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STUDIES ON (Na^+-K^+)-ACTIVATED ATPASE

XXIV. LOCALIZATION AND PROPERTIES OF ATPASE IN THE INNER EAR OF THE GUINEA PIG

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SUMMARY

(1) The properties and distribution of an ouabain-sensitive (Na^+-K^+)-ATPase system in the inner ear structures of the guinea pig were investigated with ultramicrochemical techniques. The properties of this enzyme system are in good agreement with those found in other tissues.

(2) The enzyme was present in high activity in the stria vascularis (7.95 moles/kg dry wt. per h). There was a clear decrease in enzyme activity from the base of the cochlea to the apex.

(3) The enzyme activity in the other cochlear structures was rather low (<0.5 mole/kg dry wt. per h), with the exception of the highly vascularized part of the spiral ligament (1.60 moles/kg dry wt. per h).

(4) These results strongly suggest that the (Na^+-K^+)-ATPase system in the stria vascularis plays the primary role in the maintenance of the cochlear cation gradients, while the contribution of Reissner's membrane to this process can only be very minor.

(5) The good agreement between the half maximal inhibition concentration of ouabain for the enzyme system and the cochlear potentials, indicates moreover, a dependence of these potentials on the functioning of the (Na^+-K^+)-ATPase system.

INTRODUCTION

The mammalian inner ear consists of a spirally-wound three-fold tube. The middle tube is the scala media, filled with endolymph, an extracellular fluid with a cation composition resembling an intracellular medium. The scala media is surrounded on two sides with spaces filled with perilymph, the scala vestibuli and the scala tympani (Fig. 7). The cation composition of perilymph agrees with that of extracellular fluids. The characteristic difference between the ionic compositions of the cochlear fluids has been demonstrated in various animal species[1]. The fine structure of the cochlear tissues has been extensively described by IURATO[2].

The separation between scala media and scala tympani is formed by the thick fibrous membrana basilaris upon which rests, on the endolymphatic side, the organ of Corti. The scala vestibuli and scala media are separated by Reissner's membrane,

consisting of two cell layers with microvilli on the endolymphatic side. The third side of the scala media is formed by an epithelial structure, called stria vascularis, which rests on a layer of connective tissue, the spiral ligament. The cells of the stria vascularis are characterized by a large number of mitochondria, a high activity of enzymes participating in oxydative metabolism[3,4] and deeply invaginated basal and lateral cell walls. These characteristics are commonly found in cells involved in active cation transport.

JOHNSTONE[5] demonstrated that after anoxaemia the K^+/Na^+ ratio of the endolymph decreased very markedly. RAUCH[6] found a very rapid transport of radioactive potassium from vestibular perilymph to endolymph. This transport could be inhibited by NaCN, iodoacetic acid and ouabain. No transport of Na^+ and K^+ could be demonstrated from scala tympani to endolymph through the membrana basilaris. Electrophysiological studies showed that the cochlear potentials, both the resting potential between endolymph and either perilymph or blood, as well as the microphonic potential arising after acoustic stimulation, are strongly dependent on metabolic poisons[7,8] and on changes in the cationic composition of endolymph and perilymph[9–12]. These findings indicate that the cochlear cation gradients, which are underlying these potentials, are maintained by an active transport process.

In a previous publication[13] we have demonstrated the presence of a very high activity of an ouabain-sensitive (Na^+-K^+)-ATPase system in the stria vascularis of the guinea pig and in the homologous structure in the chicken. At the same time a strongly inhibitory effect of ouabain on the cochlear microphonic potential was reported. It has been demonstrated for many tissues that this enzyme system is closely involved with active cation transport[14–16].

In this paper we report the distribution of the activity of this enzyme system over the various cochlear tissues. Since a reliable histochemical technique of the (Na^+-K^+)-ATPase system is still lacking, microdissection and ultramicro enzyme assay techniques were used for this purpose. The properties of the enzyme from stria vascularis have also been determined.

MATERIALS AND METHODS

Tissue preparation

The experiments were performed on the inner ear structures of pigmented guinea pigs (250–350 g body weight). In this animal the stria vascularis is pigmented, facilitating the recognition of this structure and its separation from the other inner ear tissues. The animals were decapitated and the whole temporal bone was quickly separated from the skull. The wall of the middle ear was removed to give wide exposure of the cochlea. The bony capsule of the cochlea was chipped away carefully and the membranous inner ear structures were isolated, proceeding from apical to basal turn. The spiral ligament and stria vascularis were dissected and frozen as described earlier[13] within 45 min after decapitation, and immediately lyophilized for 3 h in tubes with silicagel at $-25°$ and vacuum-stored in the same tubes at $-25°$ until used. In order to study the localization of the enzyme activity in different parts of the spiral ligament, this structure was longitudinally divided in three parts: part A, behind the stria vascularis and bordering the scala vestibuli, part B in the region of prominentia spiralis and sulcus externus, and part C bordering the scala tympani.

Dissection of Reissner's membrane and organ of Corti was only possible after lyophilization of the whole cochlea. After removal of the bony capsule, these frozen-dried structures could easily be discerned and dissected with the use of a fine hair under high microscopic magnification. Enzyme assays, carried out on tissue samples of the stria vascularis, isolated from fresh and frozen-dried tissue, did not reveal any significant difference in ATPase activity.

The frozen-dried structures were weighed on a Cahn-electrobalance or on a quartz-fiber balance[17] in the modification designed by BONTING AND MAYRON[18].

ATPase assays

(Na^+-K^+)-activated and Mg^{2+}-activated ATPase activities were determined with the substrate media described by BONTING AND CARAVAGGIO[14]. Medium A (complete) gives total ATPase activity. The average activity in the Media B (no K^+), C (no Na^+), D (Medium A *plus* 10^{-4} M ouabain) and E (Medium B *plus* 10^{-4} M ouabain), which are inhibitory to (Na^+-K^+)-ATPase activity, give Mg^{2+}-ATPase activity. The characteristics of the (Na^+-K^+)-ATPase activity were investigated in stria vascularis homogenates. The K^+-activation curve was obtained by adding graded amounts of KCl (0–40 mM) to Medium B, the Na^+-activation curve by adding NaCl (0–150 mM) to Medium C and the Mg^{2+}-activation curve by adding $MgCl_2$ (0–6 mM) to Media A and E. The pH–activity curves were obtained by incubation in the Media A and E, prepared with Tris–HCl buffers in a pH range from 7.4 to 9.6 and with Tris–histidine buffers in a pH range from 6.2 to 7.4. The effect of ouabain on the (Na^+-K^+)-ATPase activity was determined by adding ouabain in various concentrations (10^{-2}–10^{-9} M) to Medium A.

For the enzyme assays, the frozen-dried stria vascularis and spiral ligament were homogenized in twice distilled water in an all-glass Potter–Elvehjem tissue grinder (0.04–0.15 mg dry wt./100 μl). Aliquots of 10 μl of these homogenates were added to 150 μl incubation medium. From this mixture 10-μl aliquots were transferred to microtest tubes, incubated for 1 h at 37° and assayed for inorganic phosphate[14]. Weighed samples of the organ of Corti and Reissner's membrane (0.6–2.0 μg dry wt.) were directly placed in 10 μl medium and incubated. Final tissue concentrations in these experiments varied from 0.2 to 2.0 μg dry wt. per 10 μl medium, depending on the enzyme activity of the tissues.

RESULTS

Properties of ATPase

The relative ATPase activities of the stria vascularis in the various substrate media are shown in Table I. The four inhibitory media caused approximately the same degree of inhibition (average 59 %). Medium B gave slightly less inhibition than the other media, which is probably due to partial activation of the (Na^+-K^+)-activated ATPase by the small amount of K^+ present in the tissue. This is suggested by the low K_m value (0.87 mM) for K^+ (Fig. 3). Medium C (Na^+ free) gave slightly more inhibition than the other media. This effect has been found for several other tissues[19,20] and is probably due to a slight Na^+ dependence of the Mg^{2+}-activated ATPase.

The effect of increasing the Mg^{2+} concentration in the Media A and E is rep-

resented in Fig. 1. Both (Na^+-K^+)- and Mg^{2+}-activated ATPase were maximal at 1–2 mM Mg^{2+} at an ATP concentration of 2 mM.

TABLE I

RELATIVE ATPASE ACTIVITIES OF THE STRIA VASCULARIS IN VARIOUS SUBSTRATE MEDIA

ATPase activity in Medium A set at 100. Means with S.E. and in parentheses number of determinations.

Medium	%
A (complete)	100
B (no K^+)	44.2 ± 1.9 (22)
C (no Na^+)	37.4 ± 2.2 (5)
D (10^{-4} M ouabain)	40.4 ± 1.9 (22)
E (no K^+, 10^{-4} M ouabain)	40.5 ± 1.8 (22)
Average Media B, C, D, and E	40.6 ± 1.4

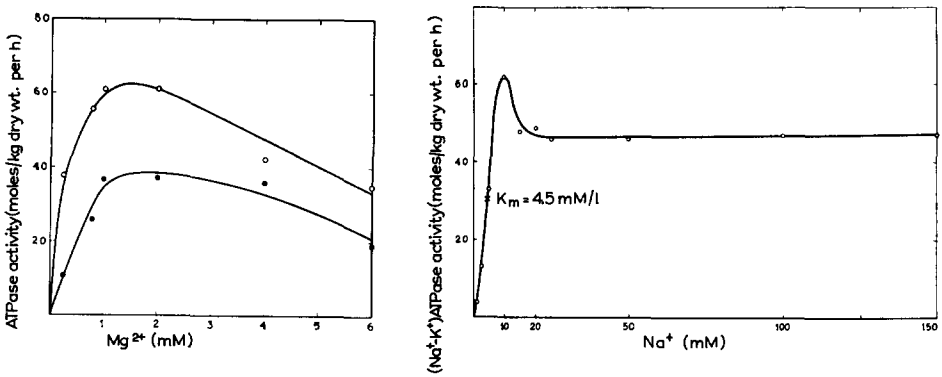


Fig. 1. Effect of Mg^{2+} concentration on (Na^+-K^+)-ATPase activity (○—○) and Mg^{2+}-ATPase activity (●—●).

Fig. 2. Effect of Na^+ concentration on (Na^+-K^+)-ATPase activity.

The activation of the (Na^+-K^+)-ATPase system by Na^+ in the presence of 5 mM K^+ is shown in Fig. 2. Maximum activity was reached at 10 mM, followed by a striking decrease between 10 and 20 mM and then remaining unchanged up to 150 mM. Half maximal activation occurred at 4.5 mM Na^+. This value is very low compared with other tissues, which may be connected with the fact that the Na^+ concentration in the endolymph (12–16 mequiv) is lower than in most intracellular fluids[6].

Fig. 3 represents the K^+-activation curve of the (Na^+-K^+)-ATPase activity in the presence of 60 mM Na^+. Maximal activation was reached at 7.5 mM and half maximal activation occurred at 0.87 mM.

The pH optimum for (Na^+-K^+)-ATPase is at 7.3 and for Mg^{2+}-ATPase at 8.7, as shown in Fig. 4.

The inhibitory effect of ouabain in various concentrations on the (Na^+-K^+)-ATPase activity is given in Fig. 5. The negative logarithm of the half-maximal inhibition concentration is 5.5, identical to the pI_{50} of 5.5 previously found by us for the inhibition of the cochlear microphonic potential[13]. At 10^{-9}–10^{-8} M ouabain there is some stimulation of the enzyme activity, which has been found in several tissues[19,32,34,36].

Distribution of the (Na+-K+)-ATPase activity in the cochlea

The ATPase activities of the stria vascularis of the various cochlear turns are listed in Table II. In these experiments the tissue concentrations in the incubation

TABLE II

ATPase ACTIVITIES IN STRIA VASCULARIS OF VARIOUS COCHLEAR TURNS

Means with S.E. and in parentheses number of determinations. Basal turn is turn No. 1, apical turn is turn No. 4.

Turn No.	*(Na+-K+)-ATPase*		*Mg2+-ATPase (moles/kg dry wt. per h)*	*Total dry weight of stria vascularis (μg)*
	Moles/kg dry wt. per h	*% of total ATPase*		
1	6.37 ± 0.40 (5)	56 ± 3	5.01 ± 0.46	13
2	7.03 ± 0.88 (7)	61 ± 4	4.32 ± 0.35	12
3	4.60 ± 0.70 (5)	57 ± 6	3.40 ± 0.58	7
4	4.35 ± 0.55 (5)	58 ± 2	3.14 ± 0.37	3

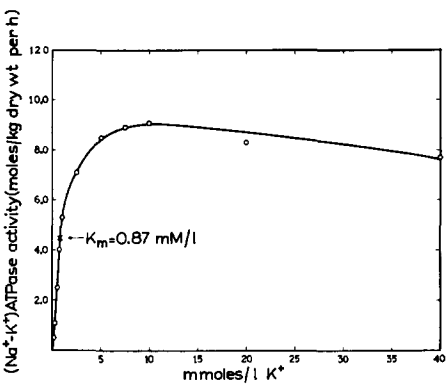


Fig. 3. Effect of K^+ concentration on (Na^+-K^+)-ATPase activity

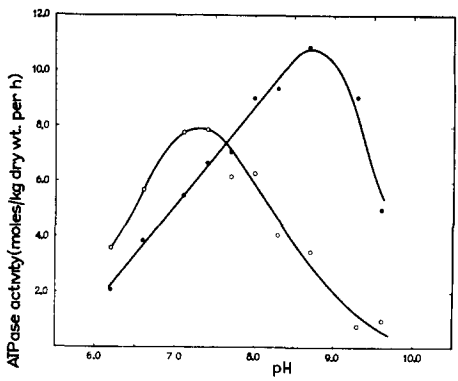
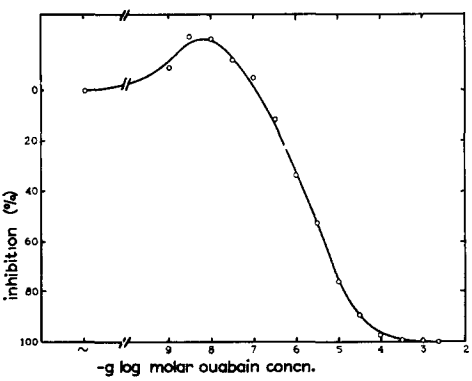


Fig. 4. Effect of pH on (Na^+-K^+)-ATPase activity (○—○) and Mg^{2+}-ATPase activity (●—●).



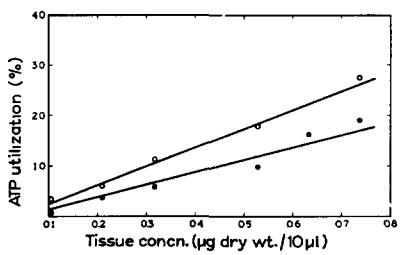


Fig. 5. Effect of ouabain on (Na^+-K^+)-ATPase activity. Negative logarithm of molar ouabain concentration causing 50% inhibition: $pI_{50} = 5.5$.

Fig. 6. ATP utilization in % of total ATP, present in incubation medium. (Na^+-K^+)-ATPase activity (○—○), Mg^{2+}-ATPase activity (●—●).

media for the third and fourth turn were considerably lower than for the first and second turn, ranging from 0.2 to 0.6 μg/10 μl. In order to exclude the possibility that this might cause the decrease of ATPase activity from first to fourth turn, shown in Table II, we determined ATPase activities for tissue concentrations varying from 0.1 to 0.7 μg per 10 μl medium. The linearity of the curve in Fig. 6 demonstrates that the decrease in enzyme activity from base to apex must be real. This effect is much more pronounced when the weight of the stria vascularis of each turn, given in Table II, is taken into account. This gives for the first turn a (Na^+-K^+)-ATPase activity of 83 moles/h, for the second 84 moles/h, for the third 32 moles/h and for the fourth turn 13 moles/h. A decrease in enzyme activity was also found in the spiral ligament and in one experiment we also observed this decrease from first to fourth turn for Reissner's membrane.

The Mg^{2+} and (Na^+-K^+)-activated ATPase activities of the different cochlear structures are given in Table III, while the distribution of the (Na^+-K^+)-ATPase

TABLE III

ATPase ACTIVITIES IN VARIOUS COCHLEAR STRUCTURES

Means with S. E. and in parentheses number of determinations.

Structure	*(Na^+-K^+)-ATPase*		*Mg^{2+}-ATPase (moles/kg dry wt. per h)*	*Mean activity of turn No.*
	Moles/kg dry wt per h	*% of total ATPase*		
Stria vascularis	7.95 ± 0.43 (16)	59 ± 1	5.57 ± 0.41	1, 2, 3
Lig. spirale				
(A) behind stria vascularis	0.41 ± 0.09 (4)	55 ± 7	0.36 ± 0.09	2, 3
(B) comprising prominentia spiralis and sulcus externus	1.60 ± 0.39 (4)	58 ± 6	1.26 ± 0.43	2, 3
(C) bordering scala tympani	0.35 ± 0.07 (4)	36 ± 7	0.62 ± 0.07	2, 3
Reissner's membrane	0.35 ± 0.06 (12)	28 ± 4	0.91 ± 0.10	1, 2, 3, 4
Organ of Corti	0.47 ± 0.27 (3)	11 ± 6	2.79 ± 0.79	2, 3

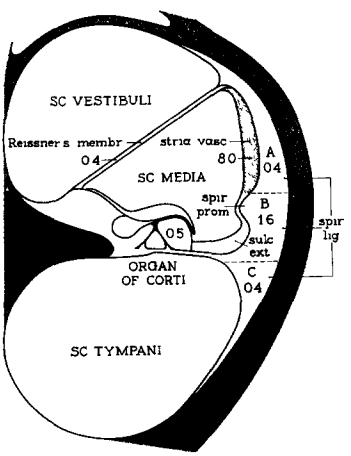


Fig. 7. Schematic cross-section of the inner ear. The amount of (Na^+-K^+)-ATPase activity is given by the numbers in the various structures (moles per kg dry wt. per h).

activity alone is schematically represented in Fig. 7. The absolute ATPase activities in Table III for the stria vascularis dissected *in toto* are 22 % higher than the sum of the absolute activities of the separate turns (Table II). This is probably due to a slight loss of activity during the extra time needed for dissection of the turns separately. Since the fourth and third turns were always isolated first, this loss could have primarily affected the results for the first and second turns. So this would tend to make the real difference between these turns and the third and fourth turns even larger. The enzyme activities for the organ of Corti and Reissner's membrane should not be influenced by the above mentioned effect, because these structures were dissected from cochleae that were lyophilized immediately after decapitation.

DISCUSSION

The (Na^+-K^+)-activated ATPase system, previously demonstrated by us in the inner ear[13], has now been studied in more detail. Its properties are quite similar to those described for this enzyme system in tissues in which cation transport against an electrochemical gradient occurs[14–16]. The stimulation by Na^+, K^+ and Mg^{2+} and the inhibition by ouabain of this ATPase system are much more pronounced than reported by IINUMA[21] for the spiral ligament–stria vascularis complex. These differences are probably due to less suitable methods used by the latter investigator, *e.g.* the use of non-homogenized, freshly dissected tissue samples without lyophilization.

The distribution of this enzyme activity in the cochlea has also been investigated. The enzyme activity of the stria vascularis is far higher than in any of the other cochlear structures and as high as in kidney tissues. This was also found to be true for other respiratory enzymes in the inner ear[3,22]. There is a clear decrease for both ATPase activities from the first to the fourth turn of the cochlea. This phenomenon has also been demonstrated for lactic and malic dehydrogenase[23] and for the O_2 consumption[24]. In addition the endolymphatic potential also decreases from base to apex[25].

The relatively high (Na^+-K^+)-ATPase activity in part B of the spiral ligament (Fig. 7) is noteworthy. This part of the spiral ligament has a very rich vascularization; it consists of very irregular cells with a high succinodehydrogenase activity[3] and large extracellular spaces. ISHII[26] recently demonstrated histochemically the presence of ATPase activity in this area and in the cells of the sulcus externus; the latter forms with the epithelial cells of the spiral prominence a continuation of the stria vascularis.

The (Na^+-K^+)-ATPase activities of the organ of Corti, Reissner's membrane and other parts of the spiral ligament are very low. On the other hand, the organ of Corti has a high Mg^{2+}-ATPase activity. NAKAI[27] demonstrated in this structure with the electron microscope, the presence of a high ATPase activity. Moreover, an active metabolism has been demonstrated in this organ[3,28]. Probably, the energy released by the Mg^{2+}-ATPase system in this organ is used for other purposes than cation transport. The organ of Corti is the site where acoustic stimuli are perceived, transduced and passed on to the nerve endings of the acoustic nerve. In view of its high (Na^+-K^+)-ATPase activity, the stria vascularis would appear to be the site of the cation pump, which maintains the ionic concentration gradient between endolymph and perilymph (Fig. 7).

The assumption by RAUCH[6] that transport of K^+ from perilymph to endolymph

would occur through Reissner's membrane, must be considered highly unlikely in view of the low (Na^+-K^+)-ATPase activity in this structure. This activity is low on a weight basis, but even more on an absolute basis. The ratio of dry weight of stria vascularis and Reissner's membrane of the whole cochlea is approx. 3:1, hence the ratio of total (Na^+-K^+)-ATPase activity is 68:1. The surface area of Reissner's membrane is about twice as large as that of stria vascularis and thus the ratio of (Na^+-K^+)-ATPase activity per unit surface area of stria vascularis and Reissner's membrane, although not quite as high as on a weight basis, is 11:1. Taking into account the multiple infoldings of the stria vascularis cell walls, the real ratio per unit active surface area must be much higher. These considerations lead us to the conclusion that the contribution of the Reissner's membrane to the active cation transport in the cochlea can only be very minor. Additional arguments for this conclusion can be derived from the fact that this membrane is avascular and has a very high electrical resistance[30].

The transport of K^+ from the vestibular perilymph to the endolymph, observed by RAUCH[6], could take place through the spiral ligament to the stria vascularis, where it would be actively secreted into the endolymph, while Na^+ would be actively removed from the endolymph by the same structure. An argument in favor of this supposition is the fact that in these experiments the spiral ligament and the stria vascularis showed a very high isotope content immediately after perilymphatic ^{42}K injection, even after interruption of the blood supply[6]. In this connection it seems unlikely that Reissner's membrane should have nearly the same Q_{O_2} as the intensively vascularized stria vascularis[31], the cells of which have a far higher number of mitochondria than the cells of the membrane. Recently, S. RAUCH (personal communication) has found a much lower O_2 consumption of this membrane than reported by CHOU[31].

The (Na^+-K^+)-ATPase system in the stria vascularis, perhaps with the aid of the cells of the sulcus externus, therefore appears to be the system which actively regulates the characteristic cation composition of the endolymph. The contribution of the other cochlear structures, judging from the (Na^+-K^+)-ATPase distribution, can be considered to be negligible. The inhibitory effect of ouabain on the stria vascularis enzyme is quite similar to that previously observed on the cochlear microphonic potential[13]. The pI_{50} for both inhibitions is identical (5.5). A similar effect of ouabain on the endolymphatic resting potential has also been demonstrated (W. KUIJPERS, unpublished observations). The stimulating effect of ouabain in low concentrations on the enzyme (Fig. 5) has been demonstrated in several other tissues[19,32,34,36], but it could not as yet be substantiated for the cochlear potentials.

In conclusion it can be stated that the (Na^+-K^+)-ATPase system in the stria vascularis appears to play a primary role in the maintenance of the cochlear gradients, while such a role seems highly unlikely for Reissner's membrane. This conclusion would be in harmony with the hypothesis of NAFTALIN AND HARRISON[33]. These authors assume that Reissner's membrane would be much less permeable to K^+ than to Na^+, and that it permits passive leakage of a perilymphatic filtrate to the endolymphatic space. From the latter space Na^+ would be removed actively, in exchange for K^+, by a Na^+ pump in the stria vascularis, leading to the typical ionic composition of the endolymph. The (Na^+-K^+)-ATPase system of the stria vascularis would be the Na^+ pump in this mechanism. In view of the strong evidence for a primary function of this enzyme system in the formation of cerebrospinal fluid[34], aqueous humor[35] and pancreatic juice[36], it would seem to be a reasonable, though not proven assumption

that the (Na^+-K^+)-ATPase system in the stria vascularis plays a similar role in endolymph formation. The two cochlear potentials, the microphonic potential and the endolymphatic resting potential, appear to depend strongly on the functioning of this (Na^+-K^+)-ATPase pump system.

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