Chapter 8

Summary and Conclusions

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In 1861, Prosper Menière documented a chronic illness characterised by disabling attacks of vertigo, hearing loss and tinnitus. The pathophysiology of this disease, which now bears his name, is largely unknown.
In 1938, Hallpike and Cairns and also Yamakawa found an endolymphatic hydrops in the temporal bones of patients with Menière's disease. Since then an endolymphatic hydrops, which is an excess of endolymph volume, has been generally accepted as the basic histopathological substrate.
Studies on experimentally induced endolymphatic hydrops in the guinea pig constitute a useful model for Menière's disease. Over the years, the most extensively studied model has been the surgical ablation of the endolymphatic sac in the guinea pig. This model results in a slowly developing chronic endolymphatic hydrops within days to weeks. A model which eliminates the secondary effects of a surgically induced chronic hydrops is the acute endolymphatic hydrops, which involves micro-injection of artificial endolymph into scala media of the guinea pig's cochlea. This acute endolymphatic hydrops model provides a useful research tool for investigating the immediate effects of a hydrops on the inner ear.
In this thesis, changes in cochlear functioning and morphology during and after induction of an acute endolymphatic hydrops are presented. Besides increasing our knowledge regarding pathophysiological mechanisms in Menière's disease, this thesis also contributes to our knowledge of fundamental processes in the inner ear.

The precise mechanism of endolymph volume regulation and pathophysiology of an endolymphatic hydrops remain enigmatic. Still, the endolymphatic sac is generally believed to represent one of the primary loci for endolymph volume regulation. In chapter 2 morphological changes of the endolymphatic sac's epithelia and luminal filling after induction of an acute endolymphatic hydrops were investigated. After microinjection of 1.1 µl of artificial endolymph into scala media of the cochlea in 10-12 minutes, the guinea pigs were terminated immediately or after different time intervals up to 2 h. The added endolymph volume of 1.1 µl corresponds with an acute endolymphatic hydrops of 23%. Post-injection examination of the specimens by light and transmission electron microscopy demonstrated no differences in endolymphatic sac morphology between injected and non-injected ears. Further, no distinct changes were observed in guinea pigs terminated after different time intervals. The endolymphatic sac's luminal homogeneous substance (HS) was always present and most often to a large extent. Ribosome rich cells and intraluminal macrophages appeared to be actively involved in degradation of HS by secreting lytic enzymes and digestion respectively. In conclusion, endolymph volume homeostasis is a complex mechanism, in which the role of HS in the endolymphatic sac remains obscure.

In chapter 3 experiments are described in which the same acute endolymphatic hydrops was induced as in chapter 2. In this study cochlear function was assessed by measuring low-level distortion product otoacoustic emissions (DPOAE). DPOAEs are widely accepted to reflect the integrity of cochlear micromechanical processes in general, and outer hair cell function (OHC) in particular.

During the experiments the most prominent $2f_1$-f_2 DPOAE at 4.5 kHz was recorded from the external ear of the guinea pig. With injection, the $2f_1$-f_2 amplitude was not influenced at first. Then after a modest decrease, the recovery frequently started within the period of microinjection. This typical behaviour might be explained by a displacement of the basilar membrane towards scala tympani. The displacement at the $2f_1$-f_2 generation site was estimated to be 19 nm. A small deflection of the OHC stereocilia and as a consequence a change in cell conductance may explain the $2f_1$-f_2 DPOAE amplitude changes. As the $2f_1$-f_2 DPOAE amplitude did not follow change in inner ear pressure, the increase of endolymph volume is likely to be responsible for the observed effects.

In chapter 4 the relation between acute inner ear pressure changes and cochlear function was investigated by measuring the $2f_1$-f_2 DPOAE amplitude. By repetitive injections of artificial perilymph into scala tympani and aspirations of native perilymph from scala tympani, inner ear pressure was manipulated without disturbing endolymph volume. The dynamics of large changes in overall inner ear pressure were followed with a delay of 1-2 s by small changes in $2f_1$-f_2 DPOAE amplitude. During injection the inner ear pressure and $2f_1$-f_2 DPOAE amplitude increased whereas during aspiration the inner ear pressure and $2f_1$-f_2 DPOAE amplitude decreased. Magnitude and sign of the $2f_1$-f_2 amplitude changes can partly be explained by a change of oval window stiffness. No explanation was found for the measured delay.

In chapter 5, in addition to the previous chapter, the $2f_1$-f_2 and f_2-f_1 distortion products in cochlear microphonics (CMDP) were also recorded during repetitive injections of artificial perilymph into scala tympani. Both these microphonic and acoustic distortion products are considered to reflect outer hair cell electromotility in the nonlinear cochlear transducer.With injection the inner pressure increased by approximately 600 Pa with concomitant small changes in CMDP and DPOAE. A small decrease in amplitude of the $2f_1$-f_2 and a small increase in the f_2-f_1 were measured in CM. This matches a shift from a symmetrical position of the operating point for hair cell transduction, leading to an increase in even-order distortion and a decrease in odd-order distortion.

In chapter 6 again an acute endolymphatic hydrops was induced by injection of 1.1 µl of artificial endolymph into scala media of the guinea pig's cochlea. During and after injection, cochlear function was assessed by measuring CMDP and DPOAE. A reversible small pressure increase and a relatively stable endocochlear potential were accompanied by a mean decrease in $2f_1$-f_2 DPOAE of only a few dB. Similar, the $2f_1$-f_2 CMDP amplitude change was minimal during and after injection. The only substantial change was measured in the f_2-f_1 CMDP amplitude. The measured range of distortion amplitudes during an acute endolymphatic hydrops could be related to small changes in the cochlear transducer operating point. The estimated basilar membrane displacement of 19 nm (see chapter 3) corresponds with a hair cell cilia displacement of a few tenths of a degree. This fits a small change in transducer conductance, for which the total range is a few degrees of hair bundle displacement.

In chapter 7 cochlear function during induction of a "catastrophic" acute endolymphatic hydrops was assessed. During cumulative microinjections of artificial endolymph the $2f_1$-f_2 and f_2-f_1

CMDP and $2f_1$-f_2 DPOAE were recorded in the guinea pig. A catastrophe occurred in the inner ear when 2.5 – 3.5 µl of artificial endolymph was injected. Morphologically a rupture of Reissner's membrane was found, most often in the apical turn of the cochlea. The catastrophe had only minor effects on the endocochlear potential, whereas it caused a marked decrease in $2f_1$-f_2 DPOAE amplitude. The $2f_1$-f_2 and f_2-f_1 CMDP amplitude increased during each injection prior to the catastrophe. After the catastrophe the f_2-f_1 CMDP amplitude decreased during each injection, possibly due to a shift of the cochlear transducer operating point position. Surprisingly, the changes in cochlear function during a catastrophic acute endolymphatic hydrops were relatively small.

In conclusion, the investigations described above all suggest a dynamically stable inner ear. The inner ear system is able to cope with substantial endolymph volume increases and inner ear pressure changes with only minimal and often temporary cochlear function loss. Even when the endolymphatic system is catastrophically blown up, cochlear function loss is only moderate.
Menière's disease is a chronic illness of which the etiology is most likely to be multifactorial. Whether an endolymphatic hydrops causes inner ear damage and clinical symptoms or that an endolymphatic hydrops is rather a marker of disordered inner ear homeostasis, remains tentative.
The findings described in this thesis demonstrate that a single acute endolymphatic hydrops has only minimal effects on cochlear function. This might be more consistent with the hypothesis that an endolymphatic hydrops is rather an epiphenomenon of Menière's disease. On the other hand, a repeated catastrophic hydrops might lead to a biochemical imbalance of inner ear fluids. Over time, this could cause inner ear damage with functional loss approximating Menière's disease.