Two-phase endolymphatic hydrops

a new *dynamic* guinea pig model
Introduction

In 1861, Prosper Menière described the classical triadic symptomatology of hearing loss, vertigo and tinnitus which he ascribed to a labyrinthine disorder. Since the discovery of hydrops in the endolympathic system of the temporal bones of patients with Menière’s disease, endolympathic hydrops has been accepted as the pathological substrate of Menière’s disease. Hydrops may arise as a result of the destabilization of natural regulation through overproduction of endolymph and/or reduced absorption of endolymph.

Reduced absorption of endolymph

The endolympathic sac is considered to be responsible for the absorption of endolymph, endolympathic pressure regulation and the degradation of waste products. Hydrops will be produced by disorders of this absorptive system. In the classical guinea pig model the hydrops is induced by destruction of the endolympathic sac and obliteration of the endolympathic duct with bone wax. However, this is a nonphysiological profound model for Menière’s disease.

Although several modifications of this animal model were developed, which produced hydrops in a variable degree, these modifications were still too destructive or could not be standardized enough to be superior in relation to this classical model.

We induced mild endolympathic hydrops through total dissection of the extraosseous part of the endolympathic sac adjacent to the sigmoid sinus in order to obstruct the venous outflow to the sigmoid sinus and produce mild fibrosis of the most distal portion of the sac. The results and implications of this study have been reported in detail in another paper.

Overproduction of endolymph

The specific chemical composition of endolymph and the generation of the transepithelial positive potential are considered to be regulated by a membrane-bound sodium-potassium activated adenosine triphosphatase (Na/K-ATPase) in the marginal cells of the stria vascularis and the dark cells of the vestibular labyrinth.

Nephrological research revealed a wide array of hormonal as well as autocrine and paracrine factors that can regulate Na/K-ATPase activity in the kidney. One of these regulators is the mineralcorticoid aldosterone, which has two important activities: (a) It is a major regulator of extracellular fluid volume, and (b) it is a major determinant of potassium metabolism.

In the guinea pig high amounts of Na/K-ATPase were detected in several inner ear structures and recent experiments have demonstrated a relationship between circulating adrenal steroids and Na/K-ATPase activity in the inner ear.
Depletion of aldosterone decreases the adenosine triphosphatase activity in the stria vascularis of the cochlea, as was demonstrated after bilateral adrenalectomy of the rat. In these experiments, morphological changes within the cellular structure of the cochlea have also been observed. Re-establishment of an endogenous level of aldosterone restores the cellular morphology and increases of the Na/K-ATPase activity.

Recent animal experiments have demonstrated that strial Na/K-ATPase levels increase as a result of enhanced aldosterone levels induced by low-sodium, high-potassium diets. Aldosterone levels may also be increased by emotional stress, which has been put forward as a precipitating factor in Menière’s disease, and which may lead to an increased secretion of adrenocorticotropic hormone (ACTH) from the hypothalamus, thus stimulating the adrenocortical production of hormones such as aldosterone.

The strial Na/K-ATPase activation by aldosterone may result in an increased secretion of potassium ions in the endolymphatic compartment and an overproduction of endolymph. This may contribute to the development of an endolymphatic hydrops, as seen in Menière’s disease.

Hypothesis

In this study, a new experimental guinea pig model is presented, which shows more resemblance to the pathophysiological process in Menière’s disease. Further investigations on this experimental model might result in a more adequate diagnosis and treatment of different stages of Menière’s disease.

In the developed experimental animal model, a reduced absorption of the endolymphatic sac is combined with an enhanced production of endolymph fluid:
1. An acute reduction of absorption is surgically induced by destruction of the venous drainage from the endolymphatic sac to the sigmoid sinus.
2. A mild increase of endolymph production is induced by administration of aldosterone, to stimulate the Na/K ATP-ase in the stria vascularis and the dark cells.

The venous insufficiency enables us to create a borderline capacity of the endolymphatic sac which is not capable of immediately restoring the increase of endolymphatic volume induced by the administration of aldosterone. The endolymphatic hydrops might be reversed as soon as the aldosterone level decreases or as soon as the outflow capacity of the endolymphatic sac is restored, imitating the dynamic fluctuant nature of Menière’s disease.

Materials and methods

Thirty healthy, female albino guinea pigs (Harlan, The Netherlands, weight 250-300 g) were divided into two groups. In group 1 (n=12) the most distal part of the endolymphatic sac of the left ear was dissected totally from the sigmoid sinus; the right ear served as a control. In the third post-operative week, this group received a sham injection of
1 ml saline 0.9% i.p. once a day during 5 days. In group 2 (n=12) the most distal part of the endolymphatic sac of the left ear was dissected totally from the sigmoid sinus; the right ear served as a control. In the third week the animals in this second group received a once-daily intraperitoneal injection of 1 ml aldosterone in a dosis of 100µg/100g/day (Sigma #A-6628) during five days.

The aldosterone affected both the operated and the contralateral non-operated ear. An additional control group consisted of 4 non-treated cochleas (n=4) and 4 aldosterone-treated non-operated cochleas (n=4).

Animal care and use were approved by the Experimental Animal Committee of the Groningen University, protocol number 0873-0594/0695, in accordance with the principles of the Declaration of Helsinki.

Surgical procedure

The operation was performed under sterile conditions with a Zeiss operation microscope in a specialized unit of the Central Animal Laboratory. Anesthesia was induced with a combination of halothane, oxygen and N₂O. During surgery temperature was stabilized using an electric heating pad. The endolymphatic sac of the left ear was visualized by an extradural posterior fossa approach. The interconnections between the sigmoid sinus and the most distal portion of the endolymphatic sac were disrupted totally and a small piece of latex was interposed between these two structures and fixated by Spogostan®. The intermediate part of the endolymphatic sac remained intact.

Fixation procedure

After three weeks all animals were killed using sublethal administration of sodium pentobarbital (60 mg/kg i.p.), and intracardiac perfusion was performed with tri-aldehyde fixative (3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% DMSO in 0.08 mol/l sodium cacodylate buffer; pH 7.4). After temporal bone dissection was performed, the cochleas were decalcified, postfixed and subsequently imbedded in Spurr’s low-viscosity resin. The part of the temporal bone with the endolymphatic sac and duct was imbedded in glycolmethacrylate.

The cochleas were cut in a midmodiolar plane. Semi-thin sections from the cochleas as well as the endolymphatic sac and duct were obtained. These were stained with toluidin blue and basic fuchsin, and examined using light microscopy.

Degree of hydrops

The light microscopical examination of the inner ear structures was performed separately by several investigators. The degree of endolymphatic hydrops was, according to Sperling et al., rated as slight, moderate or severe. The first (basal), second, and third turns of each cochlea were separately examined for their degree of hydrops.
The average hydrops score of each cochlea was determined by judgements of the three cochlear turns together, and this score was divided in 4 groups which represent the overall degree of hydrops.

**Results**

Endolymphatic hydrops

The bar-graph below demonstrates the overall degrees of hydrops and their percentages observed in the several subgroups. The findings in these subgroups will be discussed separately.

*Control*

This group represents the ears in which the endolymphatic sac was not dissected nor received aldosterone. No hydrops has been detected in any of the windings of these cochleas.

*Aldosterone*

This group represents the non-dissected ears which received aldosterone. In this group, seven out of sixteen cochleas demonstrated a slight degree of hydrops in the basal and the second windings (*Figure 1*), two of these showed slight hydrops in all cochlear windings. In case of hydrops, the basal windings were always affected. No moderate or severe degree of hydrops was noticed.

**Percentual Distribution of Hydrops**
Endolymphatic sac dissection
This group represents the ears in which the endolymphatic sac has been dissected, but which were not treated with aldosterone. A diversity in the degree of hydrops has been observed. Most cochleas showed hydrops in all cochlear windings. Intracochlear variation was present, with the most severe degrees of hydrops to be present in the apical windings. In some cochleas no hydrops was found.

Endolymphatic sac dissection + aldosterone
This group represents the ears in which both the endolymphatic sac has been dissected and aldosterone has been administered. Intercochlear variation in the degree of hydrops was also demonstrated in this group. According to Table I, the variation degree of hydrops in the dissected group seemed almost identical to the variation in the group in which both the sac was dissected and aldosterone was administered. However, the group which received an additional treatment with aldosterone was affected more severely, because the degree of hydrops was more extensive in the middle and basal turns. Intracochlear variation was also present; some cochleas showed the same degree of hydrops in all windings, some cochleas were most affected in the basal windings, and some

Figure 1. Slight endolymphatic hydrops as shown by the distension of Reissner’s membrane (arrowheads) in the basal winding of an aldosterone-treated guinea pig cochlea in which the endolymphatic sac has not been dissected. Bar=100 µm.
cochleas were most affected in the apical windings. In some cochleas no hydrops was found.

**Endolymphatic sac**
Light microscopical examination of the normal and dissected endolymphatic sac was performed to investigate the effects of distal endolymphatic sac dissection and administration of aldosterone on the remaining structures of the endolymphatic sac.

**Control**
In normal ears, the intermediate and distal parts of the endolymphatic sac served as a control. The intermediate part of the endolymphatic sac was easily identified by its high cylindrical cells, which protrude into the lumen as irregular papillae (*Figure 2a*). Within these cylindrical epithelial cells some vacuolization could be observed. The lateral intercellular spaces appeared to be undilatated. The lumen of the endolymphatic sac consisted of a population of free floating cells, predominantly rounded macrophages with large vacuoles. The epithelial lining of the distal portion of the endolymphatic sac was cuboidal, except at the extreme end, where it was squamous (*Figure 3a*).

**Aldosterone**
The cylindrical cells of the intermediate part of the endolymphatic sac had a swollen appearance compared to the control specimens (*Figure 2b*). An increase in pinocytotic activity was observed. The lateral intercellular spaces in the intermediate part showed a moderate dilatation. The epithelial lining of the distal part of the endolymphatic sac seemed undisturbed.

**Endolymphatic sac dissection**
In specimens which showed endolymphatic hydrops, the cylindrical cells showed a swollen appearance with the presence of vacuoles (*Figure 2c*), but to a lesser degree than in the aldosterone-treated ears. The cell distribution of the different epithelial cells appeared to be normal.

Despite careful dissection, in some cases, a very thin layer of endolymphatic sac tissue infrequently remained attached to the sigmoid sinus, as sometimes experienced during surgery and confirmed at histological evaluation. The loose connective tissue was replaced by scar tissue such as collagen, which was arranged in fibrils along the sigmoid sinus. This fibrous tissue seemed to separate the distal part of the extraosseous dissected endolymphatic sac from the sigmoid sinus (*Figure 3b*).

**Endolymphatic sac dissection + aldosterone**
The influence of the dissection in combination with the administration of aldosterone produced the most severe effects. The cellular structure and distribution had changed. Cylindrical cells of the intermediate part of the endolymphatic sac were replaced by
**Figure 2.** Intermediate part of the endolymphatic sac under normal and pathological conditions. Bar=20 µm.

Intermediate ES of a normal specimen. Cylindrical cells (C) protrude into the endolymphatic lumen (E). Occasionally, a vesicle has been demonstrated (arrow).

Intermediate ES of a dissected and aldosterone-treated specimen. The morphology of the epithelium has changed dramatically; most cylindrical cells have been replaced by cuboidal and flat cells (arrows). The remaining cylindrical cells (C) are extensively swollen and show degenerative aspects.

Intermediate ES of a non-dissected, aldosterone-treated specimen. Some cylindrical cells appear to be swollen. Many extracellular vesicles and intracellular vacuoles can be observed (arrows).

Intermediate ES of a dissected specimen. The cylindrical cells appear to be swollen. Some vacuoles and vesicles can be observed.
Figure 3. Distal part of the endolymphatic sac under normal and pathological conditions. Bar=50 µm.
(top) Distal ES in a normal specimen. Note the loose connective tissue (arrows) between the sigmoid sinus (SS) and the cuboidal epithelial cells of the ES (C).
(middle) Distal ES in a dissected specimen. The loose connective tissue has been replaced by scar tissue (arrows). The epithelium of the ES seems normal.
(bottom) Distal ES in a dissected, aldosterone-treated specimen. The endolymphatic lumen (E) seems to be expanded. The cuboidal epithelium has been replaced by flat epithelial cells (arrows).
cuboidal cells (Figure 2d). Only a few cylindrical cells were left and were extensively swollen. The lumen of the endolymphatic sac seemed to be expanded. A remarkable finding was the absence of staining in the lumen of both the intermediate as well as the distal part of the endolymphatic sac (Figures 2d and 3c). Cuboidal cells of the distal part of the endolymphatic sac were replaced by flat epithelial cells (Figure 3c).

Discussion

Endolymphatic hydrops

Effect of distal endolymphatic sac dissection

The extraosseous standardized dissection of the most distal part of the endolymphatic sac from the sigmoid sinus produced in most cases endolymphatic hydrops. Although this procedure is much less destructive than the classical method, it produces a variable extent of hydrops (Table I). These results and their implications were discussed in detail in an earlier paper.

Effects of aldosterone

In the non-operated aldosterone-treated cochleas, 7 out of 16 cochleas showed slight hydrops in several cochlear windings, in particular the basal windings (Figure 1). This is a remarkable finding, since in the classical model the hydrops is concentrated in the apical windings. This apical hydrops was also found in the cochleas of our study which were operated, but not treated with aldosterone.

Why does aldosterone produce hydrops which starts in the basal turns? Kuijpers & Bonting determined the localization and properties of ATPase in the inner ear of the guinea pig. They concluded that there was a clear decrease in strial enzyme activity from the base to the apex of the cochlea. As demonstrated in our study, stimulation of this enzyme activity by aldosterone did stimulate the basal turn more to develop hydrops, probably because of its relatively high enzyme activity in this part of the stria. This resulted in overproduction of endolymph which explains the slight hydrops we found. Although we stimulated the Na/K-ATPase with supraphysiological concentrations of aldosterone, it is obvious that stimulation of ATPase may contribute to the development of endolymphatic hydrops. In recent experiments, some investigators found reduced Na/K-ATPase activities in cochleas with experimental endolymphatic hydrops. These findings indicate that this decreased ATPase-activity may be compensatory as a consequence of the disturbance of endolymph homeostasis. In our model, this compensation was antagonized by administration of aldosterone.

Effects of chronic sac dysfunction in combination with acute aldosterone administration

The operated ears in table I which were treated with aldosterone did not clearly demonstrate a shift to more severe degrees of hydrops when compared with the operated ears
without aldosterone. Although not clearly expressed in table I, aldosterone increased the severity of hydrops in the middle and basal turns. As a result, the group of cochleas which were graded as severe hydrops showed a uniform pattern of severity in all cochlear windings. The absence of hydrops in some cochlea's of the dissected groups treated with aldosterone may be explained by sufficient compensatory mechanisms to maintain endolymph homeostasis. The severity of hydrops in the cochleas with moderate hydrops is unevenly distributed. It is either concentrated mostly at the top of the cochlea or at the basal windings. In contrast to the findings of the classical model in which the destruction of the endolymphatic sac was the only parameter affected, the aldosterone not only produces a slight increment of the hydrops to more severe degrees, but also a shift in severity of the endolymphatic hydrops towards the basal turns.

These findings are interesting because they may explain some of the shortcomings of the classic animal model in relation to patients with Menière’s disease. First of all, the classic hydrops model is not dynamic; the development of hydrops is progressive to severe degrees without the characteristic fluctuating pattern seen in Menière’s disease. Although very interesting electrocochleographical results were extracted from animal experiments, the dynamic pattern of fluctuations between attacks and stabilization or even restoration seen in patients cannot be imitated in the classic animal model. Horner reviewed the findings obtained from electrocochleography in human and animal experiments. Although the onset of Menière’s disease is often associated with low-frequency fluctuating hearing loss, which was confirmed in many animal experiments, some dynamic changes such as the fluctuating thresholds, and the mid frequency and high frequency hearing losses remain unexplained. Kusakari et al. found larger threshold shifts at high frequencies than at low frequencies even in early stage hydrops.

The new two-phase endolymphatic hydrops model presented in this paper, may explain the shortcomings of the earlier animal models. Dysfunction of the endolymphatic sac seems to affect the more apical part of the cochlea, resulting in a pronounced low frequency hearing loss. The influence of aldosterone is predominantly situated in the base of the cochlea, resulting in a high frequency loss. The fluctuating thresholds may be explained by the fluctuating release of aldosterone, which enhances a production of endolymph that cannot be compensated by the chronically compromised endolymphatic sac, which resulting in temporary hydrops. When aldosterone levels have been decreased, the endolymphatic outflow may be regulated sufficiently by the compromised endolymphatic sac alone, and hydrops may diminish or disappear. In some cases in our study, we found an elongated Reissner’s membrane which may indicate a previous occurrence of endolymphatic hydrops.
Endolymphatic sac

**Effect of distal endolymphatic sac dissection**
After the surgical dissection of the distal part of the endolymphatic sac from the sigmoid sinus, pathological changes were observed, including subepithelial fibrosis of the distal endolymphatic sac, and swelling and vacuolization of the cylindric epithelial cells of the intermediate part of the sac. Further details are described in an earlier paper\(^7\). The subepithelial fibrosis, which is also frequently found in Menière’s disease, may result in decreased endolymphatic outflow to the sigmoid sinus.

**Effects of aldosterone**
Administration of aldosterone also resulted in swelling of the cylindrical cells of the intermediate part of the endolymphatic sac. These cells demonstrated some vacuolization, extracellular vesicles and intercellular dilated spaces, which may indicate increased activity of endolymph absorption.

**Effects of chronic sac dysfunction in combination with acute aldosterone administration**
The epithelial lining of the endolymphatic sac showed several changes due to a combination of dissection and administration of aldosterone. Epithelial cell distribution of both distal and intermediate parts of the endolymphatic sac was disturbed extensively; cylindrical cells as well as cuboidal cells were replaced by cells with flatter characteristics. In general, this is a characteristic effect of increased pressure on epithelial cells, and this phenomenon is also found in endolymphatic sac specimens of patients with Menière’s disease.

The absence of staining in the lumen of both the intermediate as well as the distal part of the endolymphatic sac (Figure 2d), in contrary to the staining found in normal cochleas and cochleas which were dissected or treated with aldosterone alone (Figures 2a-c and 3a-b). The composition, source and function of these endolymphatic substances are not fully defined, but seem to compose of carbohydrates and proteins\(^23\). These substances seem to be related to the absorptive and secretory function of the endolymphatic sac. Their intensity in our specimens may reflect to an altered metabolic activity of the endolymphatic sac or may be a consequence of dilution due to hydrops. Contrary effects on endolymph homeostasis has been demonstrated with intravenously administered glycerol which induced a decrease of endolymphatic pressure, and and increased deposition of stainable substances within the lumen of the endolymphatic sac, probably to maintain osmotic pressure\(^24\). As a consequence, this may support our hypothesis that the endolymphatic pressure may be increased to a larger extent than the effects of dissection or aldosterone alone. The epithelial cell changes may disturb endolymphatic outflow. The reason for the presence of many vacuoles and vesicles, including their contents are unknown, but has been observed before in human specimens\(^25\). It is not known whether these changes of the endolymphatic sac in patients with Menière’s disease are a conse-
quence of congenital endolymphatic sac abnormalities which results in hydrops, or whether they may be due to the hydrops. However, these changes may indicate a disturbance of cellular function which may prevent endolymphatic outflow and contribute to the development of hydrops.

Conclusion

In conclusion, the two-phase endolymphatic hydrops may be a dynamic animal model which can explain many of the shortcomings of the classical model, and may relate to many observations seen in Menière’s disease. Although many more morphological and pathophysiological investigations with this new model have to be performed, the presence results indicate that this model may contribute to a better understanding of the pathogenesis. As a consequence, this study may contribute to a more sophisticated therapeutical approach to Menière’s disease, in particular by antagonizing endolymph production.

References

7 Dunnebier EA, Segenhout JM, Wit HP, Albers FWJ. Endolymphatic hydrops after total dissection or cauteterization of the distal portion of the endolymphatic sac. ORL 1996;58:271-276.