Injection of artificial endolymph in the guinea pig inner ear alters vestibular evoked potentials.

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Introduction

Menière disease has for a long time been associated with an endolymphatic hydrops. In experimental research, the most widely used method for the creation of an endolymphatic hydrops is surgical obliteration or dissection of the endolymphatic sac \(^{(1,2)}\). Another method is inducing endolymphatic hydrops by administration of immune complexes \(^{(3, 4)}\). Both of these methods induce a slowly developing endolymphatic hydrops.

Menière disease is characterised by episodic vertigo attacks and fluctuating, subacute, hearing losses and tinnitus. It might therefore be that the Menière attacks and the concomitant symptoms are better explained by a more sudden event like an acute endolymphatic hydrops. This can be obtained in a controllable way by microinjection of artificial endolymph in the scala media of the cochlea \(^{(5,6,7,8)}\).

Although a vertigo attack is usually the presenting symptom in Menière’s disease, little is known about the physiological changes of the vestibular organ during these attacks. In an extensive review paper Honrubia gives arguments for dysfunctioning of the vestibular system being caused by endolymphatic hydrops \(^{(9)}\). Others advocate the membrane rupture theory, leading to potassium intoxication of the vestibular system to explain the attacks of vertigo \(^{(10)}\).

In Menière patients, it is –for obvious reasons– difficult to obtain vestibular test results during a vertigo attack. During the quiet state of the disease, the vestibular test results show only small abnormalities with little clinical relevance. Furthermore, the lack of quantification and standardisation, and the fact that Menière’s disease often affects both ears, makes the interpretation of the obtained results complicated \(^{(11)}\).
About 15 years ago, the recording of short latency evoked potentials evoked by linear acceleration stimuli was introduced as a valuable method in experimental animal research \cite{12,13,14,15,16,17}.

Although the evoked responses are mainly initiated in the otolith organs \cite{18} and vertigo attacks in Menière patients are thought to originate in the semicircular canals, the method may still be useful to obtain insight in the relation between acute endolymphatic hydrops and disturbances to the vestibular system induced by expansion of the endolymphatic compartments.

Therefore, in this study, we combined injection of artificial endolymph into scala media with recordings of vestibular potentials, evoked in the injected ear and in the contralateral (control) ear.

Materials & methods

In this study 13 female albino guinea pigs with a normal Preyer reflex were used (bodyweight approximately 500 gram, Harlan, The Netherlands). Artificial endolymph injection experiments were performed in the right ear of 10 guinea pigs. Vestibular evoked potentials (VsEP) were measured in all injected ears. In five of the injected animals, VsEPs were also measured in the (uninjected) contralateral ear. Three guinea pigs were used for control measurements: In two right ears the basilar membrane was perforated until the scala media was reached, but no injection of artificial endolymph was given; and in one animal only VsEPs were measured on both sides without any further disturbance of the ears.

Animal care and use was in accordance with the principles of the declaration of Helsinki and approved by the animal experiment committee (protocol number 2325). All animals were anaesthetised before the experiment using ketalazine /xylazine intramuscularly (1 mg/kg, 0.7/0.3) and received additional doses at regularly spaced intervals. The animal was sacrificed after the last measurement without regaining consciousness.

A tracheostomy was performed to obtain a free airway during the whole procedure until sacrifice after the last VsEP measurement. To stimulate the vestibular system by acceleration pulses, a steel bolt was cemented upside down to the top of the skull of the guinea pig (figure 1a). After exposing the bony facial nerve canal near the bulla, a platinum electrode was implanted up to the first curvature. At this position only a thin bony wall separates the electrode from the vestibular nerve. A reference electrode was placed in the neck muscles. In five guinea pigs active electrodes were placed only on the
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Acceleration pulses were generated with a Brüel and Kjaer vibration exciter (type 4809) and monitored with an accelerometer, connected to a Brüel and Kjaer amplifier (type 2651). Linear acceleration pulses were applied in the direction of the earth vertical axis perpendicular to the skull. Since the inner ear is fixated in the cranial bone, the acceleration measured by the accelerometer attached to the bolt is equal to the acceleration in the vestibular system. The shaker was driven by computer–generated stimuli that consisted of Haver sine pulses, amplified by a power amplifier (Brüel and Kjaer type 2712) and computer–controlled with a programmable attenuator. The vestibular stimulus consisted of 500 alternating pulses with peak amplitude of \(40 \text{ m/s}^2\) at \(0.5 \text{ ms}\) after onset, at a rate of \(51/\text{sec}\).

Electrophysiologic responses to acoustic stimuli were also measured with the electrode in the facial canal to monitor cochlear functioning. Acoustic stimuli were computer–generated and consisted of \(1 \text{ kHz}\) Haver sine pulses that were presented at a level of \(70 \text{ dB}\) with a speaker situated \(15 \text{ cm}\) in front of the animal’s head.

The signals of the active electrodes were amplified (Disa, type 15 c01) and band–pass filtered (100 Hz–5 kHz). The first 10 ms of the electrode and accelerometer signals were recorded, averaged and processed, using a Tucker–Davis BioSig–stimulate/record system; version 2.0.
After the first VsEP measurement, the animal was transported to another set–up to inject artificial endolymph. The animal’s head was positioned to its side and kept in a stationary position by means of the steel bolt after which the bulla of the right ear was opened by a retroauriculair approach and the round window exposed. Through the round window membrane, the tip of a double–barrelled micropipette was inserted into the scala tympani, and after subsequent perforation of the basilar membrane into the scala media. DC potential at the pipette tip was measured to verify its position (figure 1b). Double–barreled micropipettes were drawn from borosilicate glass (1.5/ 0.84 mm diameter per barrel) and the tips were bevelled (Narishige EG–40). Tip diameters were around 30 µm per barrel, which is a compromise between low enough flow resistance for fluid injection and tip smallness. One barrel of the pipette was used to measure inner ear pressure and DC potential (WPI 900A micropressure system). Through the other barrel, artificial endolymph (140 mM Cl+25 mM KHCO3 ;[5, 8]) could be injected with a constant flow rate into the inner ear by applying a controllable pneumatic pressure (WPI PV830 Picopump) to the barrel end. The fluid injection rate was calculated as the total injected volume divided by the total injection time. The injected volume was measured as displacement of the fluid meniscus in the pipette, for which the inner diameter is precisely known (0.84 mm). During the injection process fluid pressures and the DC potential were recorded digitally with a rate of 20/ s.

Figure 1b  Location of the micropipette during artificial endolymph injection
SM: scala media,
SV: scala vestibuli,
ST: scala tympani,
RW: round window,
p: pipette
A typical experiment started with the measurement of vestibular evoked potentials in both ears. Then 2 μl of artificial endolymph was injected in the scala media of the right ear with an injection rate between 30–60 nl/sec. After this, VsEP–measurements in both ears were repeated immediately and 30 minutes, 1 hour and 2 hours after endolymph injection, making a total of 5 VsEP measurements per ear. The amplitude of the first positive peak of the VsEP was used as a measure for vestibular functioning. The pre–injection amplitude was used as a reference, since interindividual variation of the VsEP is high. All subsequent values were normalized with respect to this reference value. Statistical evaluation was performed using SPSS 10.5. Both the non–parametric Mann–Whitney tests for non–normally divided groups as well as independent t–tests for normally divided values were used.

Figure 2  Upper panel: fluid pressure at the tip of the micropipette in cm water; Lower panel: DC potential at pipette tip. At a the tip perforates the round window; variations in the pressure trace are caused by respiration. At b the basilar membrane is perforated. The sudden increase of DC potential confirms the tip position in the endolymph. Between c and d artificial endolymph is injected. At e the tip is withdrawn into the perilymph of scala tympani and has at f returned to its initial position in a thin fluid layer outside the round window.
Results

All 10 artificial endolymph injection experiments were successful. DC potential measurements confirmed positioning of the double-barrel pipette tip in the endolymph of scala media before artificial endolymph administration. After endolymph injection, pressures in scala media and scala tympani returned to their initial value. A typical time course for pressure and DC potential curve before, during and after injection is shown in figure 2.

Pre- and post injection VsEP measurements were acquired in all 10 animals. Two guinea pigs died after the first 2 measurements (due to an obstructed airway, respectively a suspected overdose of anaesthesia). The results from twenty-one ears were statistically evaluated (10 injected ears and 11 uninjected ears). A complete series could be obtained from 8 injected and 10 uninjected ears. An example of a series of VsEP recordings is given in figure 3.

![Figure 3](image-url)  
Averaged VsEP recordings before, immediately after and at 0.5, 1 and 2 hours after injection, measured in an injected ear (left traces) and in the contralateral uninjected ear of the same animal (right traces). The systematic increase of peak latency is explained by a slowly decreasing body temperature of the animal.
The development of the amplitude of the first positive peak (P1) of all tested ears is shown in figure 4a. Figure 4b shows the average of figure 4a for the injected and for the uninjected ears.

The averages for the injected ears (figure 4) of the first three VsEP measurements after injection (immediately, at half an hour and at one hour after injection) are not significantly different from the average values for uninjected ears. However, after two hours after injection the difference between the average values for the injected and for the uninjected ears is significant, both in the independent t–test (p=0.03), as well as in the Mann–Whitney test (p=0.03).

Figure 4a  Normalized amplitude first VsEP peak for all individual ears, measured before (i), immediately after (2) and at 0.5 (3), 1 (4) and 2 hours (5) after injection.
Figure 4b  Averages of figure 4a.
Discussion

In the injection experiments 2 µl of artificial endolymph was administered to scala media. Since the total endolymph volume of the cochlear compartment of the guinea pig inner ear is estimated to be 1.5 µl, and the total endolymph volume 4.7 µl, this is a substantial increase of endolymph volume.

The major effect of injection of artificial endolymph in scala media was the gradual decline of the vestibular evoked potentials. Because DC potentials and pressures returned to their original values after injection, rupture of membranes or gross mixing of endolymphatic and perilymphatic fluid is unlikely as an explanation for the observed VsEP–decline. In the control experiments, where the basilar membrane was punctured but no injection was performed, VsEPs remained comparable to the potentials in normal ears. This makes slow leakage through the puncture hole unlikely.

It is possible that the composition of the injected artificial endolymph does not exactly match that of artificial endolymph. This, in combination with the rather high injection rate, may explain the decline in endocochlear potential during injection, as seen in figure 3. However, the endocochlear potential recovers to almost its original value within 3 minutes, while the effects on the VsEP occur on a time scale of hours.

Rask–Andersen et al. showed that injection of artificial endolymph into scala media has a dramatic effect on the composition of the contents of the endolymphatic sac. They found that the normal intraluminal homogenous substance in the sac disappears shortly after injection and supposed that this substance plays an important role in the regulation of endolymph volume by absorptive or desorptive mechanisms. But how such a volume recovery mechanism could explain a permanent decline of VsEPs remains unclear. It can also simply be that injection of endolymph has done direct damage to vestibular sensory cells.

In a recent paper Yamauchi et al. showed that a translabyrinthine dilatational pressure is generated across the membranous labyrinth in response to an increase in endolymph volume, leading to ampullary distention and cupular deformation. Whether this dilatation pressure also affects the otolith organs (the supposed main source of the VsEP) is unclear at this moment.
Conclusion

Measurements of the short latency vestibular evoked potentials after an acute endolymphatic hydrops, induced by the administration of artificial endolymph, indicate the existence of a sub-acute, functional, damaging effect on the vestibular system. The characteristics of the damage and its possible relation with vertigo attacks in Menière’s disease are a subject of further research, in which morphological and physiological methods will be combined.
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20. Rask–Andersen H., DeMott J.E., Bagger–Sjoback D., Salt A.N. Morphological changes of the endolymphatic sac induced by microinjection of artificial endolymph into the cochlea.
