

Chapter 9

Longitudinal recording of the compound action potential in two-phase endolymphatic hydrops

Dunnebier EA, Verheul J, Segenhout JM, Wit HP, Albers FWJ.
Longitudinal recording of the compound action potential in two-phase endolymphatic hydrops.
Submitted for publication.

Introduction

Since Hallpike and Cairns and also Yamakawa in 1938 discovered hydrops of the endolymphatic system in the temporal bones of patients with Menière's disease, endolymphatic hydrops has been generally accepted as the basic histopathological substrate of Menière's disease^{1,2}.

Total surgical destruction of the endolymphatic sac in guinea pigs and obstruction of the vestibular aqueduct with bone wax is a well-established model for inducing endolymphatic hydrops as observed in temporal bones of patients with Menière's disease³. However, this is a non-physiological profound model for Menière's disease, in which the endolymphatic sac is completely destroyed. In patients suffering from Menière's disease endolymphatic sac tissue remains present, although the size frequently is reduced, suggesting a reduction in resorption capacity^{4,5}.

In our department we developed a new two-phase experimental model for endolymphatic hydrops to investigate the pathogenesis of Menière's disease⁶. In this model, absorption of endolymph is chronically disturbed by surgical dissection of the distal portion of the endolymphatic sac without damaging the intermediate part. Periodic increase of endolymph production is induced by administration of aldosterone, to stimulate the Na/K ATP-ase activity in the stria vascularis and the dark cells of the inner ear. The acute endolymph production will disturb the endolymph homeostasis which is not capable to restore immediately due to the borderline capacity of the endolymphatic sac resulting in the development or increase of hydrops⁶.

In our model of two-phase endolymphatic hydrops, delicate morphological alterations of the structures of the organ of Corti have been demonstrated by scanning electron microscopy in specimens one month after inducing endolymphatic hydrops, such as atrophy and loss of stereociliar structures which were mainly located in the low-frequency regions of the cochlea⁷. Post-mortem observations from a cochlea of a Menière patient revealed similar pathological changes of the stereociliar complex⁸. These ultrastructural changes may represent the histopathological substrate with regard to the early manifestations of inner ear dysfunction in Menière's disease.

Electrocochleographic findings, such as an enlarged summing potential/action potential ratio, may indicate the presence of endolymphatic hydrops⁹. However, there is considerable variability of electrocochleographic results and their interpretation among Menière's patients which may be described to a dynamic pathophysiology. In experimental animal models of endolymphatic hydrops, electrocochleographic results were not consistent and showed a variety of fluctuations¹⁰.

Two-phase endolymphatic hydrops, and its possible dynamic fluctuation of endolymph volume or biochemical composition of the cochlear fluids, may result in fluctuating or permanent influences on sensory cells and functional changes. The permanent implantation of electrodes enabled us to record longitudinally in a standardized setting, which

may reveal a pattern of dynamic changes of our two-phase concept which may be similar to dynamic clinical observations as seen in Menière's patients.

Materials and methods

Thirty healthy female albino guinea pigs (Harlan, The Netherlands) with a weight of 250-350 gram were used in this experiment. Animal care and use were approved by the experimental Animal Committee of the Groningen University, protocol number 1294, in accordance with the principles of the Declaration of Helsinki.

All animals were anesthetized with a 2:1 mixture of ketamine hydrochloride (50 mg/ml) and xylazine 2%. After a retroauricular incision, on each side the temporal bone was opened after which a platinum ball electrode was positioned on the round window membrane. The electrode wire, with a teflon coating of 0.1 mm (Phymep, France) was positioned subcutaneously to an exposed area on the median skull. The temporal bone defect was closed by dental cement and the retroauricular incision was closed by soluble stitches.

After exposure of the median skull, four holes were burred through the skull (diameter 1.6 mm) and stainless screws with a length of 4 mm were placed in these holes. Stainless steel dental wire was wrapped around these screws which served as two reference electrodes. These two reference electrodes as well as the two active electrodes were tinsoldered separately on a plastic-isolated electrode unit. All electrodes and the electrode unit were stabilized on the skull by dental cement. The skin was closed around this electrode unit.

After 14 days, further treatment followed according to several schedules:

- (a) normal control ears without further treatment ($n=10$)
- (b) only administration of aldosterone ($n=8$)
- (c) only dissection of the distal part of the endolymphatic sac ($n=7$), contralateral ears were not dissected and served as a control ($n=7$)
- (d) dissection of the distal part of the endolymphatic sac (day 14) followed by one episode of administration of aldosterone (day 46-50) ($n=7$), contralateral ears were not dissected and served as a control ($n=7$)
- (e) dissection of the distal part of the endolymphatic sac (day 14) followed by two episodes of administration of aldosterone (day 46-50, and 60-64) with a free interval of 10 days ($n=7$), contralateral ears were not dissected and served as a control ($n=7$)

The exact procedure of the distal endolymphatic sac dissection has been described in an earlier paper¹¹.

An episode of administration of aldosterone consisted of once-daily intraperitoneal injections of aldosterone during five days in a dose of 100 microgram aldosterone per

100 gram body weight. The animals were killed at the end of their schedule. The right ear, which was influenced by the systemically injected aldosterone, served as a control in the aldosterone-administrated groups.

Electrocochleographic measurements were performed weekly with extra measurements after endolymphatic sac dissection and during the first episode of administration of aldosterone (2 hours after injections, day 46, 48 and 50) and the second episode (day 60, 62 and 64). The animals were shortly sedated by a low dosage of the above mentioned ketamine/xylazine mixture related to their weight at the time of measurement.

The measurements were performed in a standardized setting in an isolated chamber in which the nose of the guinea pig pointed to a loudspeaker (Pro-sound G-930; 120Watt, one-way, 60-18 (k)Hz, impedance 4 Ohm) at a distance of 10 cm.

Computer generated clicks were band-pass filtered using a Krohn-Hite filter (model 3550), resulting in clicks of 1, 2, 4, 8 and 16 kHz. The filtered clicks were offered with different signal intensities. At the end of the experiment, a registration of following measurements for each frequency was made in which a fixed signal intensity with a good recordable potential was chosen to obtain similar scaling of all recordings (for example, see *Figure 1*). The height of the action potential of each recording relative to the baseline was measured in millimeters and plotted in a time-related diagram. However, to compare the relative changes of the action potentials with ears of the control side and the other groups, the first three (normal) measurements were averaged and this average was defined as a value of 1.0. All measurements for that particular registration were scaled according to this new baseline. The results of each ear were calculated and scaled by computer and plotted in separate diagrams in which the longitudinal potentials after dissection and aldosterone were visualized for different frequencies (for example, see *Figure 2*).

At the end of the experiment, the animals were killed by sublethal administration of sodium pentobarbital (60 mg/kg i.p.) and decapitation, the cochleas were removed, fixed, imbedded in Spurr's low-viscosity resin and midmodiolar sections were studied for the degree of hydrops using light microscopy⁶. The degree of endolymphatic hydrops, which was estimated by the degree of distention of Reissner's membrane in several cochlear turns, was related to the electrocochleographic registration (*Table 1*).

Results

Longitudinal follow-up and technical failures

In all 30 guinea pigs, electrodes were bilaterally implanted without technical or medical complications and adequate electrical responses (compound action potentials) were obtained immediately after surgery.

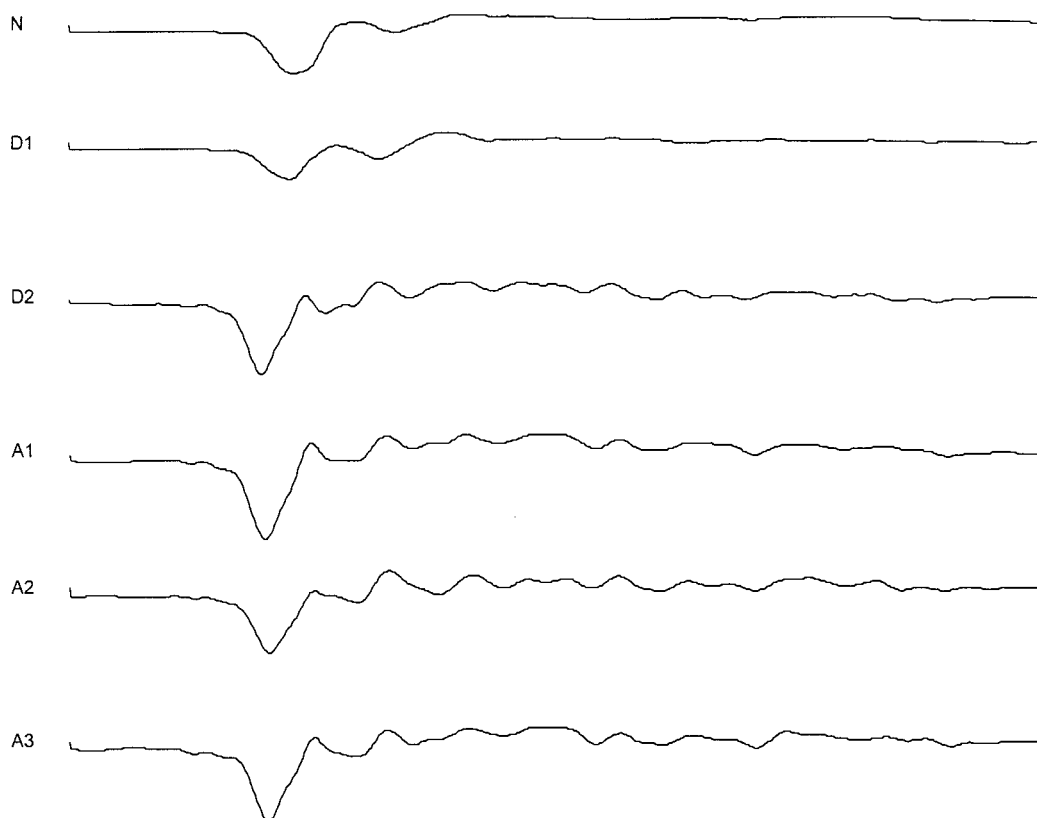


Figure 1. Some long-term 1-kHz recordings of a dissected and aldosterone-treated specimen, showing a control recording before dissection (N, day 7), a decline of the CAP shortly after dissection (D1, day 15) and its restoration four weeks hereafter (D2, day 45). Subsequent administration of aldosterone demonstrated a fluctuation (A1,2,3, respectively day 46, 48, 50). Not indicated in figure: X-axis= milliseconds, Y-axis=millivolts. A complete recording for this specimen at different frequencies has been visualized in Figure 2.

However, in the post-operative weeks 26 ears out of these 60 ears failed in their responses. Of these 26 ears, 13 ears failed to respond immediately in an early phase probably due to technical reasons. In another 13 ears electrical responses were lost during longitudinal follow-up; 4 ears (2 guinea pigs) were lost due to bilateral electrode loss after loss of the electrode unit on the skull after 3 and 6 weeks.

The other ears showed responses which could not be compared significantly due to very low (and hardly measurable) potentials.

Intracochlear and intercochlear comparisons

As indicated in the example of figure 2, the responses of the different frequencies were quite similar in this specimen. In other specimens of all subgroups, no significant changes between the different frequencies were observed. Therefore we decided to

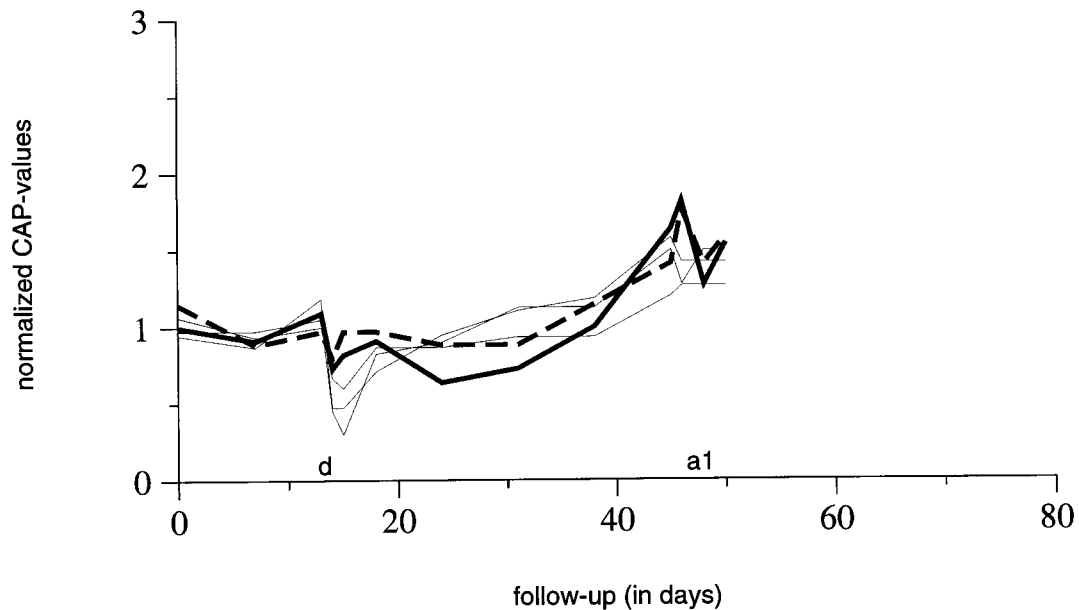


Figure 2. Normalized recordings of CAP versus length of study measured at 1kHz (thick line), 2, 4, 8 kHz (thin lines) and 16 kHz (dotted line). After three control recordings, this ear was dissected (d; day 14) and received one episode of aldosterone (a1; day 46, 48, 50). No significant changes between the different frequencies were observed during the treatment period.

compare the 1 kHz and 16 kHz responses to obtain information about the effects of the treatment on the apical and basal regions of the cochlea.

The degree of endolymphatic hydrops in a specific specimen at the end of the study was correlated to the responses (see also *Table 1*).

(a) normal untreated cochleas (n=10; 5 lost ears):

Five cochleas did not respond shortly after implantation of the electrodes, which may be considered as technical failures. The other five ears showed an enormous variation with values ranging from three times lower or higher than the median value. No sign of endolymphatic hydrops was noted in all specimens.

(b) administration of aldosterone (n=8; 2 lost ears):

Aldosterone-treated ears showed the same variability of the CAP before treatment as observed in the normal control ears. Aldosterone injections at the end of the study did not seem to change the CAP significantly, although an indication of an initial rise after the first injection and a subsequent decline of the potential after the last injections may be observed. Some of these specimens which only received aldosterone systemically showed a slight endolymphatic hydrops in the basal turn of the cochlea.

Ears in which the endolymphatic sac was contralaterally dissected (group *d* and *e*) and which received one or two periods of aldosterone at the end of the study showed the

Table 1. Degree of hydrops in successful recordings for the normal and dissected and/or aldosterone-treated subgroups.

| Treatment Modality | Lost ears | Degree of Hydrops | | | |
|--|-----------|-------------------|--------|----------|--------|
| | | None | Slight | Moderate | Severe |
| No Treatment (<i>n</i> =10) | 5 | 5 | | | |
| Aldosterone only (<i>n</i> =8) | 2 | 4 | 2 | | |
| Dissection only (<i>n</i> =7) | 3 | | 2 | 2 | |
| Dissection and 1 Episode of Aldosterone Injections (<i>n</i> =7) | 1 | 1 | 3 | 2 | |
| Dissection and 2 Episodes of Aldosterone Injections (<i>n</i> =7) | 3 | | 3 | 1 | |

same tendency of an initial rise and a subsequent decline of the CAP during each 5-day treatment with aldosterone.

(c) dissection of the endolymphatic sac:

CAP recordings were performed on cochleas after endolymphatic sac dissection (*n*=7; 3 lost ears) and the contralateral ears (*n*=7; 3 lost ears).

Dissected ears showed a decline of CAP of 20-50% the first day after dissection, which recovered to the starting value or even to higher potentials up to +50% at the end of the study (*Figure 3*). Three specimens showed slight to moderate degrees of hydrops in the apical and middle turns. In the specimen with the highest 1 kHz-CAP at the end of the study (*Figure 3*), only a slight degree of hydrops was noticed in the more basal windings, while the 16 kHz-CAP of this guinea pig showed a remarkable decline of this potential at the end of the study.

Contralateral ears, which were not dissected, showed the same decline of potential one day after dissection on the other side. Some of these potentials were restored to starting values, but some maintained to show a decline of 50% of its starting potential. No hydrops was observed in these specimens.

(d) dissection of the endolymphatic sac and administration of one episode of aldosterone:

CAP recordings were performed on cochleas after endolymphatic sac dissection and aldosterone treatment (*n*=7; 1 lost ear) and the contralateral ears (*n*=7; 4 lost ears).

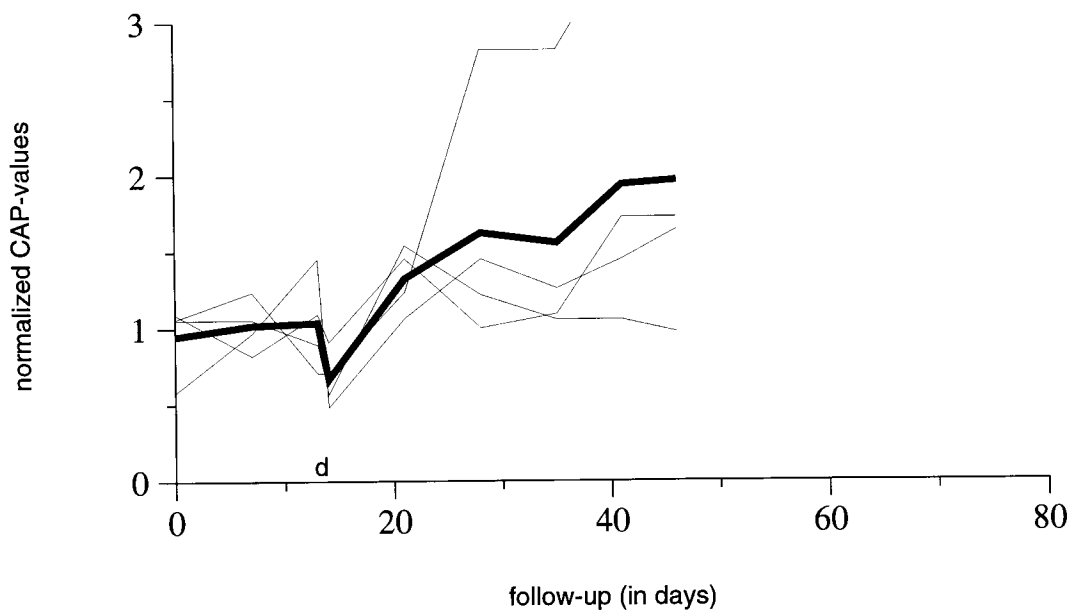


Figure 3. Normalized recordings of 1-kHz CAP versus length of study of four dissected specimens (thin lines) showing the decline of the CAP shortly after dissection (d) and subsequent restoration. An average line was estimated (thick line).

An average decline of the CAP of 30-50% after dissection which gradually recovered to values which were higher than the starting value, were observed in most specimens (Figure 4). Two ears showed increased CAP after dissection which influenced the average. Slight to moderate hydrops was noticed in the upper and middle windings of this group. One ear showed a short absence of the CAP after dissection which gradually restored to a value of 40% lower than the starting value. This specimen showed slight hydrops in all windings.

As mentioned earlier, aldosterone did not significantly alter the CAP at the end of the study (Figure 4). In contrast to an earlier study⁶, in the specimens of this study no exacerbation of hydrops was detected due to aldosterone.

All contralateral ears showed a decline of the CAP as earlier described in contralateral ears after dissection. Hydrops was not observed in these specimens.

(e) *dissection of the endolymphatic sac and administration of two episodes of aldosterone*: CAP recordings were performed on cochleas after endolymphatic sac dissection and aldosterone treatment ($n=7$; 3 lost ears) and the contralateral ears ($n=7$; 5 lost ears).

After dissection, a similar decline of the CAP was observed which restored to starting values or even higher just before administration of aldosterone (Figure 5).

As described earlier, the first as well as the second episode of aldosterone did not significantly alter the CAP at the end of the study, although a tendency of decline may be observed. The treatment free interval of 10 days between the two episodes of aldosterone

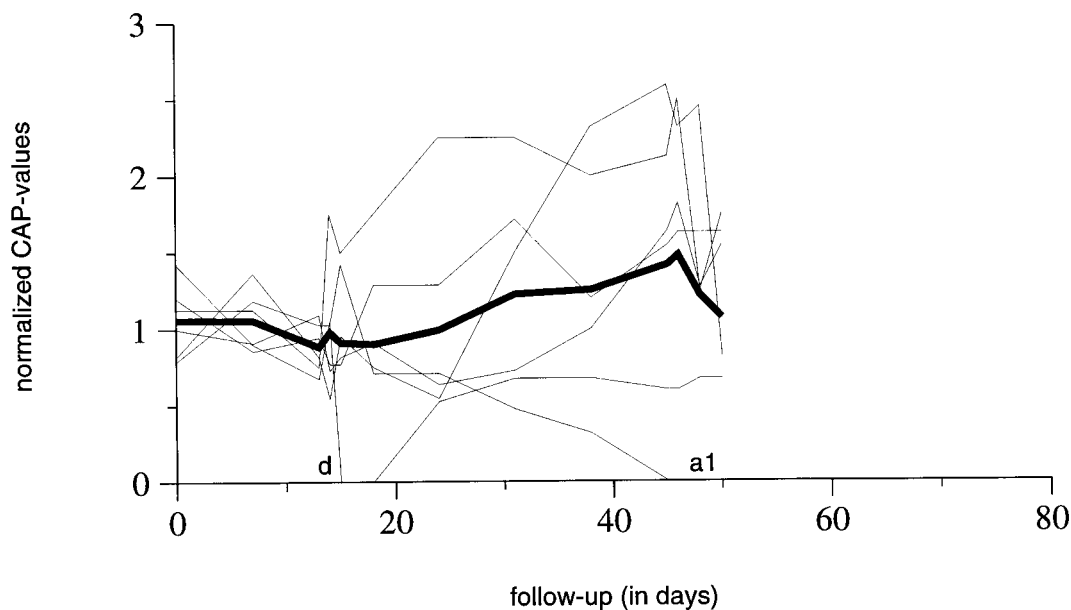


Figure 4. Normalized recordings of 1-kHz CAP versus length of study of dissected specimens followed by one episode of aldosterone treatment ($n=6$). Dissection (d) resulted in an enormous variability and fluctuancy between the specimens (thin lines). Subsequent aldosterone treatment (a1, day 46, 48, 50) demonstrated a tendency of an initial rise of the CAP followed by a decline. An average line was estimated (thick line).

showed a tendency of restoration of the CAP (Figure 5). In general, slight degrees of hydrops were noticed in these specimens (Table 1).

In contralateral ears, an average decline of the CAP of 30% after contralateral dissection, followed by a merely gradual decline to the end of the study. Aldosterone did not significantly alter the CAP and no hydrops was noticed in these contralateral specimens.

Discussion

In this study we evaluated the electrophysiological consequences of a new experimental animal model of endolymphatic hydrops which may mimic the possible underlying pathophysiology and the fluctuant nature of symptoms as observed in patients with Menière's disease.

In our opinion, the two-phase concept of endolymphatic hydrops due to an imbalance between endolymph absorption and secretion is a much more realistic model compared to the classical experimental models, which demonstrated irreversible and progressive endolymphatic hydrops and seemed not representative for the dynamic character of the symptoms observed in Meniere's disease.

The longitudinal measurements of the CAP enabled us to study the relative changes of the CAP in different phases of our two-phase endolymphatic hydrops model to dem-

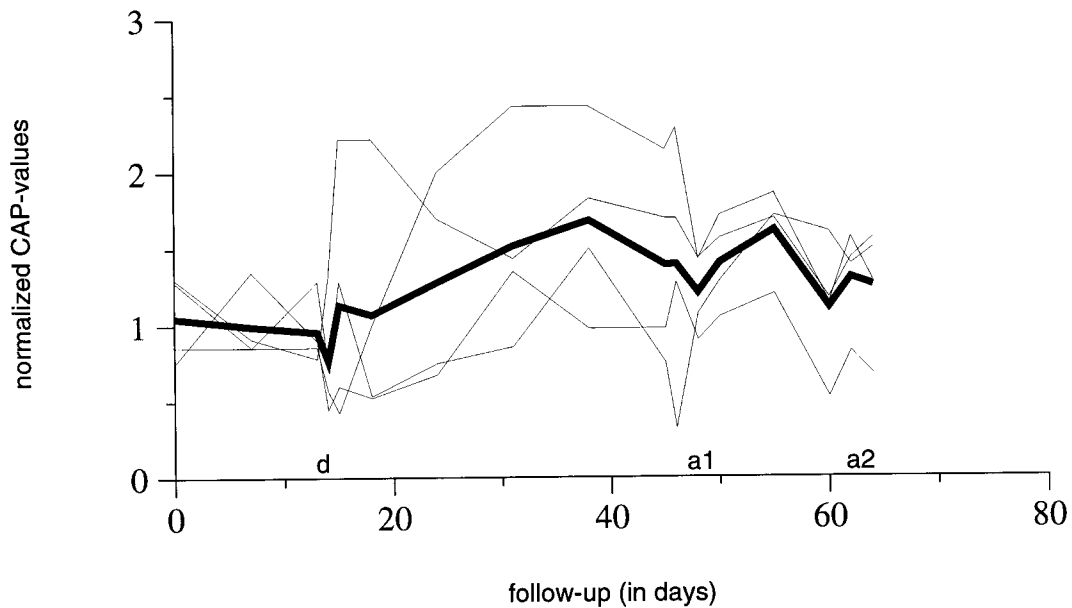


Figure 5. Normalized recordings of 1-kHz CAP versus length of study of dissected specimens followed by two episodes of aldosterone treatment ($n=4$). Dissection (d) resulted in an enormous variability and fluctuancy between the specimens (thin lines). Both aldosterone episodes (a1, day 46, 48, 50; and a2, day 60, 62, 64) demonstrated a tendency of an initial rise of the CAP followed by a decline. During the treatment-free interval (day 50 to 60) between the two episodes of aldosterone, the CAP seemed to show a tendency of restoration. An average line was estimated (thick line).

onstrate possible dynamic patterns which might be expected according to our theory. Two compromising factors on endolymph homeostasis including their interaction on sensory cell function were evaluated during a follow-up period by determining the CAP, which reflected dysfunction of sensory cells as well as the neuronal afferents. We used filtered clicks to analyze different frequencies in order to detect tonotopical changes.

Failure of response of our electrode implants were excluded from this study. Most of these failures may be ascribed to technical failures, such as loss of the electrode units and change or loss of position on the round window membrane which may be due to growth of the cochlea or chronic middle ear infection.

In *normal (untreated) cochleas* the enormous intracochlear and intercochlear variability of the CAP values indicates an unstable CAP. Perhaps this may partially be described to a natural fluctuation, but also to an unstable electrode contact due to growth of the cochlea, or the condition of the middle ear. Although sedation during the registration was related to body weight, variation in CAP may be related to depth of sedation.

The variability in our control values resulted in a major standard deviation which made it difficult to recognize delicate fluctuations of the CAP in treated ears.

Dissection of the endolymphatic sac resulted in a major post-operative decline of the CAP in all dissected groups. However, although perhaps in a minor degree, contralateral ears showed the same pattern of decline, which may indicate a general influence on both ears. The major part of this decline lasted only 2-3 days and may be ascribed to operative effects. Transitory impairment of hearing in both ears has been demonstrated after unilateral neurosurgical and neurootological procedures, which may be due to loss of cerebrospinal fluid and intracranial hypotension¹². Pressure changes may be transmitted to both ears by the communication between the CSF and perilymph fluid systems, resulting in pressure imbalances between the peri- and endolymphatic compartments and perhaps as a consequence sensory cell dysfunctions of the organ of Corti.

After dissection an enormous variation and fluctuation of the CAP was noticed which induced a high standard deviation. Averages of these measurements might only be regarded as tendencies due to the treatment. Horner and colleagues^{13,14} found threshold shifts which started at lower frequencies several weeks after endolymphatic sac dissection, but they also found fluctuations which were superposed on this progressive loss and which in some cases restored the CAP to normal¹³. In contrast to our study, they found unchanged audiograms in the non-dissected ears, but also in the first post-operative week of the dissected ears. Most dissected specimens in our study demonstrated a remarkable restoration and rise of CAP values up to +50% of its starting values. This phenomenon is unexpected and the underlying etiology is unknown.

In all groups which subsequently received *one or two episodes of aldosterone injections*, no significant changes of the CAP was observed at the time of administration. A tendency to an initial rise and subsequent decline of the CAP was present in all specimens, although this also may be described to a natural fluctuation which has been visualized more obviously by more frequent measurements at the time of aldosterone injections.

In the specimens which received two episodes of aldosterone treatment, a tendency of restoration of the CAP was observed between the two episodes (*Figure 5*).

The *degree of hydrops* in this study was merely slight (*Table 1*). In contrast to one of our earlier studies⁶, no exacerbations of hydrops due to administration of aldosterone after dissection was noticed in the present study. This slight hydrops may render the cochlea and endolymphatic system more resistant to changes in endolymphatic fluid balance which may result in a minor degree of sensory cell damage and absence of significant changes of the CAP.

Tonotopic morphological changes were found in a scanning electron microscopic evaluation of our two-phase model⁷, in which atrophy and degenerations of the sensory cell structures of the organ of Corti was observed in radial and longitudinal gradients along the cochlea. Most damaged were the outer hair cells in the apical and middle

cochlear turns. These areas correspond to the lower frequencies. In most cases, we did not demonstrate significant differences between the frequencies.

In conclusion, the aim of our study was to develop a new experimental model in which several compromising mechanisms interact, and which may mimic the fluctuations as observed in early stages of Menière's disease. Severe degrees of hydrops and severe electrophysiological damage would not have been useful in developing a dynamic model in which delicate effects of minor changes must be evaluated for their fluctuant or permanent influences on the electrophysiological processes. However, in this study moderate disturbances in endolymph homeostasis or perhaps longer follow-up periods might have demonstrated more clearly the point of imbalance in our dynamic model. The variability and the resulting high standard deviation prevented us to draw conclusions about delicate changes due to each treatment modality.

Longer follow-up periods may provide more information about the relation between the electrophysiological influence of the compromising factors, the degree of hydrops, the degree of sensory cell damage, and their fluctuant or permanent influence on sensory cell function.

References

- 1 Hallpike CS, Cairns H. Observations on the pathology of Menière's syndrome. *Proc Roy Soc Med* 1938;31:1317-31.
- 2 Yamakawa K. Über die pathologische Veränderung bei einem Menière-Kranken. *Proc 42nd Ann Meet Oto-Rhino-Laryngol Soc Japan J Otolaryngol* 1938;44:2310-12.
- 3 Kimura RS. Experimental blockage of the endolymphatic duct and sac and its effect on the inner ear of the guinea pig. *Ann Otol Rhinol Laryngol* 1967;76:664-87.
- 4 Sando I, Ikeda M. The vestibular aqueduct in patients with Menière's disease. *Acta Otolaryngol (Stockh)* 1984;97:558-570.
- 5 Albers FWJ, Weissenbruch R van, Casselman JW. 3DFT-magnetic resonance imaging of the inner ear in Menière's disease. *Acta Otolaryngol (Stockh)* 1994;114:595-600.
- 6 Dunnebier, E.A., Segenhout, J.M., Wit, H.P., Albers, F.W.J. Two-phase endolymphatic hydrops; a new dynamic guinea pig model. *Acta Otolaryngol. (Stockh)* 1997;117:13-19.
- 7 Dunnebier EA, Segenhout JM, Dijk F, Albers FWJ. Sensory cell damage in two-phase endolymphatic hydrops; a morphologic evaluation of a new experimental model by low-voltage scanning techniques. *Hearing Research* 1998, in press.
- 8 Horner KC. Cochlear and vestibular epithelia from a patient with Menière's disease: a case study. *Scann. Micros.* 1992;6:1115-1128.
- 9 Ferraro JA, Kaufman Arenberg I, Stephenson Hassanein R. Electrocochleography and symptoms of inner ear dysfunction. *Arch. Otolaryngol.* 1985;111:71-74.
- 10 Horner KC. Functional changes associated with experimentally induced endolymphatic hydrops. *Hear. Res.* 1993;68:1-18.

- 11 Dunnebier, E.A., Segenhout, J.M., Wit, H.P., Albers, F.W.J. Endolymphatic hydrops after total dissection or cauterization of the distal portion of the endolymphatic sac. *ORL* 1996;58:271-276.
- 12 Walsted A, Salomon G, Thomsen J, Tos M. Cerebrospinal fluid loss and threshold changes. *Audiol Neurootol* 1996;1:247-255.
- 13 Horner KC, Cazals Y. Rapidly fluctuating thresholds at the onset of experimentally induced hydrops in the guinea pig. *Hear. Res.* 1986;26:319-325.
- 14 Rydmarker, S., Horner, K.C. Atrophy of outer hair cell stereocilia and hearing loss in hydropic cochlea. *Hear. Res.* 1991;53:113-122.