

## Chapter 10

### **Summary and Conclusions**

In 1861, Prosper Menière described the classical triadic symptomatology of hearing loss, vertigo and tinnitus, which he attributed for the first time to a labyrinthine disorder. The underlying pathophysiology of this disabling inner ear disease was not known, but since Hallpike and Cairns, and also Yamakawa in 1938 discovered hydrops of the endolymphatic system in the temporal bones of patients with Menière's disease, endolymphatic hydrops has been generally accepted as the basic histopathological substrate of Menière's disease.

Endolymphatic hydrops may arise as a result of the destabilization of natural regulation through overproduction of endolymph and/or reduced absorption of endolymph. Total destruction of the endolymphatic sac, which is considered to be responsible for the absorption of endolymph, and obliteration of the vestibular aqueduct, resulted in endolymphatic hydrops in the guinea pig, and has been established as the classical guinea pig model for Menière's disease (Kimura, 1965). However, obliteration of the vestibular aqueduct is regarded as a non-physiological model for Menière's disease. In patients with Menière's disease, endolymphatic sac tissue still remains present, although the size of the endolymphatic sac is reduced, suggesting a reduction in resorptive capacity.

The production of endolymph is thought to be regulated by Na/K-ATPase in the marginal cells of the stria vascularis of the cochlea, as well as in the dark cells of the utricle and the cristae ampullares of the semicircular canals. In recent experiments, a relationship between circulating adrenal steroids and Na/K-ATPase activity in the inner ear was observed. Emotional stress leads to the activation of neuroendocrine effector systems, including the production of adrenal steroids such as aldosterone, and could thus increase the production of endolymph.

A borderline capacity of the ES, in combination with a periodic increase of endolymph production caused by stressful situations may be responsible for the development of Menière's disease. Indeed, manifestations of Menière's disease frequently occur during stressful experiences in patients with physiological systems under challenge due to a neurasthenic psychological profile.

We developed the two-phase endolymphatic hydrops model in guinea pigs which was based on two compromising factors; mild chronic endolymphatic sac dysfunction has been established by dissection of the distal part of the endolymphatic sac from the sigmoid sinus, in combination with periodic increase of endolymph production by stimulation of the Na/K-ATPase pumps in the stria vascularis by administration of aldosterone. This model, which seemed to represent a functional model combining multiple etiologies, and may resemble the fluctuant characteristics of Menière's disease.

*Chapter 2* describes the first stage of our two-phase model by damaging the distal portion of the endolymphatic sac by surgical dissection or cauterization with silver nitrate to produce mild dysfunction of endolymphatic outflow. The intermediate part of the sac,

which is probably the most active part, remained intact. Dissection and, in a minor degree, cauterization resulted in variable degrees of hydrops with no correlation to the inflicted damage.

One of the interesting findings in our study is that an extraosseous dissection of the most distal part of the endolymphatic sac from the sigmoid sinus, is already destructive enough to produce endolymphatic hydrops. Biopsies or endolymphatic sac surgical procedures, such as drainage procedures for Menière's disease, have been considered to be diagnostic or therapeutic, but have never been considered as harmful to the function of the endolymph homeostasis.

Our results indicate that any damage to the endolymphatic sac compromises its function, and may contribute to the development of hydrops, either asymptomatic or symptomatic. This may explain the difference between the acute positive effects of sac surgery and the poor or negative outcome of the results of long-term studies.

*Chapter 3* describes the light microscopic observations of the cochlear and endolymphatic sac structure after both of the compromising factors of our two-phase endolymphatic model were applied together. Several weeks after endolymphatic sac dissection, aldosterone was injected for further destabilization of endolymph homeostasis.

The epithelial lining of both distal and intermediate parts of the endolymphatic sac was disturbed extensively. In general, this is a characteristic effect of increased pressure on epithelial cells, and this phenomenon is also found in endolymphatic sac specimens of Menière's patients. It is not known whether these changes of the endolymphatic sac in patients with Menière's disease are the reflection of a primary dysfunction which results in hydrops, or whether they may be secondary to the hydrops.

Aldosterone as a single treatment revealed slight degrees of endolymphatic hydrops in the basal windings. However, when aldosterone was injected several weeks after endolymphatic sac dissection, endolymphatic hydrops was increased extensively due to the combination of both compromising factors together. The apical cochlear turns, which represents the lower frequencies of the hearing process, were most severely affected.

In conclusion, this light microscopic evaluation supports our two-phase model.

*Chapter 4* describes the investigation of the organ of Corti in the normal guinea pig with a sophisticated field emission scanning electron microscopic technique, which allows scanning at low voltages, to collect reference data on superficial morphology. Delicate structures were difficult to study with conventional scanning techniques, in which only high scanning voltages were available which necessitated thick coatings of gold or platinum to obtain a reasonable image. This resulted in an inferior resolution of fine surface structures which were covered by these coatings.

Several interesting delicate structures of the stereocilia, in particular fine surface structures, were detected for the first time using scanning electron microscopy. These findings include the different types of cross-links and the tip-links, the fine surface morphology of the stereocilia and their attachments and imprints in the tectorial membrane. One of the most interesting findings in this study is a network of long filamentous structures, which has been identified mainly at the top of the longest stereocilia and the undersurface of the tectorial membrane, and which may represent the glycocalyx.

*Chapter 5* describes a subsequent scanning electron microscopic study of the filaments which we found on the tips of the longest outer hair cell stereocilia, and the undersurface of the tectorial membrane in which the tips of the longest stereocilia are embedded.

In general, the glycocalyx might create a micro-environment around specialized structures. In the sensory cells of the organ of Corti, they might play a role in the frequency specificity and mechano-electrical transduction.

By the use of field emission scanning electron microscopy, optimal visualization of the filaments, which may represent the glycocalyx, was possible at low accelerating voltages of 2-3 kV. In this study, the recently demonstrated glycocalyx on the tallest stereocilia of the three rows of outer hair cells, was confirmed. There was a decrease in the amount of glycocalyx from apical to basal hair cells, and from the third to the first row of outer hair cells.

Earlier attempts to demonstrate the glycocalyx in transmission electron microscopy failed, because conventional fixation failed to preserve the constituents of the glycocalyx. In this study, non-coated specimens with their glycocalyx were studied with scanning microscopy and embedded for evaluation by transmission electron microscopy. This allowed the glycocalyx to be demonstrated also by transmission electron microscopy. To demonstrate whether the glycocalyx may also be demonstrated in other tissues using these non-coating techniques, cryofixation and observation of cat duodenum microvilli demonstrated a similar aspect of glycocalyx. This supports our observations of a glycocalyx layer on the stereociliar structures and the tectorial membrane.

*Chapter 6* describes sensory cell damage due to the two-phase endolymphatic hydrops, which was evaluated using the earlier mentioned scanning microscopical technique. Most effects were observed in radial gradients from the third to second row outer hair cells, and longitudinal gradients in which most severe effects were observed in the apical turns. Most affected were the ears which underwent distal endolymphatic sac dissection followed by administration of aldosterone.

Damage proceeded from degeneration and absence of short stereocilia of outer hair cells and even some inner hair cells in the apical turns, to stereociliary disarrangement and atrophy, followed by degeneration and absence of outer hair cells. Cuticular plates

were frequently possessed or overgrown by microvillar structures. To our knowledge, these structures were not described before. Whether these structures were degenerative changes of the cuticular plates and the stereocilia or whether they may indicate some kind of regeneration still remains unclear.

In specimens which showed severe damage, the cuticular plates could not be recognized at all, and the outer hair cells seemed to be replaced by adjacent supporting cells, compensating degeneration and loss of these hair cells.

*Chapter 7* describes a transmission electron microscopic evaluation of the ultrastructural changes of the cochlear structures in the two-phase endolymphatic hydrops.

Sensory cell damage proceeded from stereociliary disorganization to total loss of stereocilia and the formation of microvillar structures on the cuticular plates as already described in our scanning study in chapter 6. Intracellular degeneration, such as disruption of mitochondria and displacement of the cell nucleus, and deformation of the cellular outline were observed in our affected specimens.

Aldosterone as a single treatment resulted in an increased activity of the marginal cells in the stria vascularis, while dissection and aldosterone application together resulted in loss of marginal cell extensions to total loss of marginal and intermediate cells. Loss of adequate marginal cell function must result in a severe compromise of endolymph production.

Reissner's membrane showed signs of earlier distension, which may result in stretch-activated channels and increased transport and accumulation of fluid between the mesothelial and epithelial cell layers. Macrophages positioned against and between these layers may indicate a repair mechanism.

Our findings demonstrates changes which may be reversible due to the compromising effect of a single treatment, such as aldosterone which mainly interacts with the stria vascularis, and irreversible changes due to interaction of both compromising factors.

*Chapter 8* describes a transmission electron microscopic evaluation of the ultrastructural changes of the remaining parts of the endolymphatic sac.

Aldosterone treatment had no visible effect on endolymphatic sac structures, but dissected ears revealed severe deviations in the intermediate part of the sac which was unaffected by the surgical procedure of distal dissection. The epithelium of the intermediate sac was low, which is characteristic for increased pressure on epithelial cells in fluid containing compartments. The epithelium also showed dilated lateral intercellular spaces, indicating increased fluid transport, and displayed serious degenerative processes. Distally, the endolymphatic duct was completely blocked by newly formed bone, which may diminish endolymph absorption. Additional aldosterone treatment had no cumulative effect on the dissected ears. In conclusion, dissection of the distal part of the endolym-

phatic sac resulted in severe effects on remaining sac structures, which may further compromise endolymphatic outflow and result in aggravation of hydrops.

*Chapter 9* describes longitudinal recording of the compound action potential in our two-phase endolymphatic hydrops model by permanently implanted round window electrodes.

Control ears demonstrated an extensive variability of responses which resulted in a high standard deviation, in which delicate changes were difficult to detect.

Dissection of the endolymphatic sac resulted in a decline of the compound action potential in operated as well as non-operated (contralateral) specimens, probably due to a disturbance of the cerebrospinal and perilymphatic fluid compartments. Restoration of this decline was variable with a tendency to end at higher values than the starting values in the dissected ears. Administration of aldosterone produced fluctuancies which may only be regarded as tendencies. No significant changes of the compound action potential were found in all aldosterone-treated groups. The variability and the resulting high standard deviation prevented us to draw conclusions about delicate changes due to each treatment modality.

Severe degrees of hydrops and severe electrophysiological damage would not have been useful in developing a dynamic model in which delicate effects of minor changes must be evaluated for their fluctuant or permanent influences on the electrophysiological processes. However, in this study moderate disturbances in endolymph homeostasis or perhaps longer follow-up periods might have demonstrated more clearly the point of imbalance in our dynamic model. Longer follow-up periods may provide more information about the relation between the electrophysiological influence of the compromising factors, the degree of hydrops, the degree of sensory cell damage, and their fluctuant or permanent influence on sensory cell function.

## **Conclusions**

The aim of our study was to develop a new experimental model, the two-phase endolymphatic hydrops, in which several compromising mechanisms interact, and which may mimic the fluctuations as observed in early stages of Menière's disease.

Dissection of the distal part of the endolymphatic sac as well administration of aldosterone as a single treatment revealed morphological changes which, to some extent, may still be reversible. Dissection affected the remaining parts of the endolymphatic sac, as illustrated by the severe epithelial changes in the non-operated intermediate part, which may further aggravate hydrops in the cochlea. Aldosterone had a primary effect on the cochlear structures, as illustrated by the slight degrees of hydrops in the basal windings and the increased activity in the marginal cells.

Both compromising factors together produced synergistic effects on degenerative changes of the epithelia of the endolymphatic sac as well of the sensory and accessory structures in the cochlea. In conclusion, the two-phase endolymphatic hydrops model seems to represent a new and dynamic model for Menière's disease.

A certain lack of correlation between the degree of hydrops and cochlear damage was found in our studies. This may indicate that hydrops may be considered as an epiphenomenon rather than a causative factor. The endolymph volume as a parameter for the quality of endolymph homeostasis has to be disputed. Other primary factors and their influences on endolymph regulation and dysregulation probably are more directly involved in sensory cell function.

In future studies, dissection of the endolymphatic sac needs further refinement because some specimens in our study demonstrated severe degeneration of the remaining parts of the endolymphatic sac and excessive bone formation of the distal part of the vestibular aqueduct, which was more severe damage than we expected. Longer administration of aldosterone may reveal changes which may attribute to a better understanding of the process of endolymph formation and endolymph homeostasis.

In our electrophysiological studies, longer follow-up periods may provide more information about the relation between the electrophysiological influence of the compromising factors, the degree of hydrops, the degree of sensory cell damage, and their fluctuant or permanent influence on sensory cell function. The sensory cell damage did not result in detectable significant changes of the compound action potential. This can be explained by the fact that the outer hair cells were most affected while the inner hair cells, which are assumed to be a major determinant of the cochlear potentials were relatively unaffected. Electrophysiological recordings, which are able to registrate this early damage to the outer hair cells, must be developed to obtain a diagnostic tool of the early stages of Menière's disease.

Since hydrops may be an epiphenomenon, estimation of the degree of hydrops after a certain follow-up seems irrelevant. The degree of electrophysiological damage, independent from time-schedules, must be related to cochlear damage, fluid pressure changes, and electrochemical changes of endolymph homeostasis. This will provide more relevant information about the stage of the dysfunction and its reversible or irreversible status.

This point of view also serves the clinical understanding and staging of Menière's disease, because the clinical symptomatology is still the only parameter from which the severity of the disease can be estimated and accordingly treated.