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Acute endolymphatic hydrops

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Changes in CMDP and DPOAE during acute increased inner ear pressure in the guinea pig

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Introduction

The cochlea is a nonlinear mechanical transducer that transforms sound pressure waves into electrical information, the so-called mechanoelectric transduction (MET). The reverse electromechanical transduction is performed by the outer hair cell (OHC) which mechanically influences the vibration of the cochlear partition (Kim, 1986). When the cochlea is stimulated by two tones, distortion products can be measured in cochlear microphonic potentials (CMDP) and in the external auditory meatus as otoacoustic emissions (DPOAE). Both microphonic and acoustic distortion products are considered to be reflections of OHC electromotility in the cochlea. Obviously, it is of interest to find a relation between these two functional modalities of the cochlea and the OHC in particular.

In this study the most prominent distortion products, the $2f_1$- f_2 and f_2- f_1, have been observed while increasing inner ear pressure. This was done by rapid injection of artificial perilymph into the scala tympani of the guinea pig's cochlea. Measurement of specific distortion products may describe changes in gain and operating point of the non-linear cochlear amplifier. Furthermore, transfer characteristics of forward and backward transmission may be clarified by using CMDP and DPOAE (Zhang and Abbas, 1997). These data could help gaining insight into pathophysiological mechanisms involved in pathologically increased inner ear pressures.

When pressure variation is in the normal physiological range, this does not influence cochlear function as measured by compound action potentials in the guinea pig (Böhmer, 1994). In humans, during activities like coughing or sneezing, perilymphatic pressure may undergo substantial fluctuations up to 10 mm Hg (1.4 kPa).

An increased pathologic inner ear pressure may be the result of an increased intracranial pressure since the perilymphatic space is linked to the subarachnoid space via the cochlear aqueduct. Human otoacoustic emissions have been proven to be very sensitive to modifications of intracranial pressure (de Kleine et al., 2000). Another clinical example of increased inner ear pressure or volume with an accompanying change of cochlear function is the endolymphatic hydrops in patients with Menière's disease.

In a previous study (Valk et al., 2004a), we have already measured the $2f_1$- f_2 DPOAE's in response to microinjection of artificial perilymph into the cochlea. Large changes of approximately 500 Pa in overall inner ear pressure gave only a small mean change of 0.7 dB in $2f_1$- f_2 DPOAE amplitude. The magnitude of change could (partly) be explained by a change in oval window stiffness. In this study, the same experimental setup was used, with the addition of the simultaneous recording of the $2f_1$- f_2 and f_2- f_1 CMDP. We attempt to establish a better understanding of increased inner ear pressure effects on distortion products in both cochlear microphonic potentials and OAE's.

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 skin electrodes 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 . 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. 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, which is a compromise between a low enough flow resistance for fluid injection and tip smallness. One barrel of the pipette was used to measure inner ear pressure (WPI 900A micropressure system). Through the other barrel, artificial perilymph (Salt and De Mott, 1998) 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 (0.84 mm). The fluid injection rate was calculated as the total injected volume divided by the total injection time.

During the experiment the DPOAE's 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 of the measuring equipment are described 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 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.

A measurement contained a sequence of 10 microinjections of artificial perilymph of 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). 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, f_1 CM and inner ear pressure. Calculations and fits to relevant portions of the obtained recordings were made off-line with an appropriate software package.

Results

Successful repeated sequences of injection with a rate of 50 nl/s (n=10) were performed in all guinea pigs (n=8).

The mean steady state inner ear pressure measured prior to injection of artificial perilymph was 205 Pa.

To determine the rise and fall times of the curves from the measured signals, proper fits were obtained with a simple exponential function:

$$a \exp(-t/\tau) + c$$

In some cases (figure 1c, 2c, 4c) the curves needed an extra term in the formula for a proper fit:

$$a \exp(-t/\tau) / [1 + b \{1 - \exp(-t/\tau)\}] + c$$

According to Wit et al. (1999) time constants τ were calculated from the fits.

In figure 1a the averaged recorded inner ear pressure during and after injection of 0.5 μl of artificial perilymph is shown. At the start of injection the mean pressure increased to an approximately 600 Pa higher level within seconds. At the end of injection, the pressure immediately decreased,
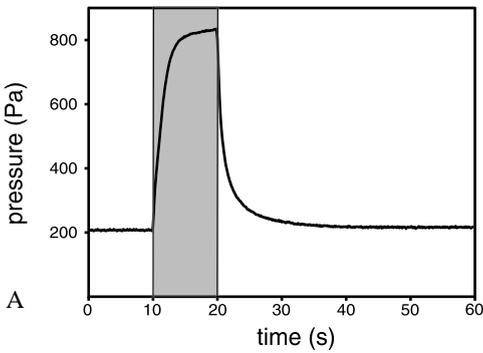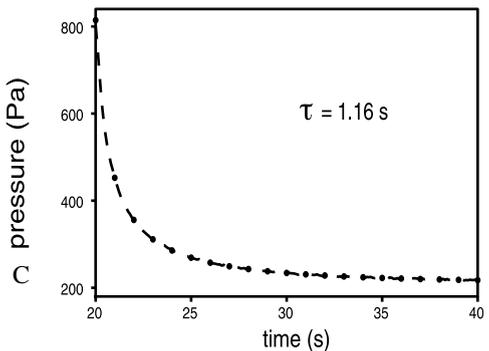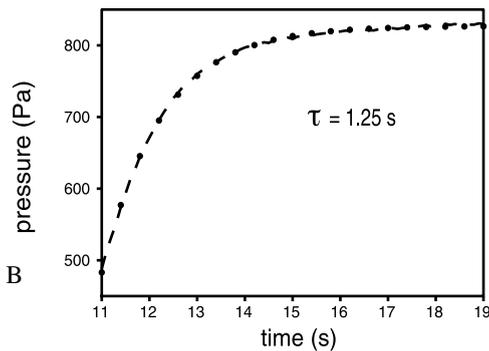

Figure 1: A. Averaged changes (n=8) in inner ear pressure (Pa) during and after injection of 0.5 μl of artificial perilymph into scala tympani. The grey area depicts the injection period.

B. The shape of the mean inner ear pressure curve at the start of injection (time axis corresponds with figure 1A). The recording (filled circles) is fitted (broken line) with an exponential function, yielding a time constant τ.

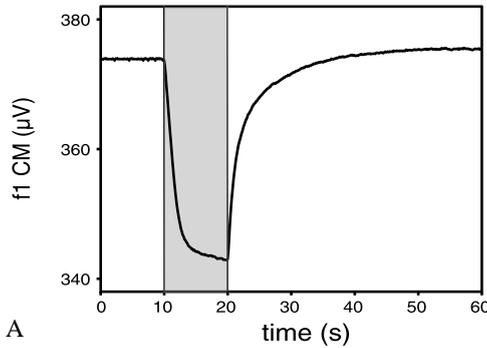C. The same is done for inner ear pressure recovery directly after injection.

Figure 2: A. Averaged changes (n=8) in f_1 CM amplitude (µV) during and after injection of 0.5 µl of artificial perilymph into scala tympani. The grey area depicts the injection period.
B. The shape of the mean f_1 CM amplitude curve at the start of injection (time axis corresponds with figure 2A). The recording (filled circles) is fitted (broken line) with an exponential function, yielding a time constant τ.
C. The same is done for f_1 CM amplitude recovery directly after injection.
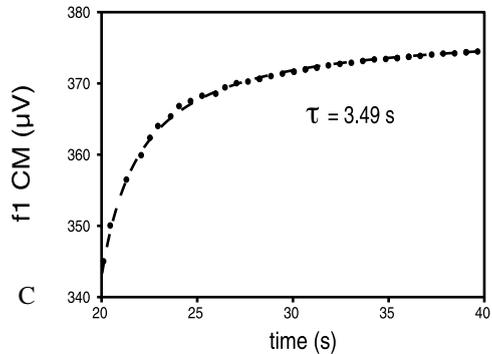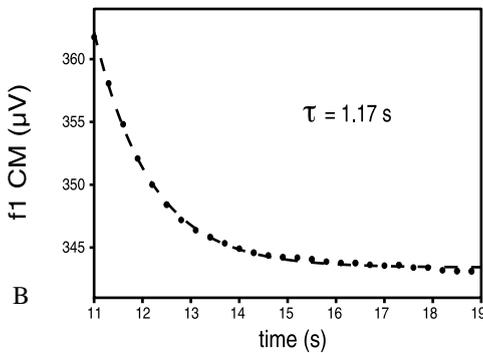
returning to its initial value within seconds. This pattern was consistently observed in all individual experiments. The time constant for increase and subsequent decrease of inner ear pressure were 1.25 and 1.16 s respectively.

The f_1 signal in the cochlear microphonics (figure 2a) showed a mirrored behaviour in respect to the inner ear pressure. Directly after the start of injection, the f_1 CM decreased by 30 µV. The corresponding time constant was 1.17 s, which is very close to the time constant for pressure increase. Conversely, at the end of injection the f_1 amplitude returned to its initial value with τ = 3.49 s, which is slow with respect to the corresponding τ for pressure.

The averaged DPOAE $2f_1$-f_2-amplitude showed a similar typical course as the inner ear pressure (figure 3a). Almost directly after the start of injection the $2f_1$-f_2-amplitude increased to reach a maximum of 0.4 dB above its original value at the end of the injection period. When the injection was instantaneously terminated, the amplitude dropped and returned to slightly above its onset level. There was a small observable time lag with respect to the pressure curve. The time constant for increase and subsequent decrease of the $2f_1$-f_2-amplitude were 1.52 and 3.43 s respectively. In figure 4a the typical temporary decrease for the mean $2f_1$-f_2 CMDP amplitude relative to the f_1 CM is shown. The time constant for decrease in amplitude at the onset of injection was 1.12 s, which corresponds with τ for pressure. The recovery is somewhat slower (2.28 s), but still faster than the time constant for $2f_1$-f_2 DPOAE recovery.

Finally, in figure 5, the averaged results of the f_2-f_1 CMDP amplitude relative to f_1 CM depict an

Figure 3: A. Averaged changes (n=8) in $2f_1$- f_2 DPOAE amplitude (dB SPL) during and after injection of 0.5 μl of artificial perilymph into scala tympani. The grey area depicts the injection period.
B. The shape of the mean $2f_1$- f_2 DPOAE amplitude (μPa) curve at the start of injection (time axis corresponds with figure 3A). The recording (filled circles) is fitted (broken line) with an exponential function, yielding a time constant τ.
*C. The same is done for $2f_1$- f_2 DPOAE amplitude recovery directly after injection.*

A

B

C
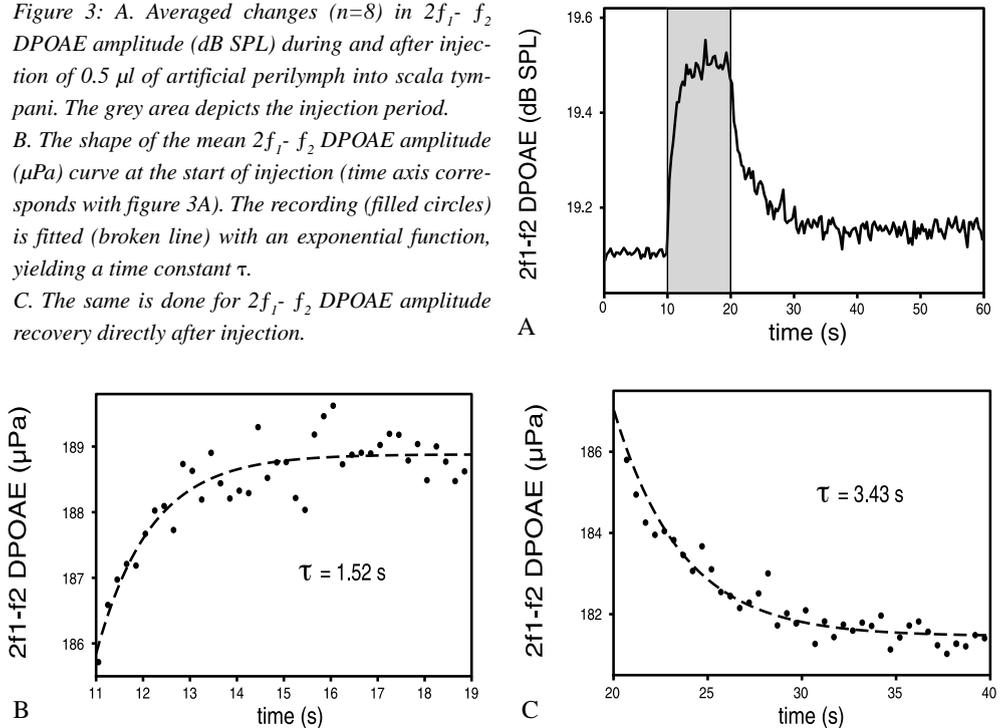
increase during injection of artificial perilymph. When the injection was terminated, the f_2- f_1 CMDP amplitude returned to the pre-injection level. Unfortunately, due to a low signal-to-noise ratio for the f_2- f_1 CMDP, the obtained curve could not be fitted properly and no time constants could be derived.

Discussion

The most striking result of this study is the minimal change in amplitude of the distortion products in both OAE and CM during an acute increase of inner ear pressure. Besides, amplitudes changed to normal when inner ear pressure returned to its initial level. These results indicate a dynamically stable inner ear which is able to cope with a pressure increase of 600 Pa with only minimal and reversible cochlear function loss. According to Böhmer (1994) this level of pressure increase would still be in the physiological range of the guinea pig ear varying between –100 and +700 Pa. The inner ear is an elastic structure completely encompassed by bone, in which pressure changes depend on the compliance of the membranous structures (Wit et al., 2000). In normal ears, hydrostatic pressure in the perilymph equals pressure in the endolymph, and pressure changes applied to one compartment are immediately transmitted to the other through the elastic walls. The perilymph is in a dynamic equilibrium with the cerebrospinal fluid via the cochlear aqueduct. An excess of perilymph will escape through the cochlear aqueduct restoring inner ear fluid level.
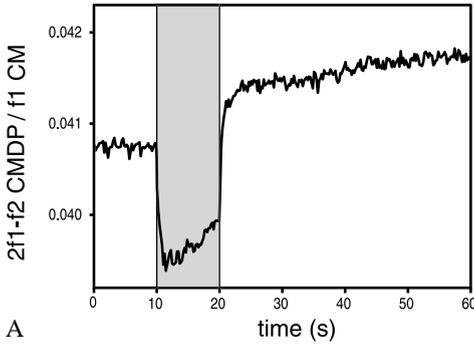
Figure 4: A. Averaged changes (n=8) in $2f_1$-f_2 CMDP relative to f_1 CM amplitude during and after injection of 0.5 μl of artificial perilymph into scala tympani. The grey area depicts the injection period.
B. The shape of the mean $2f_1$-f_2 CMDP (relative to f_1 CM) amplitude curve at the start of injection (time axis corresponds with figure 4A). The recording (filled circles) is fitted (broken line) with an exponential function, yielding a time constant τ.
*C. The same is done for $2f_1$-f_2 CMDP (relative to f_1 CM) amplitude recovery directly after injection.*
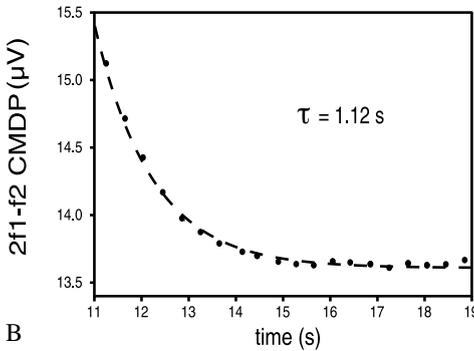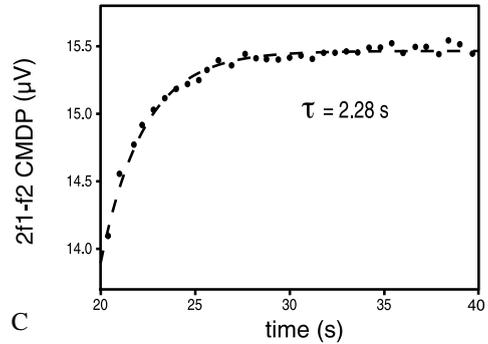
When increasing inner ear pressure, a cochlear disturbance with functional loss is expected. The observed decrease in CM f_1 amplitude thus seems obvious. An increased cerebrospinal fluid pressure also suppresses the CM. The increase in $2f_1$-f_2 DPOAE is counterintuitive, but in accordance with a previous publication in which we demonstrated the same change in cochlear function as measured by $2f_1$-f_2 DPOAE's (Valk et al., 2004b). According to Büki et al. (1996) a change in stiffness of the oval window might slightly improve middle ear transfer and thus explain the small increase in DPOAE amplitude. In contrast to the $2f_1$-f_2 DPOAE, the $2f_1$-f_2 CMDP showed a decrease in amplitude during injection. Unfortunately, in literature very little can be found about the relation between DPOAE and CMDP. With the exception of Kemp and Brown (1984), who found close parallels in the guinea pig in the behaviour of the $2f_1$-f_2 DPOAE and CMDP during and after anoxia. Furthermore, group latencies and third-tone suppression were found to be very similar, linking both signals to have a common origin.

The DPOAE and CMDP are generated in response to the same acoustic stimuli. The acoustic primaries are transferred through the external and middle ear to eventually reach the sensory epithelia in the cochlea. The cochlea as a nonlinear mechanical transducer transforms sound pressure waves into electrical information. This forward transduction process is called the mechano-electric transduction (MET). The cochlear microphonic potential is a direct result of this process and is thought to be mainly produced by outer hair cells. On the other hand, there is also a reverse electromechanical transduction in which the outer hair cell mechanically influences the vibration

Figure 5: Averaged changes (n=8) in f_2- f_1 CMDP relative to f_1 CM amplitude during and after injection of 0.5 µl of artificial perilymph into scala tympani. The grey area depicts the injection period.
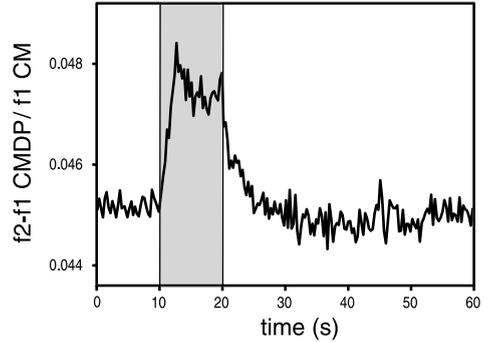
of the cochlear partition (Kim, 1986). Sound is transmitted back to the external ear where the acoustic DPOAE can be measured; the backward transmission.

The rise and fall times of the distortion products in response to changes in inner ear pressure were derived from the fitted curves (figures 2, 3 and 4). As both CMDP and DPOAE were measured, this yielded information on forward and backward transmission. As anticipated, the time constants for the CMDP were smaller than the time constants for the DPOAE. Indeed, the latter would travel back and forth the middle ear. The averaged differences were 0.4 and 1.15 s for the $2f_1$- f_2 distortion product during increase and decrease of inner ear pressure respectively.

At pressure increase all measured signals almost directly followed the change in inner ear pressure. Time constants varied from 1.17 to 1.52 s with respect to 1.25 s for the inner ear pressure curve. However, with pressure decrease there was a delay of 1-2 s for all measured signals. Measured differences cannot be accounted for by delay in measuring equipment, for this was <0.18 s. Assumed that the overall stiffness of the stapes-oval window-system governs the behaviour of the DPOAE $2f_1$- f_2 amplitude, it is expected that it would follow stiffness changes directly and not 2 s later, as observed. It is conceivable that a delayed change in the endocochlear potential (EP) could be the cause of difference in τ values. However, in experiments by Salt and DeMott (1999) the EP followed a temporary pressure change with a delay of < 0.1 s. So, the mechanisms for the observed delays in inner ear pressure recovery remain to be elucidated.

By injecting artificial perilymph into scala tympani of the cochlea, the basilar membrane is temporarily displaced towards scala media. By this displacement, the operating state of the cochlear amplifier is influenced. Bian et al. (2002) described the forward mechano-electrical transduction (MET) by a sigmoid-shaped function, which relates the displacement of the basilar membrane to the response of the hair cells. We measured a small decrease in the amplitude of the $2f_1$- f_2 distortion in CM (with respect to the amplitude of f_1 in CM) and a small increase in the f_2- f_1 in CM. Using the model by Bian et al. (2002) this leads to the conclusion that the operating point for hair cell transduction is somewhat shifted from a symmetric position. This shift leads to an increase in even-order distortion and a decrease in odd-order distortion. Along these lines of reasoning we would also expect the $2f_1$- f_2 DPOAE to decrease. This might be the case at the generation sites (Withnell et al., 2003), but this effect is then more than compensated for by an improved middle ear transfer.

Anyhow, the observed changes are small. Changes of overall inner ear fluid pressure have minor

effects on cochlear function. It may be that creating an artificial endolymphatic hydrops, by injecting into scala media instead of scala tympani, has a larger effect. This will be the tested in further research, which will hopefully give insight into diseases with pathologically changed inner ear fluid volumes, as in Menière's disease.

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