Cochlear Function and Sodium and Potassium Activated Adenosine Triphosphatase

Abstract. *The maintenance of the cation gradients between endolymph and perilymph in the cochlea requires the operation of a cation pump. An adenosine triphosphatase system activated by sodium and potassium is present in high activity in the cochlear membranes (tegmentum vasculosum and stria vascularis). The cochlear microphonic potential is inhibited by perilymphatic perfusion of ouabain and erythrophleine. Since the microphonic potential depends on the high concentration of potassium ions in the endolymph, our findings strongly suggest the operation of an adenosine triphosphatase cation pump system activated by sodium and potassium, in the generation of cochlear cation gradients.*

In various animal species, including man, a characteristic difference between the ionic compositions of the cochlear endolymph and perilymph has been demonstrated (*1*). The ionic composition of the perilymph (Na^+, 130 to 150 meq/liter; K^+, 4 to 5 meq/liter) is similar to that of other extracellular fluids, while the composition of endolymph (Na^+, 12 to 16 meq/liter; K^+, 140 to 150 meq/liter) is similar to that of intracellular fluid. The perilymph is probably a plasma ultrafiltrate (*2*). Because of its structure the stria vascularis is thought to function in the formation of the endolymph (*3*). Rauch (*4*) has demonstrated a transport of both K^+ and Na^+ from perilymph to endolymph through Reissner's membrane. Potassium ions were transported three times as rapidly as sodium ions, and both ions were reabsorbed in the stria vascularis. The K^+ transport was inhibited by ouabain.

In view of the cation gradients between perilymph and endolymph, it is understandable that various electrical potentials have been detected in the cochlea. These potentials can be divided into resting potentials and potentials that can only be observed during acoustical stimulation (*5*). To the latter group belongs the cochlear microphonic potential (CMP). This potential follows exactly the frequency of the acoustical stimulus—without true threshold, refractory period, or adaptation—and is very sensitive to oxygen deprivation. Considerable depression of the CMP occurs when the K^+ concentration in the endolymph is decreased (*6*) and also when the K concentration in the perilymph is increased (*7*). This fact proves that the cation gradient between endolymph and perilymph is required for the occurrence of the CMP.

We have tried to determine whether the cochlear cation gradients are maintained by a Na^+- and K^+-activated adenosine triphosphatase cation pump system which is sensitive to ouabain (*8*). Enzyme assays were carried out in membranous structures of the cochlea of 1-day-old chickens and of guinea pigs. In addition, the effect of ouabain and erythrophleine on the CMP was determined in the guinea pig. Tissues for enzyme assay were quickly dissected at 0°C within 45 minutes after the animals were decapitated; they were frozen on dry ice, lyophilized, and stored at −25°C until used. The whole cochlear sac of the chicken was lifted from its cartilaginous capsule after re-

Table 1. Adenosine triphosphatase (ATPase) activities of cochlear structures from chicken and guinea pig (means with standard errors).

Structure	Determinations (No.)	Mg-activated ATPase (moles/kg dry weight per hr)	Na-K activated ATPase (moles/kg dry weight per hr)	Na-K activated ATPase (% of total ATPase)
		Guinea pig cochlea		
Total membranous structure	3	0.97 ± 0.35	0.78 ± 0.34	44 ± 2
Stria vascularis	5	4.4 ± 0.44	8.2 ± 0.63	65 ± 2
		Chicken cochlea		
Whole cochlear sac	3	4.2 ± 1.0	1.9 ± 0.37	32 ± 4
Tegmentum vasculosum	3	8.1 ± 1.4	5.1 ± 0.96	39 ± 3

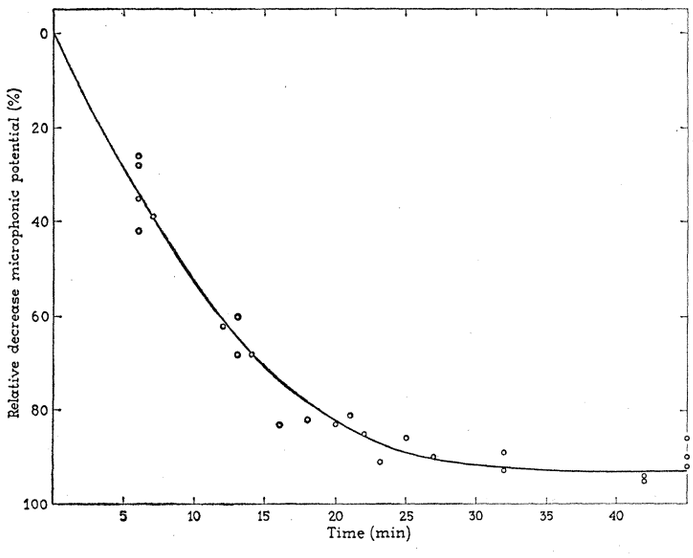

Fig. 1 (left). Time course of relative decrease of cochlear microphonic potential in guinea pig during perfusion of the scala vestibuli of the cochlea with $10^{-3}M$ ouabain in modified Krebs-Ringer solution.

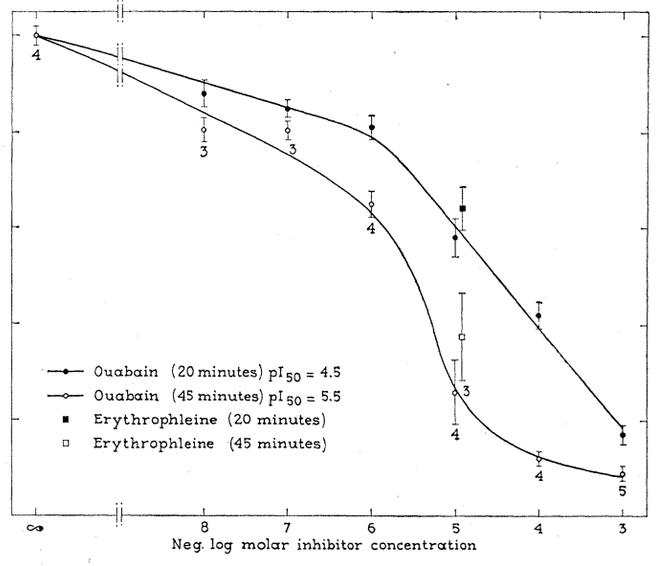

Fig. 2 (right). Relative decrease of cochlear microphonic potential upon perfusion of scala vestibuli for 20 and 45 minutes with ouabain and erythrophleine in modified Krebs-Ringer solution. Means with standard errors given with number of measurements. The pI_{50} is the negative logarithm of the concentration causing 50 percent inhibition.

moval of the medial wall of the middle ear. The tegmentum vasculosum was separated from the other cochlear structures with fine needles under 60-fold magnification. The membranous structures of the guinea pig cochlea were lifted from the bony modiolus after careful removal of the bony capsule. After the membranous structure was stretched on a glass slide, the stria vascularis from the second turn was removed from the ligamentum spirale with a hair mounted in glass. After homogenization in twice-distilled water (0.05 to 0.2 mg/100 μl, dry weight), the tissues were assayed as described by Bonting (*9*).

The CMP, obtained after acoustic stimulation by pure tones (0.2 to 10.0 kc/sec, 80 db), was recorded from a nichrome wire electrode implanted in the scala vestibuli of the first turn of a guinea pig anesthetized with nembutal, and an indifferent electrode implanted in the neck muscles. After amplification, the electric signal was displayed on an oscilloscope. The perilymphatic space (scala vestibuli) of part of the first and second turns was perfused with 10 μl of modified Krebs-Ringer solution per minute (*10*) with or without ouabain (10^{-8} to $10^{-3}M$) or erythrophleine ($10^{-5}M$). The emerging perfusate was immediately removed by suction so that entry of drug into the blood circulation would be avoided. The amplitude of the CMP was measured on the oscilloscope screen before perfusion and at various intervals after the start of perfusion.

In agreement with Iinuma's findings (*11*) we found in total membranous structures of the cochlea considerable Na^+- and K^+-activated adenosine triphosphatase activity sensitive to ouabain (Table 1). Our separate assays of stria vascularis from guinea pig and chicken tegmentum vasculosum [comprising stria vascularis and Reissner's membrane and shown to have a secretory function (*12*)] showed a remarkably high activity of this enzyme. The time course of the inhibitory effect of ouabain on the CMP is presented in Fig. 1. The inhibition of the CMP was dependent on the concentration of ouabain; erythrophleine produced a slightly smaller effect (Fig. 2). The inhibition was reversible, since after the perfusion of ouabain was discontinued, the CMP amplitude gradually increased again.

The presence of a high activity of Na^+- and K^+-activated adenosine triphosphatase that is sensitive to ouabain, particularly in the tegmentum vasculosum and the stria vascularis, and the abolition of the CMP by ouabain and erythrophleine strongly suggest the operation of a Na^+- and K^+-activated adenosine triphosphatase cation pump system in the generation of the cation gradients required for cochlear function.

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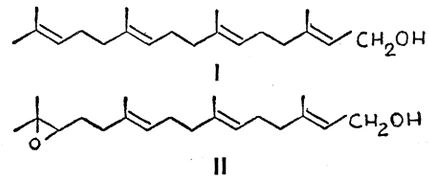
Chemoprophylactic Agent in Schistosomiasis: 14,15-Epoxygeranylgeraniol

Abstract. *The occurrence of all*-trans *(−)-14,15-epoxygeranyl-geraniol in* Pterodon pubescens *Benth. is established, and its prophylactic activity against infection by* Schitsosma mansoni *demonstrated. Two other diterpenes present in the oil are inactive.*

Certain essential oils exhibit prophylactic activity in schistosomiasis. Such a property has been attributed, for example, to North American cedarwood oil (*1*), which provides protection against the penetration of cercariae of *Schistosoma mansoni* in mice. These results stimulated the search for essential oils of Brazilian plants which might exhibit similar activity. As literature reports (*2*) indicated that it might resemble cedarwood oil in composition, attention was directed to the oil of the fruits of *Pterodon pubescens* Benth. (fam. Leguminosae Papilionoideae), commonly known as "sucupira branca." We have been unable to confirm the reported chemical similarity between the two oils, but have shown (*3*, *4*) that the *Pterodon* oil, pure or diluted, when applied topically to the tails of mice shows outstanding protective action against the penetration of cercariae of *S. mansoni* (Table 1).

Extraction of freshly collected fruits of *P. pubescens* (1 kg) with hexane gave, after evaporation of the solvent, a viscous, brown oil (100 g). Chromatography of the oil (100 g) on silica gel (2 kg), by elution with hexane and then hexane-acetone (20 : 1) gave successively geranylgeraniol (I), (−)-14,15-



epoxygeranylgeraniol (II), and a crystalline diterpene. Geranylgeraniol, purified by distillation and preparative thin-layer chromatography, was obtained as a colorless oil, b.p. 110°C at 0.01 mm; and identified by infrared (*5*) and thin-layer chromatographic comparison (*6*) with an authentic sample (*7*). The nuclear magnetic resonance (NMR) spectrum showed absorption at 1.60 [9 H (this notation indicates nine protons), singlet, three allylic methyl groups], 1.68 (6 H, singlet, two allylic methyl groups), 2.00 and 2.05 (sharp, allylic methylene), 4.15 [2 H doublet: *J* (spin-spin coupling constant) 7 hz; CH_2 OH] and 4.90 to 5.70 parts per million (ppm) (4 H, multiplet, vinyl protons), and was identical with that of geranylgeraniol from linseed oil (*5*) and from *Cedrella toona* Roxb. (*8*), as well as being compatible with an all-*trans* structure (*9*). The high-resolution mass spectrum (*10*) showed M+ (molecular ion) at 290.26094 (calculated for $C_{20}H_{34}O$, 290.26095), and M−H_2O at 272.25042 (calculated for $C_{20}H_{32}$, 272.25039). Principal fragmentation peaks were observed at *m/e* (ratio of mass to charge) 221 (M−69), 203 (M−69−18), 191 (M−69−30), 189, 161, 147 to 149, and 135 to 137. Geranylgeraniol proved inactive toward cercariae of *S. mansoni* (Table 2). (−)-14,15-Epoxygeranyleraniol (II), purified by distillation and preparative thin-layer chromatography, was obtained as a colorless oil