Na⁺,K⁺-ATPase and Ca²⁺-ATPase activities in the cochlear lateral wall following surgical induction of hydrops

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Abstract

Na⁺,K⁺-ATPase and Ca²⁺-ATPase activities have not been studied quantitatively in the cochlea affected by endolymphatic hydrops. The present study was designed to measure quantitatively the Na⁺,K⁺-ATPase and Ca²⁺-ATPase activities in the cochlear lateral wall and the threshold of auditory brainstem response (ABR) for guinea pigs in the early stages (=2 months) of experimentally induced endolymphatic hydrops. A significant negative association was demonstrated between Ca²⁺-ATPase activity and the change in ABR threshold for hydropic cochleae ($P=0.014$), but not for control cochleae ($P=0.123$), although no such significant association was revealed between Na⁺,K⁺-ATPase activity and any change in ABR threshold for both hydropic cochleae ($P=0.751$) and control cochleae ($P=0.352$). A significant increase in Ca²⁺-ATPase activity in the cochlear lateral wall was observed for the hydropic ear, in which normal ABR thresholds were maintained, as compared to the control ear. On the contrary, a mild decrease in Ca²⁺-ATPase activity in the cochlear lateral wall was observed for the hydropic ear, in which ABR thresholds increased significantly. The present findings suggest that alterations of Ca²⁺-ATPase activity in the cochlear lateral wall may implicate disturbed calcium-homeostasis in the inner ear, resulting in hearing dysfunction in the early stages of experimentally induced endolymphatic hydrops. © 2001 Elsevier Science B.V. All rights reserved.

Key words: Ca²⁺-ATPase; Na⁺,K⁺-ATPase; Microcolorimetric assay; Endolymphatic hydrops

1. Introduction

Operative obliteration of the endolymphatic sac and duct results in marked endolymphatic hydrops at rates approaching 100% for the guinea pig, with maximum hydrops developing in 6–8 weeks following insult (Kimura, 1967; Konishi et al., 1981; Kumagami and Miyazaki, 1983; Albers et al., 1987a; Yazawa, 1990;

Ichimiya et al., 1994a). Experimentally induced endolymphatic hydrops has been known as a model for Meniere's disease. The only consistent morphological finding amongst patients with Meniere's disease and for the animal model is an enlargement of the endolymphatic space, or so-called endolymphatic hydrops. Although sensorineural hearing loss is a nearly constant auditory disorder for human Meniere's disease, and for experimentally induced endolymphatic hydrops in test animals such as guinea pigs, the pathophysiological mechanism leading to such hearing loss has not yet been fully elucidated. Although progressive degeneration of the sensory hair cells, spiral ganglia, stria vascularis and Reissner's membrane has been demonstrated by electron microscopic studies for experimentally induced hydrops in guinea pigs, only minor morphological changes could be observed in these tissues in the early stages of

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Abbreviations: ABR, auditory brain stem response; SPL, sound pressure level; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulfonic acid; EGTA, ethylene glycol bis(amino-ethylether) tetraacetate; OD, optical density; ELISA, enzyme-linked immunosorbent assay; CM, cochlear microphonics; EP, endocochlear potential; AP, action potential; PM, plasma membrane; ER, endoplasmic reticulum

such experimentally induced hydrops (Kimura, 1967; Kumagami et al., 1981; Kumagami and Miyazaki, 1983; Cohen and Morizono, 1984; Albers et al., 1987a,b; Kusakari et al., 1987; Ruding et al., 1987; Nadol et al., 1995). On the other hand, electrophysiological and electrochemical alterations in the inner ear as well as the elevation of the auditory threshold have always been observed in the early stages of such experimentally induced endolymphatic hydrops (Silverstein and Takeda, 1977; Konishi et al., 1981; Kumagami et al., 1981; Kumagami and Miyazaki, 1983; Aran et al., 1984; Cohen and Morizono, 1984; Kusakari et al., 1987; Meyer zum Gottesberge, 1988; Sziklai et al., 1989, 1992; Salt and DeMott, 1994a). Therefore, in the early stages of experimentally induced endolymphatic hydrops, the electrophysiological changes in cochlear function have not been shown to correlate to morphological changes in the cochlear hair cells and stria vascularis.

The obvious discrepancy between electrophysiological changes of cochlear function and morphological changes in the early stages of experimentally induced endolymphatic hydrops would suggest that, in addition to degenerative changes to the cochlea, there also exists dysfunction of the tissues of the inner ear responsible for ionic homeostasis and osmotic pressure (Meyer zum Gottesberge, 1988; Salt and DeMott, 1994a). The relative activity levels of the enzymes, which are related to the transport of ions and fluid, may play a role in alterations to the ionic composition and osmotic pressure of the inner-ear fluid in the early stages of endolymphatic hydrops. Whilst histological changes, electrophysiological changes and ionic concentration changes in the experimentally induced hydropic cochleae have been extensively studied, comparatively little has been reported relating to concomitant changes to the activities of ion-exchange enzymes in the cochleae by enzyme cytochemical studies (Hsu and Lin, 1993; Nishiyama et al., 1994) and immunocytochemical studies (Ichimiya et al., 1994a; Keithley et al., 1995; Nadol et al., 1995).

The presence of Na^+, K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall has been presumed to play an important role in ionic homeostasis of the inner ear, which is crucial to cochlear function (Kuijpers and Bonting, 1969; Kerr et al., 1982; Mees, 1983; Yoshihara and Igarashi, 1987; Yoshihara et al., 1987; Iwano et al., 1989; Schulte and Adams, 1989; Albers et al., 1991; Ichimiya et al., 1994b; Gratton et al., 1995, 1997; Curtis et al., 1997; Årup et al., 1999; Hsu et al., 2000). Although changes in ion concentration and immunohistochemical and ultracytochemical studies of Na^+, K^+ -ATPase and Ca^{2+} -ATPase in the cochlea have been studied amongst experimentally induced hydropic animals (Ichimiya et al., 1994a; Nishiyama et al., 1994; Van Benthem et al., 1994; Nadol et al., 1995), chrono-

logical changes in Na^+, K^+ -ATPase and Ca^{2+} -ATPase activity in the inner ear have not yet been studied quantitatively during the evolution of experimentally induced endolymphatic hydrops.

In order to clarify the role of Na^+, K^+ -ATPase and Ca^{2+} -ATPase in ionic homeostasis and the auditory function of hydropic ears, the present study was undertaken to quantify, using a microcolorimetric assay, the chronological alterations of the specific activities of Na^+, K^+ -ATPase and Ca^{2+} -ATPase in the cochlear lateral wall of guinea pigs in the early stages (= 2 months) of experimentally induced endolymphatic hydrops.

2. Materials and methods

2.1. Animals

A total of 32 healthy, adult male, Hartley albino guinea pigs with normal hearing, weighing 220–300 g each, were used. Thresholds of auditory brainstem response measured for test animals prior to the experiment being conducted ranged from 20–30 dB sound pressure level (SPL) for both ears of all animals included in this study. For the induction of endolymphatic hydrops, surgical obliteration of the endolymphatic sac and duct of the right ear was performed via an intradural posterior fossa approach as described by Kimura and Schuknecht (1965), such surgical procedure being performed under sterile conditions, the left ear remaining intact to be used as a control. The animals were divided into seven groups allowing for various survival periods, as described in Table 1.

2.2. Auditory brainstem response test

An evoked potential system (Nicolet, Bravo, Madison, WI, USA) was used to measure the various auditory brain stem response (ABR) thresholds. The testing procedure was as described previously (Hsu et al., 2000). The ABR thresholds were measured prior to surgery and prior to subsequent animal sacrifice for all experimental animals. The extent of auditory dysfunction was assessed by determining the difference between the initial and final ABR thresholds for both the surgically modified ears and control ears.

2.3. Tissue collection

The animals were decapitated immediately after the final ABR measurement whilst test animals were under deep anesthesia by way of an intraperitoneal injection of pentobarbital (35 mg/kg body weight). Following decapitation, the temporal bones were quickly removed within 10 s, opened to expose the otic capsule, quick-

Table 1

Mean ABR thresholds (dB SPL, mean $\pm$ S.D.) for hydropic ears and control ears of each experimental group

Group	<i>n</i>	Survival (days)	ABR thresholds			
			a	a'	b	b'
1	5	1	26.00 $\pm$ 4.18	25.00 $\pm$ 3.54	27.00 $\pm$ 2.74	39.00 $\pm$ 4.18*‡
2	5	4	27.00 $\pm$ 2.74	26.00 $\pm$ 2.24	29.00 $\pm$ 2.24	26.00 $\pm$ 2.24
3	5	7	26.00 $\pm$ 5.47	26.00 $\pm$ 5.47	26.00 $\pm$ 6.52	27.00 $\pm$ 5.70
4	4	14	27.50 $\pm$ 2.89	26.25 $\pm$ 6.29	26.25 $\pm$ 2.50	31.25 $\pm$ 4.79
5	5	28	29.00 $\pm$ 2.24	28.00 $\pm$ 4.47	28.00 $\pm$ 2.74	41.00 $\pm$ 7.42*‡
6	4	42	27.50 $\pm$ 2.89	26.25 $\pm$ 7.50	30.00 $\pm$ 2.50	31.25 $\pm$ 10.31
7	4	56	26.25 $\pm$ 2.50	22.50 $\pm$ 5.00	27.50 $\pm$ 2.89	40.00 $\pm$ 8.16*‡

a: Initial measurement of control ear prior to surgery. a': Final measurement of control ear prior to animal sacrifice. b: Initial measurement of hydropic ear prior to surgery. b': Final measurement of hydropic ear prior to animal sacrifice. *Significant difference between b and b' ($P < 0.01$, paired *t*-test). ‡Significant difference between a' and b' ($P < 0.01$, paired *t*-test).

frozen by immersion in liquid nitrogen and then stored at -70°C until microdissection was performed subsequently. Using microdissection techniques, the lateral wall of the individual cochleae was collected, preserved in 0.6 ml dilution buffer (mannitol 310 mM, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulfonic acid (HEPES) 25 mM) in a 2-ml microcentrifuge tube and stored at -10°C until assayed for enzyme-specific activity. Immediately prior to the measurement of enzyme-specific activity, the lateral wall specimens of the individual cochleae were homogenized by ultrasonication (VC-600, Sonics and Materials Inc., Danbury, CT, USA).

The care and use of the animals reported on in this study were approved by the National Taiwan University's Animal Care and Use Committee.

2.4. Microassay of lateral wall specimens

The microassay procedure was performed according to the protocol of a technique described previously (Hsu et al., 2000), of which, only a brief description is provided here. Assays of the activities of $\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ -ATPase and Mg^{2+} -ATPase were initiated in the reaction mixture containing NaCl (354 mM), KCl (14.4 mM), MgCl_2 (3.6 mM), NaHCO_3 (37.5 mM), ethylene glycol bis(amino-ethylether) tetraacetate (EGTA, 1.5 mM), glucose (33.3 mM) and ATP (9 mM), with or without ouabain (1 mM), respectively. The lateral wall homogenates (10 μl) of the individual cochlea were incubated at $37 \pm 0.5^{\circ}\text{C}$ for 40 min in the above-detailed reaction mixture. The optical density (OD) was determined at 630 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Dynatech, MR-7000, Ashford, Middlesex, UK). The $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity was assayed by adding ouabain (1 mM) and CaCl_2 (3.1 mM) and omitting EGTA in the reaction mixture described above. The enzyme-specific activity was expressed as $\mu\text{mol P}_i$ liberated/mg protein/h.

2.5. Statistical analysis

Statistical comparisons between control ears and surgically modified ears were performed using the Student's *t*-test. Two-tailed *P* values smaller than 0.05 were considered to constitute statistically significant indicators of difference between comparison populations. Regression models were used to analyze the association between enzyme activities and ABR thresholds. The statistical package SPSS 8.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis.

3. Results

3.1. Obliteration of endolymphatic sac and duct

The successful surgical destruction of the right endolymphatic sac and duct was confirmed for all experimental animals following dissection of the temporal bone.

3.2. Hearing thresholds of ABR

The mean ABR threshold for each study group is

Table 2

Mean of Na^+, K^+ -ATPase activity ($\mu\text{mol P}_i/\text{mg protein/h}$, mean $\pm$ S.D.) in the cochlear lateral wall for hydropic ears and control ears of each experimental group

Group	<i>n</i>	Survival (days)	Na^+, K^+ -ATPase activity	
			control ear	hydropic ear
1	5	1	16.67 $\pm$ 3.41	13.17 $\pm$ 3.50
2	5	4	12.45 $\pm$ 0.77	13.70 $\pm$ 2.50
3	5	7	13.83 $\pm$ 1.82	12.29 $\pm$ 1.49
4	4	14	14.40 $\pm$ 2.49	15.30 $\pm$ 3.01
5	5	28	13.84 $\pm$ 0.83	13.87 $\pm$ 2.15
6	4	42	15.80 $\pm$ 3.13	14.50 $\pm$ 3.74
7	4	56	14.15 $\pm$ 1.89	13.31 $\pm$ 1.51

No significant difference between control ear and hydropic ear of each group ($P > 0.05$, paired *t*-test).

Fig. 1. The means of Na^+, K^+ -ATPase-specific activities, as well as changes in ABR threshold, for hydropic ears (A) and control ears (B) of the seven study groups are displayed as a function of survival time (days). The means of Ca^{2+} -ATPase-specific activities, as well as changes in ABR threshold, for hydropic ears (C) and control ears (D) of the seven study groups are displayed as a function of survival time (days).

summarized in Table 1. Hearing acuity did not vary with survival time for control ears while a fluctuation in hearing thresholds measured by ABR was observed for the surgically modified ears during evolution of endolymphatic hydrops. The difference between the mean final ABR threshold for hydropic ears and that for control ears was notable for study groups with a survival time of 1 day ($P=0.009$), 4 weeks ($P=0.033$) and 8 weeks ($P=0.006$) subsequent to surgical induction of endolymphatic hydrops.

3.3. Na^+, K^+ -ATPase-specific activity

The mean values of Na^+, K^+ -ATPase-specific activity in the cochlear lateral wall of control ears and hydropic ears for all study groups are summarized in Table 2. Na^+, K^+ -ATPase-specific activity levels reveal no difference between hydropic cochleae and control cochleae

for all study groups ($P=0.517$, 0.102 , 0.365 , 0.168 , 0.484 , 0.776 , 0.226 , respectively). The means of Na^+, K^+ -ATPase-specific activities, as well as changes in ABR threshold, for hydropic ears and control ears for all study groups are displayed as a function of survival time (Fig. 1A,B). In Fig. 2A, no significant association is indicated between Na^+, K^+ -ATPase activity and a change in ABR threshold for both the hydropic ears ($P=0.751$) and the control ears ($P=0.352$).

3.4. Ca^{2+} -ATPase-specific activity

The mean values for the Ca^{2+} -ATPase-specific activity for the cochlear lateral wall for control ears and for hydropic ears for all study groups are summarized in Table 3. The mean of the Ca^{2+} -ATPase-specific activity, as well as changes in ABR threshold, for hydropic ears and control ears for all study groups are displayed as a

Fig. 2. Associations between enzyme activity and changes in ABR threshold. Regression lines, the black lines corresponding to hydroptic ears and the gray lines to control ears, were used to summarize these associations. Only for the hydroptic ears, did the Ca²⁺-ATPase activity show significant negative association with changes in ABR threshold. (A) Na⁺,K⁺-ATPase activity. (B) Ca²⁺-ATPase activity.

function of survival time (Fig. 1C,D). A significant increase in Ca²⁺-ATPase activity in the cochlear lateral wall was always apparent for the hydroptic cochleae as compared to the control cochleae at 4 days ($P=0.019$), 1 week ($P=0.017$), 2 weeks ($P=0.031$) and 6 weeks ($P=0.019$) subsequent to surgical induction of hydrops, whilst no significant change in ABR threshold was shown between the hydroptic ear and the control ear ($P=1, 0.784, 0.253, 0.463$, respectively). Contrasting this, a mild decrease of the Ca²⁺-ATPase activity in the cochlear lateral wall was demonstrated for the hydroptic cochleae as compared to the control cochleae at 1 day ($P=0.394$), 4 weeks ($P=0.592$) and 8 weeks

($P=0.316$) post-surgery, whilst a significant increase in ABR threshold was shown between the hydroptic ear and the control ear ($P=0.008, 0.033, 0.006$, respectively). In Fig. 2B, the significant negative association between Ca²⁺-ATPase activity and the change in ABR threshold for the hydroptic ears is revealed ($P=0.014$), but not so for the control ears ($P=0.123$). It is strikingly observed that those hydroptic cochleae with normal ABR thresholds show elevated levels of Ca²⁺-ATPase activity.

4. Discussion

The present study results reveal the fluctuating levels of Ca²⁺-ATPase activity in the cochlear lateral wall of surgically modified ear during the early stages of evolution of surgically induced endolymphatic hydrops. Although the underlying mechanism of fluctuation of Ca²⁺-ATPase activity is not exactly known, the alterations of Ca²⁺-ATPase activity in the hydroptic cochlea may be attributable to the fluctuating characteristics of the pathogenesis of early endolymphatic hydrops. A significant increase in the specific activity of Ca²⁺-ATPase was observed as early as four days following the surgical induction of endolymphatic hydrops ($P=0.019$) and a significant negative association has been shown between Ca²⁺-ATPase activity in the cochlear lateral wall and changes in ABR threshold for

Table 3

Mean of Ca²⁺-ATPase activity (μmol Pi/mg protein/h, mean ± S.D.) in the cochlear lateral wall for hydroptic ears and control ears of each experimental group

Group	n	Survival (days)	Ca ²⁺ -ATPase activity	
			control ear	hydroptic ear
1	5	1	13.06 ± 1.01	12.21 ± 2.61
2	5	4	15.21 ± 1.78	19.42 ± 4.19*
3	5	7	14.90 ± 1.88	19.15 ± 3.39*
4	4	14	14.66 ± 1.12	17.41 ± 2.31*
5	5	28	13.04 ± 1.17	12.67 ± 1.06
6	4	42	13.38 ± 2.84	19.74 ± 3.47*
7	4	56	13.86 ± 1.75	12.68 ± 0.23

*Significant difference between control ear and hydroptic ear of each group ($P<0.05$, paired *t*-test).

hydropic ears as compared to control ears ($P=0.014$). On the other hand, no significant difference of Na^+, K^+ -ATPase activity in the cochlear lateral wall was noted between the hydropic cochleae and control cochleae for all study groups for the period from one day to eight weeks following surgical induction of endolymphatic hydrops ($P=0.517, 0.102, 0.365, 0.168, 0.484, 0.776, 0.226$, respectively). In the early stages of experimentally induced endolymphatic hydrops, therefore, Ca^{2+} -ATPase activity in the cochlear lateral wall may be involved in ABR threshold changes, but Na^+, K^+ -ATPase activity would apparently not.

Contrasting the previous studies of reduced immunoreactivity of Ca^{2+} -ATPase in the cochlear lateral wall of hydropic ears of guinea pig (Ichimiya et al., 1994a; Nadol et al., 1995), the quantitative observations of the present study revealed significant increase in Ca^{2+} -ATPase activity in the cochlear lateral wall of guinea pig in the early stages of experimentally induced endolymphatic hydrops. This discrepancy may be attributable to the semi-quantitative measurement provided by immunostaining and the quantitative measurements of a biochemical assay such as ours. Previous immunohistochemical studies have not revealed a decline in the Na^+, K^+ -ATPase activity of the cochlear lateral wall of the guinea pig until six months after the induction of endolymphatic hydrops (Ichimiya et al., 1994a; Nadol et al., 1995). Ultracytochemical studies have also revealed a decline in the Na^+, K^+ -ATPase activity in the cochlear lateral wall of guinea pig at 11 weeks and also at one year following surgical induction of endolymphatic hydrops (Nishiyama et al., 1994; Van Benthem et al., 1994). This reduction in Na^+, K^+ -ATPase activity was always connected with degenerative changes in the stria vascularis of hydropic cochleae following long-term subject survival (Nishiyama et al., 1994; Van Benthem et al., 1994). The relatively late and minor changes in Na^+, K^+ -ATPase activity of experimentally induced endolymphatic hydrops provide support for the hypothesis that alterations in Na^+, K^+ -ATPase activity level do not mediate the primary pathophysiological process resulting in Meniere's disease and represent a secondary pathophysiological event (Van Benthem et al., 1994).

It has generally been found across a variety of studies that hydrops develops within days and significant decreases in the electrophysiological measurements are observed in the first several weeks subsequent to the surgical induction of endolymphatic hydrops in guinea pig. Reduced cochlear microphonics (CM) (Konishi et al., 1981; Kumagami et al., 1981; Kumagami and Miyazaki, 1983; Kusakari et al., 1987; Klis et al., 1990), reduced endocochlear potential (EP) (Konishi et al., 1981; Cohen and Morizono, 1984; Sziklai et al., 1992; Salt and DeMott, 1994a,b) as well as the

elevation of the threshold of the compound action potential (AP) (Aran et al., 1984; Horner and Cazals, 1986; Böhmer and Dillier, 1990; Horner, 1991; Sziklai et al., 1992; Salt and DeMott, 1994a,b) have all been demonstrated in the early stages of experimentally induced endolymphatic hydrops in guinea pig. Despite the variability in the electrophysiological response of hydropic animals, the most consistent finding associated with the development of hydrops was found to be an elevation of AP threshold in the 2–4 kHz region by Salt and DeMott (1994a,b). They observed that the elevation of AP threshold was generally more variable for hydropic ear at from four days to four weeks following surgical obliteration of the endolymphatic sac than in the later stages of surgically induced endolymphatic hydrops. They also found that auditory dysfunction in the hydropic cochlea did not correlate directly with the degree of endolymphatic hydrops or the passage of time following obliteration of endolymphatic sac and duct. In the present study, auditory function was evaluated with changes of click-evoked ABR thresholds. We are surprised to observe that a significant increase in ABR threshold was observed in the surgically modified ear as compared to the control ear on the first postoperative day ($P=0.009$) whilst no change in specific activity for both Ca^{2+} -ATPase and Na^+, K^+ -ATPase was noted in the cochlear lateral wall of the surgically modified ear ($P=0.394, 0.517$, respectively). We infer, thus, that this auditory dysfunction is due to hemorrhage and trauma of the brainstem and dura over the operculum deliberately created during obliteration surgery of the endolymphatic sac and duct. Moreover, Walsted et al. (1991 and 1994) observed a significant loss of hearing immediately subsequent to neurosurgery, with recovery proceeding over a 1-week period, and they inferred that a transient perilymphatic hypotonia resulted directly from loss of the cerebrospinal fluid and decreased cerebrospinal fluid pressure during surgery. We also found that the caliber of the hearing in the control ears did not change with increasing survival time whilst a variation in the ABR threshold for the surgically modified ears was observed during the evolution of endolymphatic hydrops. Further, alteration in final ABR threshold for the hydropic ears was closely correlated with the level of Ca^{2+} -ATPase activity in the cochlear lateral wall, but not the passage of time following surgical induction of hydrops. Salt and DeMott (1994a,b) have observed that the elevation of AP threshold correlated more closely with the endolymphatic $[\text{Ca}^{2+}]$ level than it did with the endolymph volume for some experimental hydropic ears of guinea pigs. Their data, in conjunction with the present results, support the concept that disturbed Ca^{2+} homeostasis of the inner ear contributes to cochlear dysfunction during the evolution of endolymphatic hydrops, even if it may not be

the primary factor responsible for the generation of hydrops.

Endolymphatic hydrops was observed in the cochleae of guinea pigs, even for those animals sacrificed 1 or 2 days following the obliteration of the endolymphatic sac (Kimura, 1967; Sakagami et al., 1995), and was always observed in the guinea pigs surviving for more than 2 weeks subsequent to surgical induction (Kumagami and Miyazaki, 1983; Albers et al., 1987a). Although some studies have reported an altered concentration of Na^+ , K^+ and Cl^- ions in hydropic cochleae at from 6 to 8 weeks subsequent to surgical induction of endolymphatic hydrops (Silverstein and Takeda, 1977; Sziklai et al., 1992), the majority of previous studies have noted no change to the endolymphatic and perilymphatic concentrations of Na^+ , K^+ and Cl^- ions in the early stages of the experimentally induced endolymphatic hydrops (Konishi et al., 1981; Cohen and Morizono, 1984; Sziklai et al., 1992). By contrast, disturbed calcium-homeostasis in the inner ear was observed in the early stages of experimentally induced endolymphatic hydrops (Meyer zum Gottesberge-Orsulakova and Kaufmann, 1986; Meyer zum Gottesberge, 1988; Salt and DeMott, 1994a,b; Wang and Zhang, 1994; Zhang et al., 1995). Disturbed calcium-homeostasis was reflected by the increase of endolymph $[\text{Ca}^{2+}]$ as well as intracellular $[\text{Ca}^{2+}]$ for the secretory cells of the inner ear. A time-dependent increase in intracellular $[\text{Ca}^{2+}]$ was shown for the vestibular light cells of the guinea pigs during the first week following surgical induction of hydrops and a very prominent increase in intracellular $[\text{Ca}^{2+}]$ was observed at 3 months subsequent to surgical induction of hydrops (Meyer zum Gottesberge-Orsulakova and Kaufmann, 1986; Meyer zum Gottesberge, 1988). A striking correlation between increased endolymph $[\text{Ca}^{2+}]$ and decreased EP was observed at 12 months following surgical induction of hydrops in guinea pig (Meyer zum Gottesberge and Ninoyu, 1987; Meyer zum Gottesberge, 1988). Salt et al. (1989) demonstrated an endolymph Ca^{2+} gradient along the length of the cochlea and proposed that the Ca^{2+} gradient be partly related to the gradient of EP, this Ca^{2+} gradient causing Ca^{2+} to be driven out of the cochlea through the ductus reuniens. On the basis of these observations, Salt and DeMott (1994a,b) suggested that the endolymphatic sac may play a role in the homeostasis of endolymph Ca^{2+} , they also found that endolymph $[\text{Ca}^{2+}]$ tended to increase with time after surgical induction of endolymphatic hydrops for the guinea pigs. These authors also noted that the degree of elevation of endolymph Ca^{2+} was very small in the very early stages of hydrops evolution, whilst a significant increase was observed at 16 weeks ($P < 0.05$) following the surgical induction of endolymphatic hydrops, when endolymph volume was no longer in-

creasing. Moreover, Wang and Zhang (1994) and Zhang et al. (1995) observed a significant increase ($P < 0.01$) in endolymph $[\text{Ca}^{2+}]$ in the hydropic cochlea of the albino guinea pig at 1–3 months following obliteration of the endolymphatic sac and duct. Disturbed Ca^{2+} homeostasis in the inner ear, leading to increased endolymph $[\text{Ca}^{2+}]$, has been proposed to be involved in the pathogenesis of endolymphatic hydrops by these authors. It has been generally recognized that endolymph $[\text{Ca}^{2+}]$ plays an important role in the mechano-electrical transduction performed by cochlear hair cells (Ichimiya et al., 1994b; Curtis et al., 1997; Årgrup et al., 1999), this increased endolymph $[\text{Ca}^{2+}]$ probably causing abnormal stimulation of the physiological processes of outer hair cells and modifying the transduction processes in the sensory hair cells and neural transmitter release (Meyer zum Gottesberge, 1988; Salt and DeMott, 1994a).

Under normal conditions, extracellular $[\text{Ca}^{2+}]$ is in the range of 10^{-3} to 10^{-5} M whilst $[\text{Ca}^{2+}]$ in the cytoplasm is in the range of 10^{-7} to 10^{-8} M (Ikeda et al., 1987; Meyer zum Gottesberge, 1988; Salt et al., 1989; Årgrup et al., 1999), this gradient of Ca^{2+} across the cell membrane being maintained by a balance between Ca^{2+} influx through the plasma membrane (PM) channels and Ca^{2+} efflux through a PM Ca^{2+} -ATPase and Na^+ - Ca^{2+} exchanger (Friedman and Gesek, 1995; Årgrup et al., 1999). Enzyme cytochemical studies have revealed high levels of Ca^{2+} -ATPase activity along the folded PM of the stria marginal cells and the apical and lateral PMs of the spiral prominence, and much less activity in the organ of Corti of the guinea pig (Yoshihara and Igarashi, 1987). Immunocytochemical studies have also revealed significantly greater levels of PM Ca^{2+} -ATPase and endoplasmic reticulum Ca^{2+} -ATPase (ER Ca^{2+} -ATPase) in the stria vascularis than in the spiral ligament and the organ of Corti of the guinea pig (Curtis et al., 1997; Årgrup et al., 1999). It has been suggested that endolymph Ca^{2+} enters the apical aspects of the stria marginal cells and is transported out at the basolateral aspects by PM Ca^{2+} -ATPase whilst cytosolic free Ca^{2+} may be sequestered into the ER as storage by ER Ca^{2+} -ATPase (Curtis et al., 1997; Årgrup et al., 1999). Meyer zum Gottesberge (1988) has suggested that the destruction of the endolymphatic sac reduced the overall surface epithelium available for the transcellular transport of endolymph Ca^{2+} , resulting in an increased endolymph $[\text{Ca}^{2+}]$. Alternatively, Ikeda et al. (1987) and Ikeda and Morizono (1988) have demonstrated an electrochemical potential gradient for Ca^{2+} between perilymph and endolymph, such a gradient reflecting the existence of an active transport mechanism for Ca^{2+} from perilymph to endolymph, these authors suggested that the mechanism of increased endolymph $[\text{Ca}^{2+}]$ is attributable to the in-

creased influx of Ca^{2+} from perilymph due to the depression of the EP.

Therefore, we think that increased endolymph Ca^{2+} ions, following surgical obliteration of the endolymphatic sac, enter the apical parts of the stria marginal cells down the electrochemical gradient via Ca^{2+} channels, but must be transported out at the basolateral parts of the cells by PM Ca^{2+} -ATPase or Na^{+} - Ca^{2+} exchanger activity, in order to maintain the low intracellular $[\text{Ca}^{2+}]$. Auditory function will be preserved as long as the increased activity of Ca^{2+} -ATPase in the cochlear lateral wall is sufficient to transport out the excessive Ca^{2+} ions and maintain the low intracellular $[\text{Ca}^{2+}]$. The present results are consistent with this notion inasmuch as the data exhibit a significant increase in Ca^{2+} -ATPase activity in the cochlear lateral wall of hydropic ears with a normal ABR threshold as compared to the control ears. We also infer that a reduced or insufficient Ca^{2+} -ATPase activity in the cochlear lateral wall of hydropic ears will impair the transport of cytosolic Ca^{2+} to the extracellular fluid and/or sequestration of cytosolic Ca^{2+} into the ER, followed by intracellular overloading with Ca^{2+} . This again may result in an increase in the endolymph $[\text{Ca}^{2+}]$, which in turn results in an increase in the osmotic pressure. Therefore, based on our present study results, an increased endolymph $[\text{Ca}^{2+}]$ as well as changes of ABR threshold for the guinea pig with experimentally induced hydrops may be closely related with a level of Ca^{2+} -ATPase activity insufficient to transport out the overloading intracellular $[\text{Ca}^{2+}]$ in the cochlear lateral wall.

The apparent normality of auditory thresholds in albino animals cannot be taken to indicate that other aspects of cochlear function will be unaffected by the albino mutation. There are quantitative differences in endolymphatic calcium and EP between pigmented and albino guinea pigs (Gill and Salt, 1997). A number of previous studies have suggested that melanin may play a role in Ca^{2+} homeostasis of endolymph (Meyer zum Gottesberge, 1988; Gill and Salt, 1997). In albino animals, melanocytes are present but contain no melanin. We use albino guinea pigs in the present study, and we do not know if the present results could be replicated in pigmented animals. To verify this, further study in hydropic cochleae of pigmented animals is required.

In conclusion, alterations of Ca^{2+} -ATPase activity in the cochlear lateral wall were demonstrated quantitatively in the early stages of experimentally induced endolymphatic hydrops in albino guinea pigs. Alterations of Ca^{2+} -ATPase activity may implicate disturbed calcium-homeostasis of the inner ear, such disturbances possibly resulting in hearing dysfunction in the early stages of endolymphatic hydrops. With an increased under-

standing of the mechanisms involved in the regulation of $[\text{Ca}^{2+}]$, it may become possible to treat inner-ear disorders induced by disturbed Ca^{2+} homeostasis.

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