dissection site. The animal was grounded.

The concentration of sodium and potassium in cochlear fluids was measured by modification of the method of Young and Schögel (14). For analyses of sodium in endolymph and potassium in perilymph, samples of approximately 900 nl were used, the precise volume depending upon the calibration of the transfer pipette. For analyses of the potassium in endolymph and sodium in perilymph, samples of approximately 250 nl were used, again the precise volume depending upon the calibration of the transfer pipette. These transfer pipettes were made from quartz capillary tubes (0.5 mm ID) pulled to a tip (0.75 mm OD), which was fire-polished. At arbitrary points on the shank of the pipette, a constriction was made by heating the quartz. A small piece of soft glass was fused over the constriction to provide a calibration mark. These pipettes were coupled to a 1 μl Hamilton syringe (Hamilton Co., Whittier, Calif.) used to aspirate fluid into the pipette. These pipettes were calibrated with 3 M nicotinamide, analyzed spectrophotometrically. The samples of endolymph and perilymph were added to 0.7 ml of a solution composed of 75% acetone and 25% glacial acetic acid. A Zeiss spectrophotometer with a MM12 double glass prism monochromator was used with the Zeiss emission flame attachment (Carl Zeiss, Inc., N. Y.). An oxygen-acetylene flame was used. Recovery of 96–104% of the sodium and potassium was found in artificial perilymph and endolymph.

Three experimental protocols were employed.

I. Perilymph and endolymph were obtained from mongrel dogs before and 10 to 40 min after the intravenous injection of ethacrynic acid (1–2 mg/kg body wt). An infusion of isotonic saline was maintained to replace losses of salt and water. In these experiments single-barrelled pipettes were used.

II. Endolymph was obtained and the EP was measured in mongrel dogs before and after intravenous injection of ethacrynic acid (1–2 mg/kg body wt). In four of these six animals double-barrelled pipette-electrodes were used. Similar studies were performed in Hartley strain guinea pigs with doses of ethacrynic acid of 2–50 mg/kg. Control endolymph aspiration was omitted in the guinea pig experiments.

III. The basilar membrane of the guinea pig was punctured and the EP was monitored. The animal was then switched from room air to a mixture of 90–95% nitrogen and 5–10% oxygen. This led to a prompt depression of the EP. After varying periods of hypoxia, endolymph was aspirated into the pipette. After a suitable volume of endolymph (approximately 1.5 μl) was obtained, the hypoxic gas mixture was replaced by room air. The electrocardiogram of the animal was monitored and remained within normal limits except for S-T segment depression. Any persistent QRS change was prevented by increasing the inspired P_{O_2}.

RESULTS

The normal composition of cochlear fluids obtained from the guinea pig and the dog is shown in Table I. Perilymph is similar in cation composition to interstitial fluid whereas the concentration of potassium (meq/liter) is 148 and 142 in endolymph obtained from the dog and guinea pig, respectively. The respective sodium concentrations of endolymph are 5.8 and 5.9.

Table II shows the effect of a single intravenous dose of ethacrynic acid (1–2 mg/kg body wt) upon the composition of cochlear fluids 10 or 40 min after injection. Although the composition of perilymph is unaffected by ethacrynic acid, endolymph composition is

TABLE I

The Normal Concentration of Sodium and Potassium of Cochlear Fluids in Dog and Guinea Pig

	Na	K
	meq/liter	
Perilymph		
Dog (n = 13)	150 (137–160)	5.8 (5.5–6.2)
Guinea pig (n = 5)	140 (138–153)	5.9 (5.8–6.4)
Endolymph		
Dog (n = 13)	5.8 (4.1–6.6)	148 (141–166)
Guinea pig (n = 6)	5.6 (5.2–5.8)	142 (138–149)

TABLE II

The Sodium and Potassium Concentration of Cochlear Fluids before and 10 or 40 min after the Intravenous Administration of 1–2 mg/kg of Ethacrynic Acid to 13 Dogs

	Perilymph		Endolymph	
	Na	K	Na	K
		meq/liter		
Before	150	5.8	5.8	148
After	149 (137–158)	5.7 (5.0–6.4)	134 (107–153)	24.3 (13–55)

Because of multiple sampling there were 28 analyses of endolymph after drug was given.

strikingly altered as manifested by a marked fall in the concentration of potassium and a reciprocal increase in the sodium concentration. Multiple samples of endolymph were obtained in several dogs after the administration of ethacrynic acid and in all cases revealed in similar changes.

The relationship between the cation composition of endolymph and the EP before and after the administration of ethacrynic acid is shown by Table III. In four of the six animals double-barrelled pipette electrodes were used to measure the EP and aspirate fluid simultaneously. In the five control ears the usual cation composition previously described was observed. The EP varied from + 48 to + 85 mV. 10 or 40 min after the injection of ethacrynic acid, the same or contralateral scala media was studied. The previously described fall in potassium concentration and rise in sodium concentration was observed, but these changes were associated with no consistent or large change in the EP.

Table IV shows the unresponsiveness of the scala media of the guinea pig to ethacrynic acid. At the doses of ethacrynic acid used there was no change in the concentrations of sodium or potassium in endolymph or in the EP.

Fig. 1 demonstrates the protocol wherein the effect of altered EP on the composition of endolymph was studied in guinea pigs. At point A the tip of the double-barrelled pipette-electrode was placed in perilymph overlying the basilar membrane. The tip was advanced through the basilar membrane at point B where the negative injury potential of the cells of the basilar membrane is demonstrated. The appearance of the positive potential marks the entrance of the tip into the scala media. At point C the ventilation gas was changed from room air to a gas mixture of 5–10% oxygen and 90–95% nitrogen. In less than 1 min the EP fell to approximately half the control value. 27 min later, at point D, endolymph was aspirated through the pipette arm of the system. The collection was terminated at point E and the animal restored to room air at point F, after which there was a prompt rise of the EP to the approximate control value. Table V shows the effect of varying periods of hypoxia and depressed EP on the concentration of sodium and potassium in endolymph. Regardless of the duration or magnitude of the fall of the EP, the sodium and potassium concentrations

TABLE III

The Concentration of Sodium and Potassium in Endolymph and the EP before and after the Intravenous Administration of 1–2 mg/kg of Ethacrynic Acid to Dogs

Control				Ethacrynic acid		
Na	K	EP	Time	Na	K	EP
meq/liter		*mV*	*min*	*meq/liter*		*mV*
5.8	136	+70	40	140	19.4	+65
6.4	166	+75	40	114	55.0	+75
5.8	149	+61	10	122	22.4	+56*
5.8	156	+48	40	122	23.2	+54*
5.8	145	+85	10	126	22.3	+75*
—	—	—	40	107	27.2	+50*
Mean 5.9	150	+68	—	122	28.2	+63

* Double-barrelled pipette electrodes.

TABLE IV

The Effect in the Guinea Pig of Varying Doses of Intravenously Administered Ethacrynic Acid upon the Concentration and the EP

EP_1	Dose	Time	EP_2	Na	K
mV	*mg/kg*	*min*	*mV*	*meq/liter*	
+90	2	45	+90	4.8	146
+75	2	40	+75	4.5	145
+80	20	45	+55	4.1	147
+60	20	30	+60	5.2	147
+65	50	35	+70	4.5	144
+55	50	40	+50	4.5	146

EP_1 and EP_2 indicate the EP before and after administration of the drug.

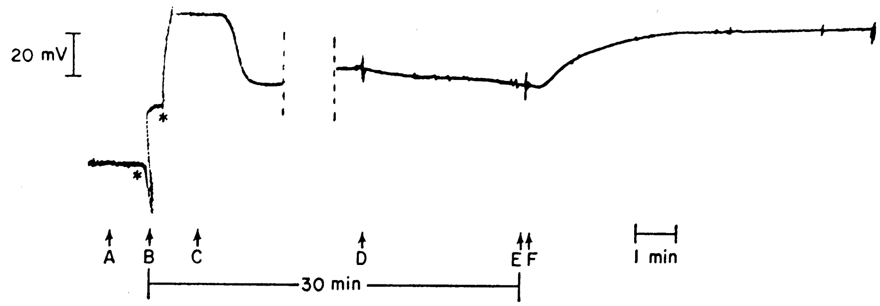

FIGURE 1 Example of the study of the effect of hypoxia on the EP and the concentration of sodium and potassium in endolymph. Points A, B, and C refer to the site of the pipette-electrode in scala tympani, within the basilar membrane and within the scala media, respectively. At point C the animal was ventilated with an hypoxic gas mixture. Endolymph was aspirated between points D and E. The animal was restored to room air at point F. The sensitivity of the amplifier between the asterisks was 2/5 of that indicated in the figure.

of endolymph are similar to that of control guinea pigs (Table I).

DISCUSSION

The normal cation composition of perilymph and endolymph described here is in agreement with previously reported values, except perhaps for the sodium concentration of endolymph obtained from the dog and guinea pig, respectively. Johnstone, in a recent review of this subject (5), concluded that the sodium concentration of endolymph was less than 2 meq/liter. This conclusion was based on his results in the guinea pig and Bosher and Warren's results in the rat (3). Johnstone ascribed the earlier-reported higher values to contamination of endolymph by perilymph during the collection procedure. The reproducibility of the values presented here, in addition to the collection of endolymph through one barrel of a pipette-electrode concomitant with the measurement of a normal EP, suggests that contamination is not an important factor in these data.

TABLE V

The Effect in the Guinea Pig of Varying Periods of Hypoxia on the EP and the Concentration of Sodium and Potassium in Endolymph

	Hypoxia				
20% O_2 EP_1	Duration	EP	Na	K	20% O_2 EP_2
mV	*min*	*mV*	*meq/liter*		*mV*
+70	30	+44	4.7	148	+59
+85	14	+24	5.2	144	+85
+67	30	+25	5.2	141	+75
+55	7	+10	5.2	144	+62
+85	27	+50	5.2	148	+70
Mean +72	20	+31	5.1	145	+71

EP_1 and EP_2 indicate the EP before and after administration of the drug.

The uncertainty as to whether the normal sodium concentration of endolymph is 1 or 5 meq/liter has little effect on the interpretation of the present data as they bear upon the relationship between sodium concentration gradients, between endolymph and interstitial fluid and the EP. The data show that both the positive polarity and magnitude of the EP are independent of the potassium or sodium concentration gradients between the scala media and interstitial fluid. These studies provide no insight into the mechanism whereby ethacrynic acid exerts its effect on cochlear fluids except to divorce the EP from the sodium and potassium gradients. Large repeated injections of ethacrynic acid (30–50 mg/kg) may cause a marked reduction of the EP as shown by Prazma, Thomas, Fischer, and Preslar (15), who used guinea pigs as the experimental animals. Silverstein and Yules (16) found an increase in the sodium concentration of endolymph after ethacrynic acid was given to cats. Table IV shows similar lack of effect of a single dose of ethacrynic acid in the guinea pig. The difference between the response of the cochlea of the dog to ethacrynic acid and that of the guinea pig and cat is similar to the species specificity demonstrated by the renal effect of this drug (17). Because such species specificity is not absolute, in that doses 50 times that which produce a saluretic response in the dog kidney are effective in the rat kidney (18), it remains surprising that ethacrynic acid does not affect the cochlea of the cat or guinea pig to a greater extent.

The demonstration in the dog of an unchanged EP at a time when the sodium concentration gradient was the opposite to that of the control values would appear to rule out the possibility that a sodium diffusion potential participates in the generation of the EP.

It has been suggested (5–7) that the EP may be due to an inwardly directed electrogenic potassium pump. The persistence of the EP at a time when the potassium gradient is the reverse of the control value (Table III) is not compatible with such an hypothesis unless one implicated an effect of ethacrynic acid on the permeability of cochlear membranes to sodium and potassium. The data in Table V are also not compatible with an electrogenic potassium pump, in that the potential can be altered markedly with no change in the concentration of potassium or sodium in endolymph. It would seem unlikely that a marked interference with the potential generating function of an electrogenic pump could occur without an alteration of its transporting function as manifested by a change in the concentration of ion involved.

Other investigators have shown changes in sodium and potassium concentration of endolymph after depression of the EP, but in these studies either complete anoxia (5) or occlusion of the blood supply (19) to the cochlea were the techniques used to cause a reduction of the EP. Deterioration of metabolically dependent concentration gradients would not be unexpected in an organ totally deprived of blood or oxygen. In the experiments shown in Fig. 1 and Table V the animal was hypoxic only to the extent that induced a diminished EP. Virtually complete recovery of this potential when the animal was ventilated with room air suggests no permanent injury of the cochlea.

These data do not allow conclusions as to the genesis of the EP except to ascribe it to mechanisms independent of the sodium and potassium concentration gradient.

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