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Acute endolymphatic hydrops

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Chapter 7

The catastrophic acute endolymphatic hydrops in the guinea pig

Valk WL, Wit HP, Albers FWJ.
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Introduction

In 1861, Prosper Menière described the classical triadic symptomatology of disabling attacks of vertigo, fluctuating hearing loss and tinnitus which he attributed to a labyrinthine disorder. In 1938, Hallpike and Cairns presented histological findings of temporal bones from patients with Menière's disease, in which the common pathological feature was a distended Reissner's membrane. Ever since, an excess of endolymph volume, or a so-called endolymphatic hydrops, is believed to represent the histopathological substrate of Menière's disease. However, there is also evidence that Menière's disease may exist without an endolymphatic hydrops (Fraysse et al., 1980). Furthermore, an endolymphatic hydrops may be present without symptoms of Menière's disease (Vasama and Linthicum, 1999).

The endolymphatic and the perilymphatic compartments are separated by the basilar membrane and Reissner's membrane, of which the latter has a relatively high compliance. A rupture of Reissner's membrane has been described as the cause for episodic attacks of Menière's disease. Subsequent diffusion of endolymphatic potassium into the perilymph surrounding the nerve branches to the vestibular and cochlear areas might cause the typical symptoms as seen in Menière's disease (Dohlman, 1980).

Studies on experimentally induced hydrops have proven to be a useful model for Menière's disease. The most extensively studied model was presented first by Kimura and Schuknecht (1965). They produced a chronic endolymphatic hydrops after surgically obliterating the endolymphatic sac and duct. In this model an endolymphatic hydrops can be demonstrated by post-mortem observation of a distended Reissner's membrane after several weeks to months. Another experimental model is the acute endolymphatic hydrops, which involves the injection of artificial endolymph into scala media of the guinea pig's cochlea (Valk et al., 2004, 2005). Again, in this acute model an endolymphatic hydrops can be confirmed by a distended Reissner's membrane (Valk et al., 2005). When a relatively small endolymphatic hydrops was induced by microinjection of 1.1 µl of artificial endolymph into scala media of the guinea pig's cochlea, this resulted in a small reversible change in cochlear function as measured by the $2f_1$-f_2 distortion product otoacoustic emission (DPOAE) (Valk et al., 2004) and distortion products in cochlear microphonics (CMDP) (unpublished observations). When a larger hydrops of 3.0-3.5 µl was induced a catastrophe occurred (Wit et al., 2000). Most probably, a permanent leak was created somewhere in the walls of the membraneous endolymphatic system.

In the present study repetitive microinjections of 0.5 µl of artificial endolymph with a rate of 50 nl/s were performed to document the limits of the endolymphatic system when coping with an acute endolymphatic hydrops. During the experiments cochlear function was assessed by measuring both DPOAE and CMDP. The measurement of CMDP, in particular of the f_2-f_1 and $2f_1$-f_2 distortion product amplitudes, may provide information about the cochlear transducer function (Bian et al., 2002) and changes in its operating point. The results provide knowledge regarding the pathophysiological mechanisms operational in a catastrophic acute endolymphatic hydrops.

Materials and methods

Experiments were performed in 9 guinea pigs (Harlan Laboratories, The Netherlands; body weight 350-450g) with a positive Preyer reflex. Animal care and use were in accordance with the principles of the declaration of Helsinki and approved by the animal experiment committee (protocol number 3047/3103).
General anesthesia was induced by intramuscular administration of ketamine/xylazine (60/3.5 mg/kg). Maintenance doses of the anesthetic were administered every hour. Muscle relaxation was obtained with succinylcholine (2.5 mg/kg). The animals were artificially ventilated through a tracheostoma (Columbus Instruments, model 7950). Body temperature was maintained at 38 degrees Celsius with a heating blanket. Heart rate was monitored by two skin electrodes, which were placed on both sides of the thorax. The animal's head was kept in a stationary position by means of a steel bolt fixed to the skull with dental cement. Following a retroauricular incision, the bulla and external auditory canal were exposed. Subsequently, the bulla was opened equalizing middle ear pressure to normal air pressure (Zhang and Abbas, 1997). By opening of the bulla, the round window was exposed. Through the round window membrane, the tip of a double-barreled micropipette was inserted into scala tympani. After subsequent perforation of the basilar membrane the micropipette was advanced into scala media (figure 1). DC potential at the pipette tip was measured to verify its position.
The double-barreled micropipettes were drawn from borosilicate glass (1.5/0.84 mm diameter per barrel) and the tips were bevelled (Narishige EG-40). The total tip diameter was around 60 μm. One barrel of the pipette was used to measure inner ear pressure (WPI 900A micropressure system). Through the other barrel, artificial endolymph (25 mM $KHCO_3$ and 140 mM KCl (Salt and DeMott, 1997)) was injected with a constant flow rate by applying a controlable pneumatic pressure to the barrel end. The injected volume was measured as the displacement of the fluid meniscus in the pipette, for which the inner diameter is precisely known.
During microinjection the $2f_1$-f_2 DPOAE was continuously measured using an Etymotic ER-10C

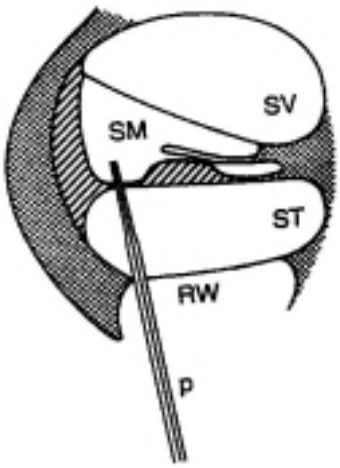

Figure 1: Location of the double-barreled micropipette during artificial endolymph injection (SM: scala media, SV: scala vestibuli, ST: scala tympani, RW: round window, P: pipette)

DPOAE probe system. The two primary frequencies were set at 6 kHz (f_1) and 7.4 kHz (f_2), meaning a f_2 / f_1 -ratio of 1.23. The low-level intensities were set at respectively 65 dB SPL (L1) and 55 dB SPL (L2). Full details on the measuring equipment are described elsewhere (Valk et al., 2004). Cochlear microphonic potentials were recorded with two differential Ni-Cr electrodes with a diameter of 50 µm. One electrode was placed in scala tympani through the bony wall of the basal turn of the cochlea. The other was placed in scala vestibuli next to the oval window. The signals were routed via an amplifier to a spectrum analyzer and two lock-in amplifiers (SRS, model SR830 DSP). The lock-in amplifiers displayed the amplitude of the $2f_1$-f_2 and f_2-f_1 CMDP which were referenced to an electronic $2f_1$-f_2 and f_2-f_1 distortion product derived from the original primaries.

In the experimental series (n=7), measurements contained a sequence of at least 10 microinjections of artificial endolymph during 10 s each with pauses of 40 s. The rate of microinjection was 50 nl/s. The repetitive microinjection was controlled with a precision electronic timer (Stanford DG535). Additionally, in two guinea pigs an outlet was made in the round window. This was done to reduce potentially disturbing physiological pressure fluctuations as caused by breathing and heartbeat.

During an experiment, National Instruments LabVIEW® was used for recording of the following output signals: amplitude and phase of the $2f_1$-f_2 DPOAE, $2f_1$-f_2 CMDP, f_2-f_1 CMDP, endocochlear potential (EP) and inner ear pressure. Calculations and fits to relevant portions of the obtained recordings were made off-line with an appropriate software package.

After injection, the animals were sacrificed by an intracardial injection of pentobarbital. From the series in which repetitive injections were performed without an outlet, 6 guinea pig inner ears were processed for histology. The specimens were processed for light microscopy (n=3) or for orthogonal-plane fluorescence optical sectioning (OPFOS) microscopy (Voie, 2002)(n=3). The contralateral non-injected ear served as a control.

Results

An illustrative result of a measurement in which 10 repetitive microinjections of artificial endolymph were performed is given in figure 2. During the repetitive 10 s pulses, the injection rate was 50 nl/s. Hence, the total injected volume of artificial endolymph was 5.0 µl.

The pressure profile shows an increase with each following injection until a typical maximum is reached at the fifth injection. After the maximum pressure peak the profile becomes relatively regular, increasing by 500 Pa from baseline. This behaviour was consistently observed in all experiments and was interpreted as follows: each time fluid is injected into scala media, endolymphatic pressure increases. With each injection the endolymph volume is further enlarged with a concomitant further increase in pressure. The membranes surrounding the endolymphatic compartment stretch until a maximum is reached and a permanent leak is created. This “catastrophe” in the inner ear occurred when 2.5 – 3.5 µl of artificial endolymph (5-7 injections) was injected. After the catastrophe the endolymphatic and perilymphatic compartment are connected and have the same pressure, yielding the regular pressure profile during injections and subsequent fluid escape through the cochlear aqueduct.

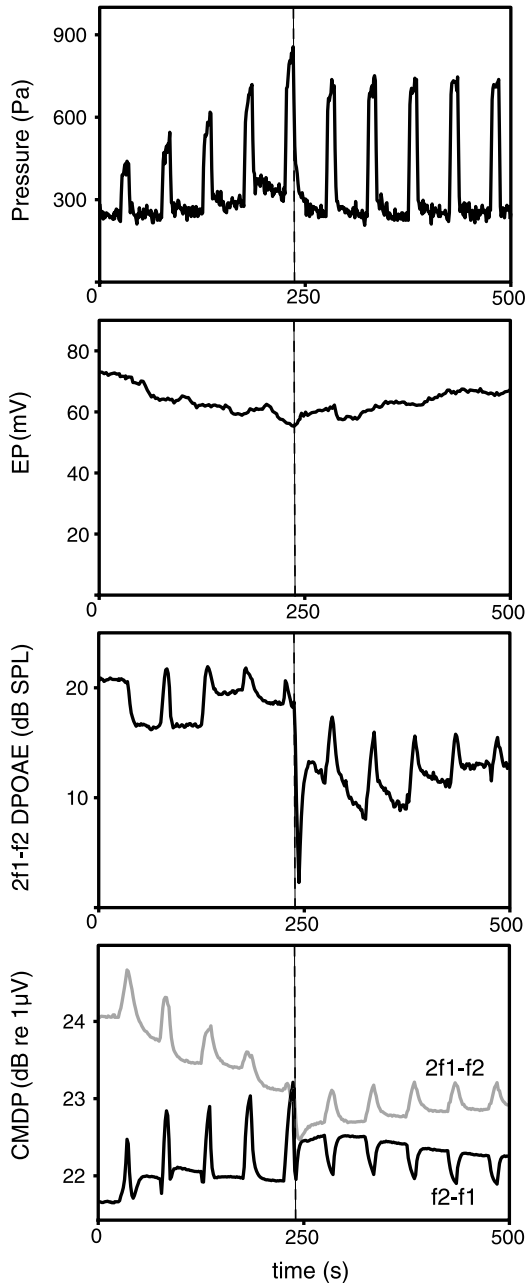

Figure 2: Typical results from an experiment in which 10 microinjections of artificial endolymph of 10 s each with pauses of 40 s were performed. The rate of injection was 50 nl/s. Panel 1 (upper panel): pressure profile measured in scala media. Panel 2: endocochlear potential (EP). Panel 3: $2f_1$-f_2 DPOAE amplitude. Panel 4 (lower panel): $2f_1$-f_2 and f_2-f_1 CMDP amplitude. The dashed vertical line corresponds with the catastrophe (see main text).

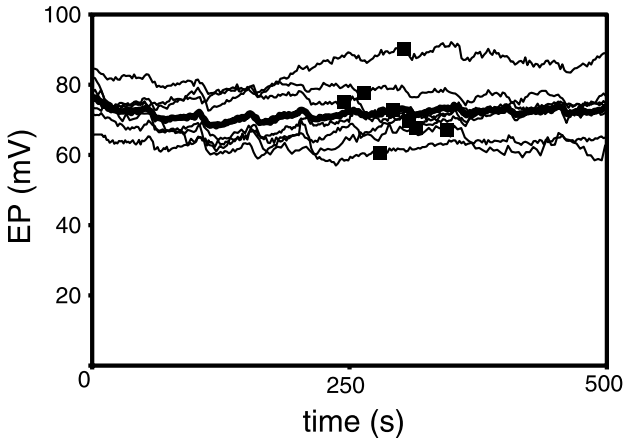

Figure 3: The endocochlear potential (EP) of all individual experiments (n=7). Dots mark the catastrophe. The thick line represents the mean EP. The time scale corresponds with figure 2.

In figure 2 (second panel) a typical EP profile is shown. In figure 3 the EP of all experiments (n=7) are individually depicted. The average EP prior to the injections was 75.8 mV. The endolymph injections had only minor effects on the EP both before and after the catastrophe, although an injection related regular profile can be recognised in its course (mean EP, thick line figure 3).
In the third panel of figure 2 the $2f_1$-f_2 DPOAE appears to increase during each injection after an initial decrease at the first injection. The catastrophe is also clearly depicted by a dramatic decrease in $2f_1$-f_2 DPOAE amplitude. After the catastrophe the $2f_1$-f_2 DPOAE partly recovers and follows the pressure profile in a regular way. Unfortunately, due to a low signal-to-noise ratio, the f_2-f_1 DPOAE could not be measured properly.
The CMDP (lower panel of figure 2) showed a remarkable consistent course. The baseline $2f_1$-f_2 CMDP amplitude steadily decreased, but during each injection the amplitude increased. The latter was also seen for the f_2-f_1 CMDP amplitude. After the catastrophe the $2f_1$-f_2 CMDP amplitude still increased during each injection, whereas the f_2-f_1 CMDP amplitude decreased during injection.
In two additional experiments, an outlet was made in the round window. The only observed effect of this was a smaller pressure increase during injections. The other recordings showed no remarkable changes.
In all the “after catastrophe” specimens that were processed for light microscopy and OPFOS, a rupture of Reissner’s membrane was found, most often in the apical turn of the cochlea (figure 4). Sometimes a rupture of Reissner’s membrane was found in more than one turn. In figure 5 the rupture of Reissner’s membrane is reconstructed in 3D with OPFOS (Voie, 2002). No ruptures were found in the membraneous vestibular part of the inner ear.

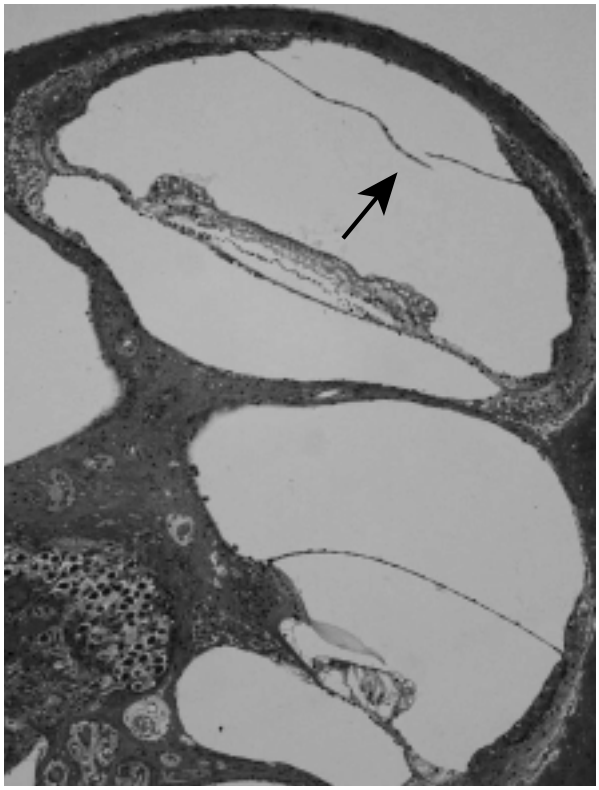

Figure 4: Light microscopic photograph of the guinea pig's cochlea, showing a rupture of Reissner's membrane in the apical turn (arrow).

Discussion

Cochlear sensitivity was assessed by measuring CMDP and DPOAE during a rapidly growing and large acute endolymphatic hydrops. In a previous study (chapter 6) 1.1 µl of artificial endolymph was microinjected into scala media of the guinea pig's cochlea. The $2f_1$-f_2 CMDP and $2f_1$-f_2 DPOAE changed minimally whereas the f_2-f_1 CMDP increased substantially. The measured range of distortion amplitudes could be related to small changes in the cochlear transducer operating point. An added volume of 1.1 µl corresponds with an acute endolymphatic hydrops of 23%, as the total endolymph volume in the inner ear of the guinea pig is 4.7 µl (Shinomori et al., 2001). The inner ear thus seemed able to cope with this degree of an acute endolymphatic hydrops, without substantial cochlear function loss. In the present study cochlear sensitivity was observed during induction of a more rapid and larger endolymph volume increase. In previous research it was shown that when injection volume exceeded 3.0-3.5 µl, a catastrophe occurred (Valk et al., 2004; Wit et al., 2000). This event was interpreted as the creation of a permanent leak somewhere in the walls of the membraneous endolymphatic system.

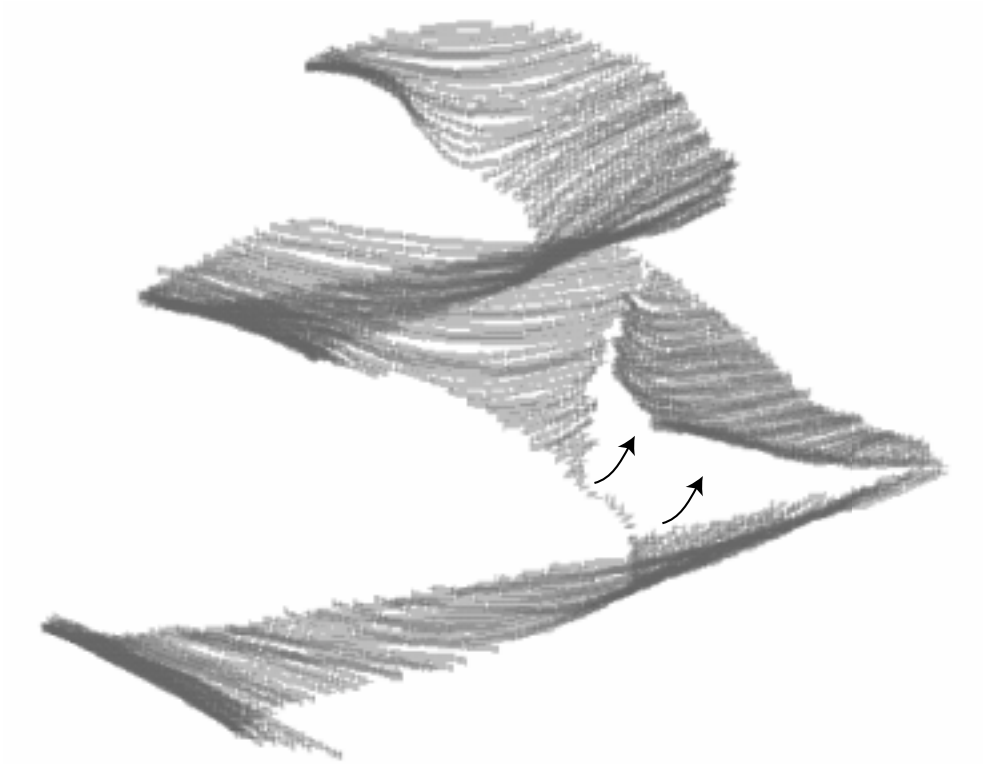

Figure 5: 3D reconstructed image of Reissner's membrane acquired by OPFOS (Voie, 2002). A rupture of the membrane is seen as a lifted flap (arrows).

In the present study, the catastrophe occurred when 2.5 – 3.5 µl of artificial endolymph (5-7 injections) was injected. This degree of hydrops, corresponding to 53-74% of the original volume, resulted in a substantial drop in $2f_1$-f_2 DPOAE amplitude, whereas the EP was not markedly influenced.

The simultaneously measured CMDP provide information about the cochlear transducer function. By injection of artificial endolymph into scala media, the basilar membrane is temporarily displaced towards scala tympani. This results in a typical CMDP behaviour during the injections. Both $2f_1$-f_2 and f_2-f_1 CMDP increased after the start of injection and decreased at the end of injection. Remarkably, the f_2-f_1 CMDP showed an opposite pattern after the catastrophe; during each injection the f_2-f_1 CMDP decreased temporarily. The f_2-f_1 behaviour can be described by the model of Bian et al. (2002, 2004) and Choi et al.(2004) for the generation of distortion products if it is assumed that the operating point of the cochlear transducer moves further away from zero during injection before the catastrophe and towards zero after it. But this model then predicts much smaller changes in $2f_1$-f_2 than in f_2-f_1, which is not observed. So the exact explanation for the changes in the CMDP can not be given. The most important is that the observed CMDP amplitudes and amplitude changes after a catastrophe were as small as before it.

The catastrophe is associated with the creation of a permanent leak somewhere in the membrane-

ous endolymphatic compartment (Wit et al., 2000). The endolymphatic and the perilymphatic compartments are separated by the basilar membrane and Reissner's membrane, of which the latter has a higher compliance. In earlier work we showed that after induction of an acute endolymphatic hydrops Reissner's membrane is distended (Valk et al., 2005).
In the presently investigated specimens a rupture of Reissner's membrane was found, most often near the helicotrema of the cochlea (figure 4). In a few specimens a rupture of Reissner's membrane was found in more than one of the higher turns. Remarkably, no ruptures were found in the vestibular part of the inner ear.
The episodic attacks of Menière's disease with its typical symptoms have been ascribed to a rupture of Reissner's membrane (Dohlman, 1980). Flock et al. (2003) suggested that micro-lesions in Reissner's membrane could alter the electro-chemical environment of the organ of Corti, which would cause hearing loss and tinnitus during endolymphatic hydrops. Pyykko et al. (2004) hypothesized that a viral infection breaks the labyrinthine barrier and triggers an autoimmune-like reaction. The endolymphatic hydrops would then be caused by leakage of the labyrinth and during attacks by rupture of membranes. These theories predict dramatic effects of membrane rupture. But the observed changes in cochlear function parameters, as shown in figure 2 and 3, were small. Even when the endolymphatic system is blown up by injection of artificial endolymph (catastrophe), the cochlear functional loss turns out to be less than expected. To what extent these findings of a (first) catastrophic endolymphatic hydrops relate to Menière's disease remains tentative. However, these results might be more consistent with the hypothesis that an endolymphatic hydrops is rather a marker of disordered inner ear homeostasis, than the cause of the clinical symptoms of Menière's disease. This assumption is supported by results of a recent extensive human temporal bone study (Merchant et al., 2005).

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