Acute endolymphatic hydrops

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Chapter 6

Changes in distortion of two-tone cochlear microphonic and otoacoustic emission signals during an acute endolymphatic hydrops in the guinea pig

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Introduction

Menière's disease is a chronic illness characterised by disabling attacks of vertigo, fluctuating hearing loss, tinnitus and often a sensation of aural fullness. In 1938, Hallpike and Cairns presented histological findings from Menière's patients, in which the pathological feature was a distended Reissner's membrane. Since then an endolymphatic hydrops, which is an excess of endolymph volume, has been generally accepted as the histopathological substrate of Menière's disease. Various etiologic factors for this endolymphatic hydrops have been proposed, including functional or anatomic obstruction of endolymphatic flow, malabsorption of endolymph, genetic anomalies, vasodilatation, allergy, viral infection and autoimmunity (Paparella and Djalilian, 2002).

Studies on experimentally induced hydrops have been performed extensively over the years. In the chronic model, the endolymphatic sac is surgically ablated, which results in an endolymphatic hydrops within several weeks to months (Kimura and Schuknecht, 1965). An experimental model without the secondary effects of the surgical ablative model is the acute endolymphatic hydrops model, which was first introduced by Kitahara et al. (1982). By injection of artificial endolymph into scala media of the cochlea an acute endolymphatic hydrops is created. A disturbance of the mechanical and electrolytic balance presumably accounts for the symptoms in Menière's disease. By studying the cochlear electrophysiology during an acute induced endolymphatic hydrops, underlying pathophysiological processes might be clarified. Sirjani et al. (2004) showed that even though endolymph volume disturbances cause only minor elevation of action potential (AP) thresholds, measures of cochlear microphonic distortion products (CMDP) were markedly influenced.

Further, in a previous study (Valk et al., 2004a) the $2f_1$-f_2 distortion products otoacoustic emissions (DPOAE) amplitude reversibly decreased by only 2.6 dB on average in response to microinjection of artificial endolymph. In another study, artificial perilymph injections with concomitant changes of overall inner ear fluid pressure had only minor effects on DPOAEs (Valk et al., 2004b).

In the present study 1.1 µl of artificial endolymph was microinjected into scala media of the guinea pigs cochlea with simultaneous measurement of acoustic and microphonic distortion products. The measurement of CMDP, in particular of the f_2-f_1 and $2f_1$-f_2 amplitudes, provides information about the cochlear transducer function (Bian et al., 2002) and changes in its operating point. By investigating both acoustic and electric modalities, a relation between cochlear function (with outer hair cell function in particular) and an acute endolymphatic hydrops might be found. This might gain insight into diseases with pathologically changed inner ear fluid volumes, as Menière's disease.

Materials and methods

Experiments were performed in 8 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 40 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. In all guinea pigs, 1.1 µl of artificial endolymph was injected with a constant rate in 12 minutes. 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 DPOAEs were continuously measured using an Etymotic ER-10C 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 given elsewhere (Valk et al., 2004ab).
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 monitoring spectrum analyzer and a digital audio processor (PCM-F1, Sony). Subsequent data acquisition was on a Betamax recorder (SL-F30, Sony). Offline Fourier analysis of the recorded CM signal yielded amplitudes of the f_1 CM, $2f_1$-f_2 CMDP and f_2-f_1 CMDP. 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, endocochlear potential (EP) and inner ear pressure.

Results

In all guinea pigs 1.1 µl of artificial endolymph was successfully injected with a mean constant rate of 92 nl/min. In figure 2, the averaged simultaneous recordings of inner ear pressure, EP, $2f_1$-

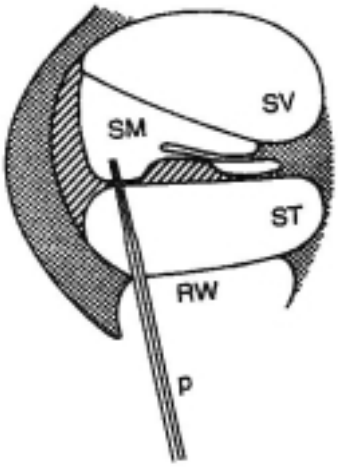

Figure 1: Location of the double-barreled micropipette during artificial endolymph injection (SM: scala media, SV: scala vestibuli, ST: scala tympani, RW: round window, P: pipette).

f_2 DPOAE, $2f_1$-f_2 and f_2-f_1 CMDP during and after this acute endolymphatic hydrops are depicted. During the injection period the inner ear pressure remained stable at an approximately 23 Pa higher level than before injection.

The average EP prior to manipulation of endolymph volume was 78.4 mV. During and after injection of artificial endolymph, the EP remained relatively stable. Typically, the EP tended to increase by a few millivolts at the start of injection.

The $2f_1$-f_2 DPOAE amplitude was 16.1 dB SPL prior to injection. The $2f_1$-f_2 DPOAE amplitude in the different guinea pigs prior to injection covered a wide range; 10-24 dB SPL. After the onset of injection the $2f_1$-f_2 DPOAE amplitude typically started to decrease after a delay of a few minutes. The amplitude reached a minimum of almost 3.4 dB below the pre-injection value. Subsequently, a recovery was observed which often occurred within the injection period. Unfortunately, the f_2-f_1 DPOAE could not be measured due to an unfavourable signal-to-noise ratio.

The simultaneous recorded $2f_1$-f_2 and f_2-f_1 CMDP relative to the f_1 CM amplitude are depicted together in the lower panel of figure 2. There was only a minor decrease in f_1 CM amplitude during and after injection. The $2f_1$-f_2 CMDP appeared to be stable during injection with only minor positive or negative changes. Typically, the f_2-f_1 CMDP increased at the onset of injection. At the end of injection, the f_2-f_1 CMDP amplitude was mostly at a higher level (6 out of 8) than before injection.

Discussion

The purpose of the present study was to quantify changes in CMDP and DPOAE as a measure of cochlear function during an acute endolymphatic hydrops. A volume increase of 1.1 µl amounts to an endolymphatic hydrops of 23% of the initial total endolymph volume in the inner ear of the guinea pig, which is 4.7 µl (Shinomori et al., 2001). As in a previous study (Valk et al., 2004a) this degree of an acute hydrops caused only a small decrease in $2f_1$-f_2 DPOAE amplitude. 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 (figure 2).

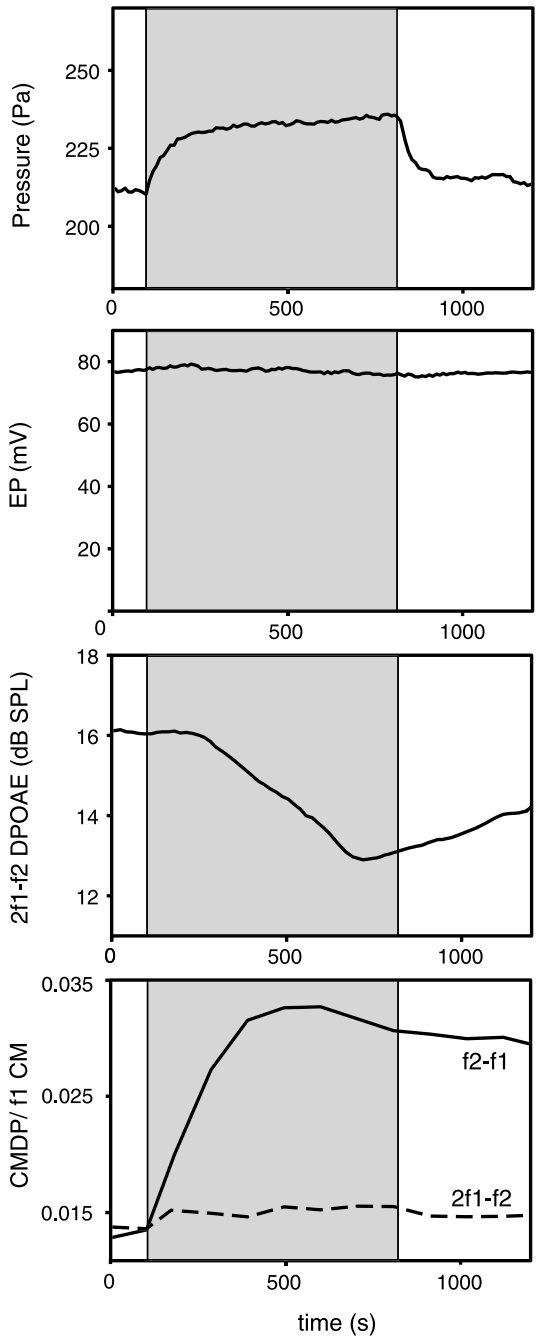

Figure 2: Averaged changes (n=8) in pressure (upper panel), endocochlear potential (second panel), $2f_1$-f_2 DPOAE amplitude (third panel) and $2f_1$-f_2 and f_2-f_1 CMDP amplitude relative to the amplitude of f_1 CM (lower panel). The grey area depicts the injection period.

An acute induced endolymphatic hydrops presumably alters cochlear mechanics by changing basilar membrane (BM) position and impeding its motion (Braun, 1996). The BM displacement towards scala tympani at the $2f_1$-f_2 generation site was estimated to be only 19 nm when the endolymph volume was increased by 1.1 µl (Valk et al., 2004a). A small permanent deflection of the outer hair cell stereocilia and as a consequence a change in cell conductance might be responsible for the observed changes in the distortion products.

Distortion is a consequence of the non-linear shape of the cochlear transducer input-output curve (Bian et al., 2002). We assumed this curve to be approximated by $f(x) = 1 / (1 + \exp(x+x_0))$, in which x(t) is the input signal (t=time) and x_0 defines the transducer operating point (figure 3a). Distortion components for a two-tone input signal, with frequency and amplitude ratio's as used in the experiment, were calculated. Distortion depends on the amplitude of the input signal and on x_0. The amplitude was chosen as a relative magnitude (amplitude of distortion product divided by amplitude of f_1) to yield the f_2-f_1 and $2f_1$-f_2 CMDP as measured (see figure 2). The dependance of distortion product amplitudes on x_0 for this choice of input signal amplitude is given in figure 3b. During and after artificial endolymph injection, the relative f_2-f_1 amplitude changed from 0.012 to 0.032, while $2f_1$-f_2 remained more or less constant between 0.012 and 0.013 (figure 2). These changes approximately correspond with the changes shown in figure 3b for a change in x_0 between 0.20 and 0.55. The consequence of this x_0 change for the position of the transducer curve is shown in figure 3a. Also shown in this figure is the range of x (t) -values corresponding with the choice for the stimulus signal amplitude. It can be concluded from this figure that the measured range of distortion amplitudes during and after injection of 1.1 µl of artificial endolymph can be covered by a relatively small change of the transducer operating point x_0.

An estimated basilar membrane displacement of 19 nm corresponds with a hair cell cilia displacement of a few tenths of a degree (Dallos, 2003). As the change in transducer conductance covers a range of a few degrees of hair cell cilia displacement (Kros et al., 1995) the transducer curve shift as shown in figure 3a is realistic.

Our results are in accordance with those of Sirjani et al. (2004), who injected volumes up to 1.2 µl with injection rates of 80, 200 and 400 nl/min. Most specifically, CM distortion and transducer operating point showed substantial changes, of which the direction and magnitude varied. To model their results these authors needed an operating point shift of about 0.1, which in their units is 5% of the stimulus peak-to-peak amplitude. The shift with respect to stimulus amplitude shown in figure 3a is of the same order of magnitude (15%).

By using electrocochleography Jin et al. (1990) showed a rise of summating potential (SP) amplitude, a decrease of action potential (AP) amplitude and an increase of the SP/AP ratio. Clinically, in patients with Menière's disease an elevated SP/AP ratio is often measured (Conlon and Gibson, 2000; Ferraro and Tibbils, 1999) and explained by asymmetric vibration of the basilar membrane as a result of endolymphatic hydrops (Gibson, 2000). SP depends critically on the position of the operating point of the cochlear transducer (van Emst et al., 1997).

On the other hand, both DPOAE's and CM were not found to be useful for differentiating patients with hydrops from those without (Fetterman, 2001).

In conclusion, the physiological response of the cochlea to an acute endolymphatic hydrops can be related to small changes in the cochlear transducer operating point.

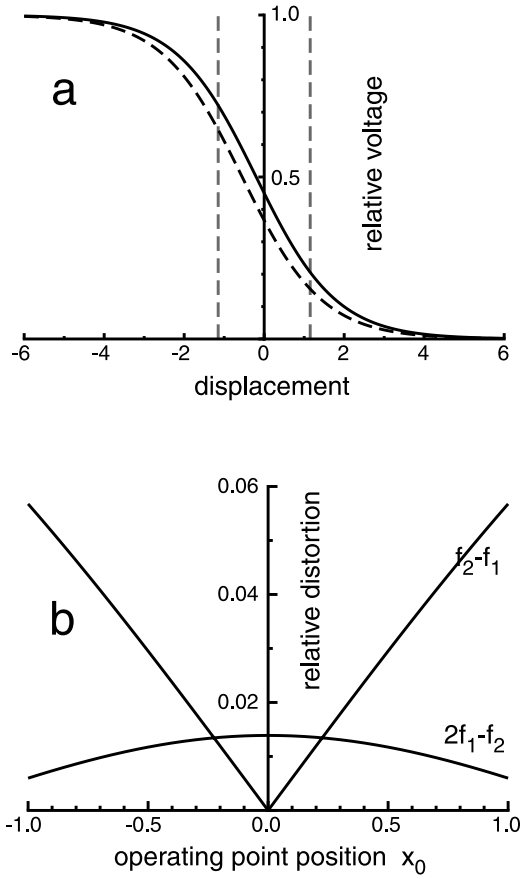

Figure 3:

a. Assumed cochlear transducer input-output curve, f(x) = 1/(1 + exp (x+x_0)) for x_o = 0.20 (solid line) and x_0 = 0.55 (dashed line). Vertical dashed lines mark the maximum amplitude of the f_1+f_2 stimulus signal x(t).

b. Magnitude of distortion products (relative to the amplitude of f_1) as a function of transducer operating point position x_0, for an input signal with maximum amplitude as shown in figure 3a

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