

## Osmotically induced motility of outer hair cells: implications for Menière's disease

D. Dulon<sup>1</sup>, J.-M. Aran<sup>1</sup>, and J. Schacht<sup>2</sup>

<sup>1</sup>Laboratoire d'Audiologie Expérimentale, INSERM U 229, Hôpital Pellegrin, Université de Bordeaux II 33076 Bordeaux, France

<sup>2</sup>Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109, USA

**Summary.** Osmolarity changes in inner ear fluids have long been considered to be contributing factors to Menière's disease. Our present study demonstrates that small changes in the osmolarity of a surrounding in vitro medium induce fast contractions (hypo-osmotic solution) or elongations (hyperosmotic solution) in isolated outer hair cells of the guinea pig. These changes were reversible upon returning cells to iso-osmotic conditions. Up to five cycles of shape change could be sustained by these cells without obvious detriment to their morphology. These findings suggest that fluctuant changes in osmolarity of inner ear fluids can result in similar fluctuant changes of hair cell shape. Since the outer hair cells may control cochlear micromechanics and function by their active motility, osmotically induced abnormalities of cell dimensions and motility may contribute to the audiological manifestations of fluctuant hearing loss.

**Key words:** Outer hair cells – Osmolarity – Motility – Menière's disease

### Introduction

The hair cells of the sensory epithelium of the organ of Corti are bathed by two fluids of widely different ionic compositions. The cuticular plate with the stereocilia is exposed to the endolymph (or an endolymph-like fluid), while the lateral and basal membranes of the cells are surrounded by the perilymph-like cortilymph. Endolymph (high  $K^+$ , low  $Na^+$ ) not only has a different composition from perilymph but also has a higher osmolarity. (In the rat, perilymph is

289 mosmol while endolymph varies from 317 to 329 mosmol [7]). Consequently, any changes in the ionic composition or osmolarity of the fluids bathing the hair cells can have pathological effects on cochlear function.

Damage to hair cells by high  $K^+$  concentrations has long been recognized [6] and Zenner et al. [11] were able to show in elegant experiments that  $K^+$  induced reversible contractions in outer hair cells in vitro. In contrast, less attention has been paid to osmotic influences, presumably because these influences have been thought to lead to irreversible destruction of the cells affected [6, 11]. However, it seems important to consider this question in greater detail, since osmotic disturbances may underlie some inner ear dysfunctions.

An osmotic imbalance in the endolymph has been postulated to cause such disorders as tinnitus and fluctuating hearing loss, inclusive of Menière's disease [2, 5]. An altered osmolarity of the endolymph would equally lead to an altered osmolarity of the perilymph due to water movements across the endolymph/perilymph barriers. The fact that such osmotic agents as glycerol are able to cause a temporary reversal of clinical audiological dysfunction is in good support of an osmotic component in Menière's disease [1]. We now report osmotically induced reversible length changes of isolated outer hair cells and discuss their potential importance in inner ear disorders.

### Materials and methods

Pigmented guinea pigs (280–340 g) were decapitated without anesthesia and the temporal bones quickly removed. The bullae and the bony walls of the cochleae were immediately opened and the upper turns of the organ of Corti removed. These were kept in Hank's balanced salt solution (HBSS) without  $NaHCO_3$  and buffered to pH 7.4 with 5 mM sodium

HEPES. The solution was then adjusted to 300 mosmol with NaCl. After collagenase treatment (0.1 mg/ml HBSS for 15 min), the tissues were placed in HBSS supplemented with 5% fetal calf serum and outer hair cells were isolated microsurgically.

For the present experiments, the isolated cells were transferred into 50  $\mu$ l of HBSS in a Petri dish (Nunc; A/S Nunc, Roskilde, Denmark) and allowed to settle for 5 min in order to attach themselves to the bottom of the dish. The cells were then continuously superfused with the selected medium at a rate of 50  $\mu$ l/min by means of a peristaltic pump (MS 4/8; Isomatec, Zürich, Switzerland). The osmolarity of the HBSS was lowered with water or increased by addition of NaCl, and was controlled before and during the experiments with a micro-osmometer (Roebing, West Berlin).

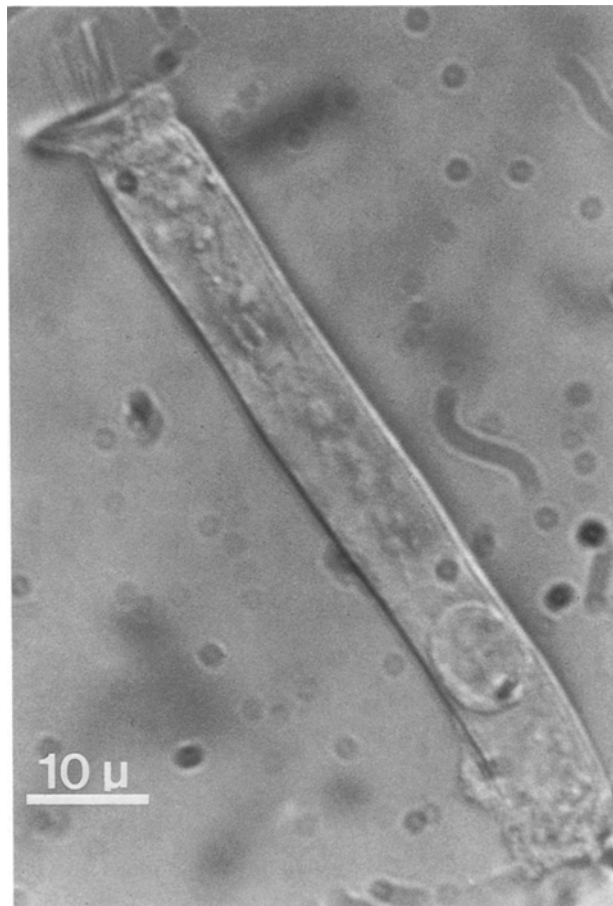
The morphology of the cells was continuously monitored via a video camera system attached to an inverted microscope (Fluovert; Leitz, Wetzlar, FRG) and the images were recorded for analysis with a U-matic video cassette recorder (VO-5800 PS; Sony Corporation, Japan).

## Results

Outer hair cells can easily be distinguished from other cells of the organ of Corti by their specific morphology. They have a long cylindrical shape with a length of 60–80  $\mu$ m (hair cells of the upper turns [8]) and a diameter of around 10  $\mu$ m. In the isolated cells (Fig. 1), the cuticular plate and the bundles of stereocilia are clearly seen at the apical end of the cells while remnants of synaptic endings are frequently found attached to their base. Hair cells with cytoplasmic granulations, high nucleus, swelling or other indications of deterioration [8] were excluded from our present study. Hair cells can maintain their viability *in vitro* for several (4–6) h, as judged by their morphology, ability to exclude trypan blue, and intracellular potential [4].

When subjected to a change of osmolarity in the surrounding medium, outer hair cells reacted with a rapid change in their shape. In decreasing osmolarity, the cells contracted within seconds (Fig. 2A and B). This contraction was reversible, and the cells returned to their initial length when they were re-exposed to iso-osmotic HBSS (Fig. 2C). Conversely, the cells reversibly elongated upon exposure to hyperosmotic HBSS. The changes in lengths were paralleled by changes in cell volumes as indicated by a concomitant change of the diameter of the cell: an increase in volume accompanied the contraction and vice-versa.

Several cycles of such shape changes could be sustained by the hair cells without apparent degeneration (Fig. 3). The cells were able to support an intact morphology for 15 min at osmolarities between 280 and 330 mosmol and to recover their original shapes when exposed again to iso-osmotic HBSS. Prolonged exposure to hypo- or hyperosmolarity, however, re-



**Fig. 1.** Example of an outer hair cell isolated from the upper turn of the guinea pig cochlea (as described in Materials and methods). Note the regular cylindrical shape, smooth cytoplasm and low position of the nucleus. Interference contrast. Bar: 10  $\mu$ m

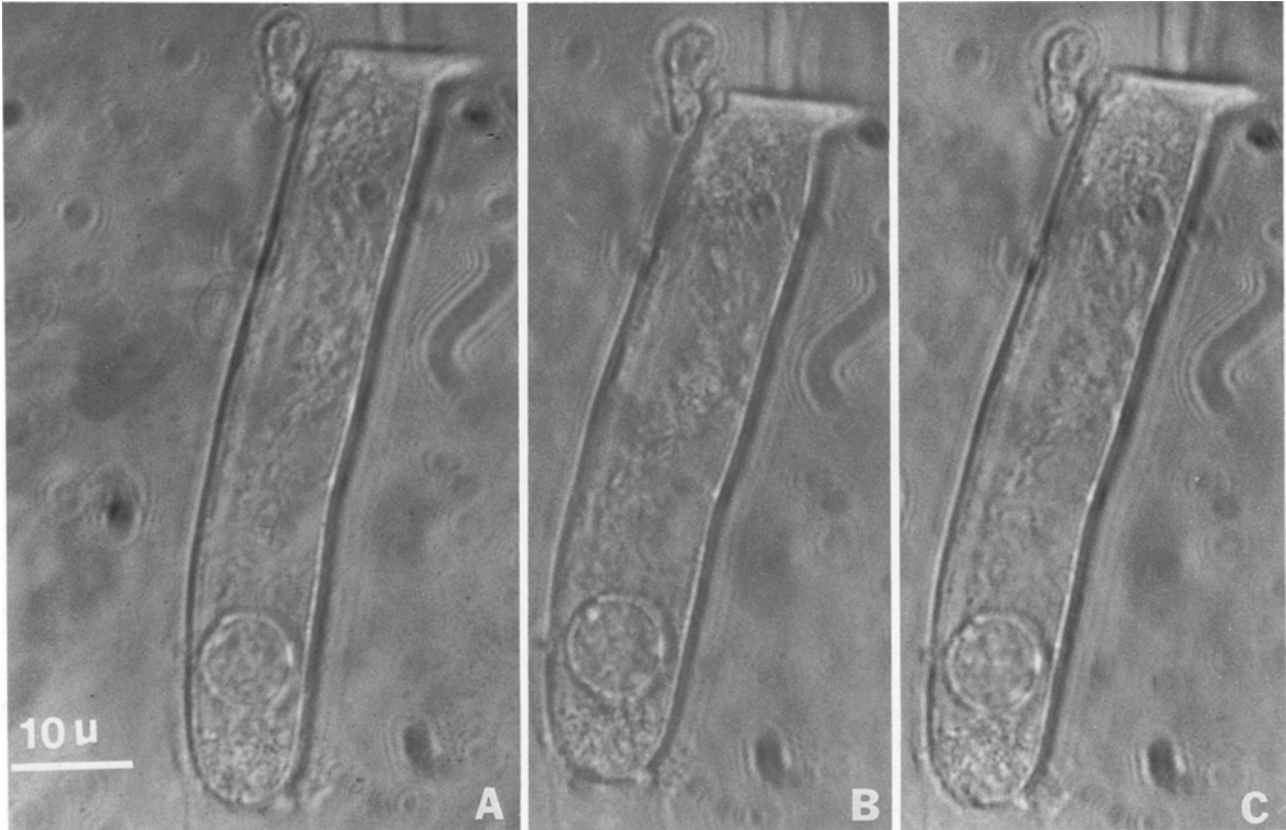
sulted in irreversible shortening and granulation of the cytoplasm, as well as rounding and eventual destruction of the cells.

The isolated hair cells were highly sensitive to even small variations in osmolarity. A change of only 3% (from 300 to 290 mosmol) led to a change in length of  $2.4\% \pm 1.2\%$  ( $n = 5$ ). Contractions averaging  $11.3\% \pm 2.2\%$  ( $n = 29$ ) were found for a 10% difference in osmolarity.

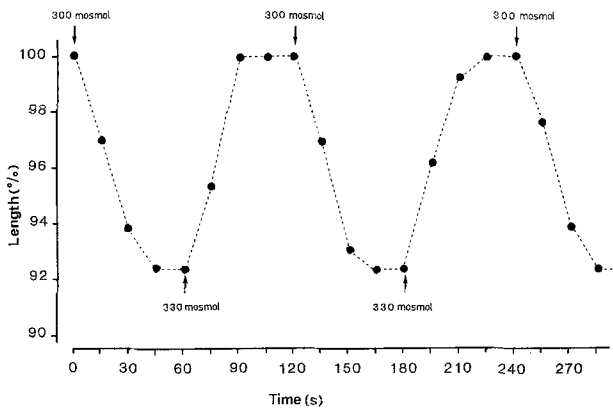
These changes in length were fast, ranging between 200 and 500 nm/s. The rate of recovery was equally rapid, and there was no difference in rate with the number of cycles of contractions and elongations that a cell had undergone.

## Discussion

As currently understood, outer hair cells regulate the micromechanics of the basilar membrane and there-



**Fig. 2A–C.** Osmotically induced contractions of an isolated hair cell subjected to media of different osmolarities (as described in Materials and methods). Bar: 10  $\mu$ m. **A** Cell in Hank's balanced salt solution (HBSS), 300 mosmol. **B** Same cell, 1 min after exposure to hypo-osmotic (280 mosmol) HBSS. **C** Same cell, 1 min after re-exposure to the original 300 mosmol HBSS



**Fig. 3.** Cycles of osmotically induced shape changes as the result of cell being alternately exposed to HBSS of 330 mosmol and 300 mosmol. Arrows indicate the time at which the solution of the indicated osmolarity was added. The time-course was constructed from video-recordings of the cellular movements

by the normal response of the ear to sound. The cells are thought to accomplish this by their motile properties, which bring about contractions (whether isometric or isotonic in vivo is not known) in response to an

appropriate physiological stimulus. This motility is energy-dependent and based on the presence of contractile proteins [3, 9].

In addition to being responsive to physiological stimuli, the outer hair cells are highly susceptible to alterations in their environment: ionic or osmotic variations will change their physical dimensions. Such pathophysiological, passive shape modifications would interfere with the normal action of the outer hair cells and thus with the proper function of the basilar membrane. This interference would lead to auditory dysfunctions that are either transient or permanent (the latter condition if irreversible damage occurs).

Our observations of an osmotically induced motility of outer hair cells may explain some of the clinical observations in Menière's disease. Changes of hair cell shape in response to osmotic fluctuations would be fast, could be sustained for some time and yet be reversible unless the osmotic derangement persists. Thus, the osmolarity model shows components that appear as in vitro analogies to the disease: the transient nature of the attacks, their recurrences, and the eventual permanent loss of hearing.

Zenner [10] recently proposed reversible,  $K^+$ -induced contractions of outer hair cells as a hypothetical basis for Menière's disease. This attractive model is based on equally compelling in vitro analogies to the in vitro situation. Our model does not argue against  $K^+$  intoxication as a mechanism in Menière's disease. On the contrary, the two models are highly compatible. Menière's disease has complex etiologies and manifestations, and both osmolarity changes and  $K^+$  intoxication may occur at different stages or forms of the disease.  $K^+$  depolarization of hair cells can explain aberrant stimulation of afferent fibers during an attack, i.e., the occurrence of tinnitus. On the other hand, the osmotic model does not need to postulate a rupture of Reissner's membrane or  $K^+$  leakage into the perilymph, but simply a fluctuant change in the osmolarity of inner ear fluids. Moreover, it explains why glycerol would be an effective antidote during such an osmotic disequilibrium.

The attractiveness of the two models of osmotic imbalance and  $K^+$  intoxication is that they are based on different disturbances of the homeostasis of inner ear fluids and yet arrive at essentially identical conclusions, namely the involvement of the outer hair cells. This lends further credence to the hypothesis that the motile and micromechanical properties of outer hair cells are a primary target in Menière's disease.

*Acknowledgement.* This research was supported by a grant from the Deafness Research Foundation, research grant NS-13792 from the National Institutes of Health and an INSERM fellowship to JS.

## References

1. Angelborg C, Klockhoff I, Larsen HC, Stahle J (1982) Hyperosmotic solutions and hearing in Menière's disease. *Am J Otol* 3:200–202
2. Dohlmann GF (1976) On the mechanism of the Menière attack. *Arch Otorhinolaryngol* 212:301–307
3. Flock A, Cheung HC, Flock B, Utter G (1981) Three sets of actin filaments in sensory cells of the inner ear. *J Neurocytol* 10:133–147
4. Gitter AH, Zenner HP, Frömter E (1986) Membrane potentials and ion channels in isolated outer hair cells of guinea pig cochlea. *ORL* 48:68–75
5. Godlowski Z (1972) Hyperosmosis of endolymph as primary pathogenic mechanism of Menière's disease and its clinical management. *Acta Otolaryngol (Stockh) [Suppl]* 299:1–36
6. Goldstein AJ, Mizukoshi O (1967) Separation of the organ of Corti into its components. *Ann Otol Rhinol Laryngol* 76:414–426
7. Sterkers O, Ferrary E, Amiel C (1984) Inter- and intracompartmental osmotic gradients within the rat cochlea. *Am J Physiol* 247:F602–F606
8. Zajic G, Schacht J (1987) Comparison of isolated outer hair cells from five mammalian species. *Hear Res* 26:249–256
9. Zenner HP (1980) Cytoskeletal and muscle-like elements in cochlear hair cells. *Arch Otorhinolaryngol* 230:82–92
10. Zenner HP (1986)  $K^+$ -induced motility and depolarization of cochlear hair cells. Direct evidence for a new pathophysiological mechanism in Menière's disease. *Arch Otorhinolaryngol* 243:108–111
11. Zenner HP, Zimmermann U, Schmitt U (1985) Reversible contraction of isolated mammalian cochlear hair cells. *Hear Res* 18:127–133

Received January 9, 1987 / Accepted February 2, 1987