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Reversible contraction of isolated mammalian cochlear hair cells *

H.P. Zenner, U. Zimmermann and U. Schmitt

Department of Otolaryngology, University of Würzburg, D-8700 Würzburg, F.R.G.

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Outer hair cells were isolated from the guinea pig cochlea using a micromechanical non-enzymatic procedure. Depolarization of outer hair cells in the presence of 25–125 mM K^+ was accompanied by a longitudinal contraction of the isolated cells. A decrease of $[K^+]$ to 5.4 mM interrupted contraction and induced a relaxation. Individual hair cells were able to undergo as many as 5 cycles of contraction and relaxation. External Ca^{2+} was required for relaxation of the contracted hair cells. The contractile event led to the production of a visible cytoplasmic network between the supranuclear area and the cuticular plate.

cochlear hair cell, isolation, K^+ intoxication, contraction

Introduction

Many experimental procedures that require a homogeneous cell population cannot be applied to mammalian inner ear research. Biochemical, virological and immunological approaches to hair cells are frequently prevented because experimental material is contaminated by non-hair cells. With enzymatic isolation of hair cells, chemical analysis of these cells became available [7,16]. Enzymatic cell separation was used for the isolation of hair cells from the bull-frog sacculus of which viability was demonstrated electrophysiologically [12]. In studies of isolated mammalian hair cells, however, it has been particularly exasperating to provide a proof of viability: single outer and inner hair cells could be prepared [2,19], but intracellular recordings did rarely show a cellular potential of more than −6 to −12 mV [2]. In the present study we describe a method for the study of living isolated mammalian outer hair cells (OHCs) which has overcome some of the complexities in dealing with the heterogeneous organ of Corti.

Recently electrophysiological giga seal whole cell recordings of single OHC prepared by this approach revealed negative cell potentials ranging from −32 to −67 mV [6].

In vivo the apical part of mammalian hair cells is exposed to the unique K^+-rich environment of the endolymphatic space. The fluctuating hearing loss of Ménière's disease is thought to be accompanied by endolymphatic hydrops and by a leakage of membranes lining the endolymphatic space, leading to K^+ intoxication of hair cells [3,8,13]. It has also been postulated that damage of hair cells can occur by entry into the Nuel space of K^+-rich endolymph through holes in the reticular lamina resulting from certain types of noise trauma [1].

Recently we could show, that exposure of the lateral and basal membrane parts of living OHCs to increasing bath K^+ concentrations resulted in a sustained reversible depolarization of the cell [6]. Here, we report that during depolarization of the cell membrane in the presence of 25–125 mM K^+/Cl^- a sustained contraction of OHC was induced. This was followed by relaxation in the presence of artificial perilymph containing 5.4 mM K^+/Cl^-. By alternating these procedures OHCs were made to undergo as many as five cycles of contraction and relaxation. External Ca^{2+} was not required for the initial contraction but was essential for relaxation. Following repeated contrac-

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tion/relaxation cycles the cytoplasm of individual OHCs exhibited a filamentous network.

Materials and Methods

Preyer$^+$ colored guinea pigs of either sex weighing 200–300 g were decapitated. Temporal bones were removed from the skull and rapidly cooled to near 0°C. Then the bulla was opened, the cochlear surface preparation (in most cases from the apical turn) was dissected and rapidly incubated in Hank's solution ($CaCl_2$ 1.25 mM; D-(+)-glucose 5.5 mM; KCl 5.4 mM; KH_2PO_4 0.35 mM; $MgSO_4$ 0.81 mM; NaCl 137 mM; Na_2HPO_4 0.4 mM) or artificial perilymph at 22°C. Artificial perilymph consisted of L-arginine 1.15 mM, L-asparagine 0.38 mM, L-cystine 0.2 mM, L-glutamine 2.05 mM, L-glutamate 0.135 mM, glycine 0.13 mM, L-histidine 0.1 mM, L-hydroxyproline 0.15 mM, L-isoleucine 0.38 mM, L-leucine 0.38 mM, L-lysine 0.22 mM, L-methionine 0.1 mM, L-phenylalanine 0.09 mM, L-proline 0.17 mM, L-serine 0.285 mM, L-threonine 0.17 mM, L-tryptophan 0.025 mM, L-tyrosine 0.11 mM, L-valine 0.17 mM, D-(+)-glucose 11 mM, $Ca(NO_3)_2$ 0.42 mM; asparagate 0.15 mM; KCl 5.4 mM; $MgSO_4$ 0.4 mM; NaCl 103.5 mM; Na_2HPO_4 5.6 mM; $NaHCO_3$ 23.8 mM.

Media and amino acids were from Boehringer, colchicine from Sigma. Unless otherwise indicated media were adjusted to an osmolarity of 300 mOsm using a Gonotec osmometer.

Unfixed cochlear surface preparations were mounted in a 65 mm petri-dish (Falcon) on a microscope stage and were immobilized by a holder. The specimens were observed by Leitz phase contrast optics (×100–×320), allowing OHCs to be identified. Closed heat polished capillaries possessing tips of 4–40 μm were mounted on a micromanipulator. These were used to open the surface preparation in the area of the tunnel of Corti allowing an easy access to remove the hair cells. In other cases, OHCs were directly isolated from the intact cochlear surface preparation. In artificial perilymph isolated hair cells were kept for 6–9 h, in Hank's solution for 3–6 h.

To control viability of a freshly prepared OHC population, in many cases some of the isolated OHCs were used in separate electrophysiological whole cell recordings [6].

For contraction experiments a single immobilized OHC was incubated in 20 μl of Hank's solution. Contraction was initiated by addition within 3–5 s of 200 μl modified Hank's solution with appropriate increased $[K^+]$ and reduced $[Na^+]$ through replacing Na^+/Cl^- by K^+/Cl and thus keeping the $[Cl^-]$ constant. To induce relaxation the medium was replaced by unmodified Hank's solution. Cells were observed during the whole procedure. Observations were made with planapochromatic bright field or phase contrast optics (Leitz) supplemented by a real time video system (Philips) or an electronically controlled film camera (Bauer) with 16 fps–4 fpm. This set-up allowed time dependent measurements of cell length and width with a resolution of 0.4–0.8 μm under real time and time lapse conditions as well as single frame analysis. Passive movement of OHCs during exchange of media was avoided by immobilization of the cells. OHCs were immobilized by electrostatic forces between the living cell and the bottom of the petri-dish. Pretreatment with 50 μg/ml polylysine improved adherence but decreased survival time. Osmolarity of the medium was controlled before and after each experiment. Bath temperature was monitored and kept constant at room temperature throughout the experiment.

Results

Incubation of OHC in a high $[K^+]$ medium (25–125 mM K^+/Cl^-) led to a remarkable longitudinal contraction of the isolated cells. Any consistent change in length or movement of stereocilia was not observed. The force generating the contractile event appeared to be limited to the region between nucleus and cuticular plate. A decrease of $[K^+]$ to the normal value of 5.4 mM interrupted contraction and induced a relaxation, although the cells only occasionally returned to the full prestimulation length (Fig. 1). After relaxation the cell could be restimulated to contract (Figs. 1, 2). Individual OHCs were able to undergo as many as 5 cycles of contraction and relaxation. Following repetitive cycles of contraction and relaxation most OHCs produced a random three-dimensional cytoplasmic network between cuticular plate and the

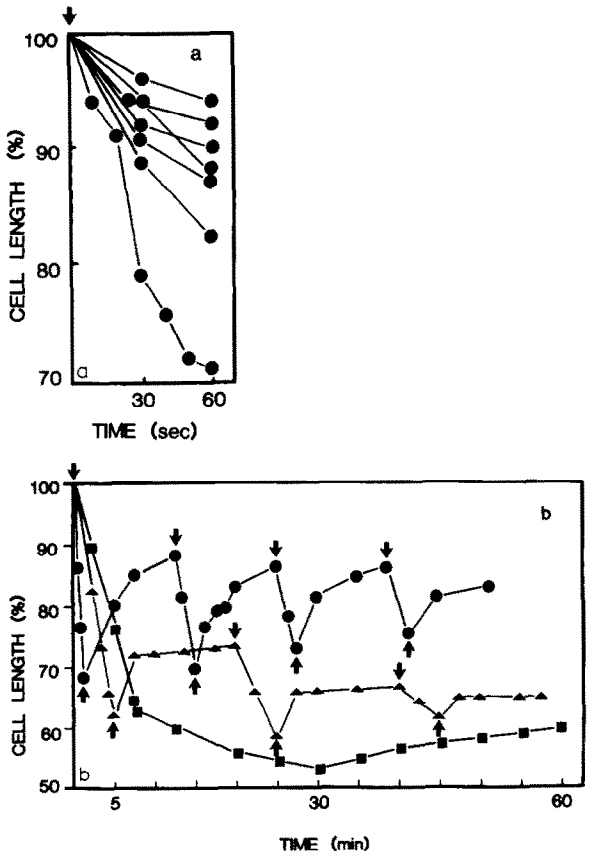


Fig. 1. Single frame analysis of contraction and relaxation of living OHCs following different stimulation times (●, 1 min; ▲, 5 min; ■, permanent depolarization). Observations were made with video-assisted × 320 phase-contrast optics. Measurements from 36 OHC were made directly on the monitor screen in 24 different experiments. (a) Values from 7 individual cells during the first 60 s of contraction. (b) Average values of the degree of cell contraction are plotted as percentage relative to their initial sizes (30–40 μm length). Arrows indicate (↓) initiation of contraction by high [K^+] (125 mM) or (↑) initiation of relaxation by low [K^+] (5.4 mM).

supranuclear area (Fig. 2h). Control experiments using K^+-gluconate instead of K^+/Cl^- to modify [K^+] revealed similar cyclic contractions of OHCs. Colchicine (1×10^{-6}–1×10^{-3} M) did not inhibit the contractile response.

Velocity and degree of the mechanical response were functions of the K^+ concentration and the exposure time. In the present study the average velocity of the contractile responses of OHCs in the presence of 125 mM K^+/Cl^- was 1.3×10^{-7} m/s (1.3 Å/ms) during the first 30 s. Following 10 s of depolarization OHCs from most preparations (apical turn) showed an average shortening of 1.3 μm. No mechanical response was to be seen when K^+ concentrations below 10 mM were used.

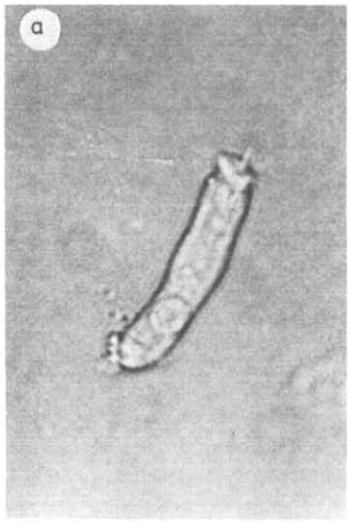
Since long-term and repeated exposures of hair cells to elevated [K^+] may occur during attacks of Ménière's disease [3,8,10] or noise trauma [1], the effects of various time dependent as well as repeated depolarizations of OHCs induced by high [K^+] were investigated. Continuous incubation of OHCs at various concentrations of K^+ (25–125 mM K^+/Cl^-) induced a permanent contraction which regularly reached a maximum within 5–20 min after [K^+] increased. At maximal contraction the cells attained a mean final length of 23 ± 5 μm (Fig. 1). Depolarization periods up to 60 s mostly allowed a subsequent relaxation if [K^+] was rapidly decreased to the normal value of 5.4 mM. In contrast, K^+ intoxication of OHC_s for 5 min and longer inhibited a return of the cells to full pre-stimulation length (Fig. 1). Following contraction continued incubation of OHCs in high [K^+] often caused a slight increase of cell length after 15–30 min, the cell died, and no further K^+-induced contraction/relaxation was possible (Fig. 1).

TABLE I

EFFECT OF MEMBRANE DEPOLARIZATION ON OHC LENGTH

Single frame analysis from 32 different single cell experiments. Stimulation was achieved as in Fig. 1 with a depolarization time of 1 min or by permanent depolarization. Values are μm ± S.E.

Initial length (μm ± S.E.) ($N = 32$)	Length after depolarization time of		Length after relaxation ($N = 32$)	Length after permanent depolarization ($N = 16$)
	30 s ($N = 8$)	60 s ($N = 32$)		
36 ± 1	32.1 ± 3.2	31.4 ± 2.6	34.1 ± 1.7	23.4 ± 5.4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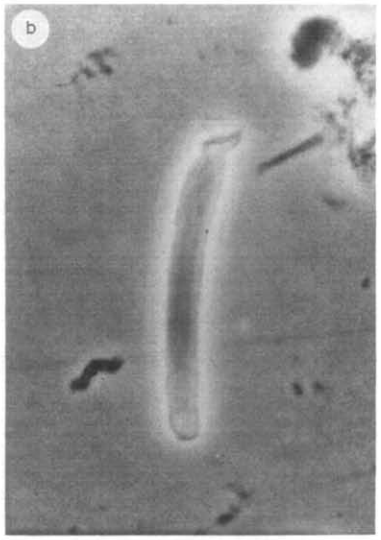
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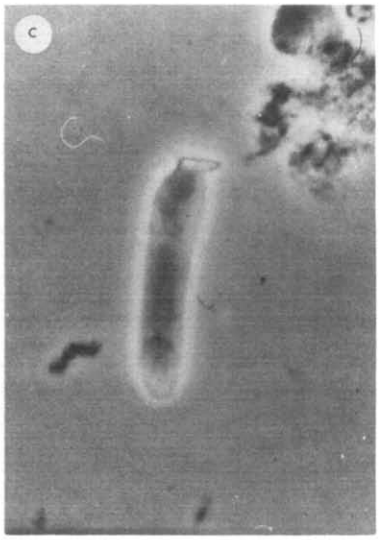
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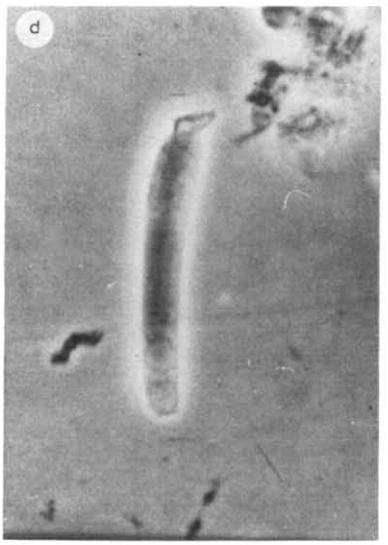
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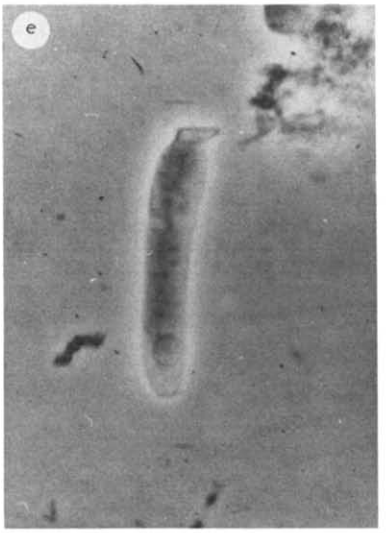
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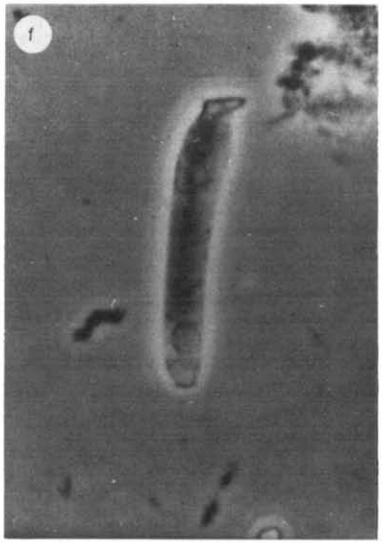
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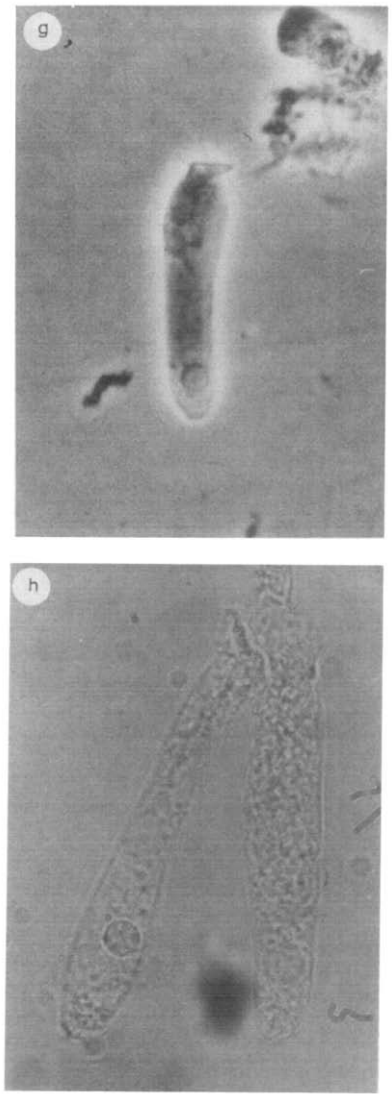

Fig. 2. (a) Living isolated outer hair cell (OHC) from the guinea pig cochlea (apical turn). (b, c, d, e, f, g) Single frames of a living OHC monitored continuously during cycles of contraction and relaxation. b, Just before stimulation; c, 1 min after initiation of contraction; d, 1 min after initiation of relaxation (i.e. 2 min after b); e, 1 min after initiation of second contraction; f, 1 min after initiation of second relaxation; g, fifth contraction; h, visualization of a cytoplasmic network in an OHC following various cycles of contraction.

In contrast to the strictly bidirectional longitudinal nature of OHC responses to K^+, changes of osmotic pressure produced a clearly different pattern. In hypotonic media (270 mOsm) most of the cells became rounded within 10 min of exposure, and changes of $[K^+]$ had no longer an effect on cell length. The cuticular plate and the hair bundle frequently remained stable longer than the body. OHCs were more resistant to hypertonic (330–360 mOsm) than to hypotonic media but eventually they shrank, lost their birefringence and formed round cell ghosts exhibiting cytoplasmic Brownian motion.

In the absence of Ca^{2+} or with a concentration of EGTA sufficient to chelate all the extracellular Ca^{2+} (3 mM EGTA) the sustained OHC contraction was not significantly affected. Furthermore, exposure of OHCs to 10^{-5}–10^{-4} M of the Ca^{2+}-ionophore A 23 187 did not lead to the mechanical longitudinal bidirectional response. Thus, external Ca^{2+} was not required for the first OHC contraction in the presence of elevated $[K^+]$. External Ca^{2+}, however, was essential for relaxation. EGTA (3 mM) or a Ca^{2+} concentration below $1–3 \times 10^{-4}$ M prevented relaxation of the contracted OHC to the original cell length.

Discussion

In the present study isolation of living mammalian OHCs is reported. As enzymatic isolation of hair cells may destroy and inactivate integral cell membrane proteins, in this study guinea pig OHCs were prepared micromechanically. This avoids enzymatic digestion of OHC surface proteins and leaves the cell membrane intact. In separate experiments addition of collagenase cleaned the cells for giga-seal patch clamp studies. Thus, we recently could demonstrate negative cell potentials ranging from -32 to -67 mV [6] and the presence of intact ion channels in the basolateral membranes of this single mammalian OHC preparation [6]. Furthermore, OHCs excluded 1% trypan blue.

The sustained contractions observed following long exposure may be similar to events that take place in Ménière's disease or/and in certain noise traumas. OHCs were able to recover from K^+ exposures of less than 60 s. Long exposure, however, to high $[K^+]$ will certainly produce cell damage, e.g. resulting from an enhanced Na influx and depletion of cellular energy stores. This may correspond to an increasing severity of Ménière's disease: in early stages with short attacks the

hearing returns to normal. As attacks, however, become longer and more frequent the deafness tends to remain.

The remarkable extent of contraction of OHCs was visualized under cell culture conditions. There may be considerable restraints imposed by the adjacent cells in vivo, which are not present with an isolated cell preparation. In contrast to other epithelial cells, however, OHCs are surrounded by large extracellular spaces, resulting in cell attachment only at the base and apex of the OHCs which could allow rather free movement of the cells.

In eucaryotic cells the properties of shape and movement depend on the complex network of protein filaments in the cytoplasm. Among the possible proteins responsible for motile functions in the OHC cytoplasm between cuticular plate and nucleus small amounts of actin [15,18], tubulin [18] and myosin [4] were demonstrated in this area. But in contrast to the cuticular plate they were found in less organized and presumably less stable arrangements. Thus, for actin and tubulin a dynamic process of assembly and disassembly has been proposed in cochlear hair cells [18]. The absence of myofibrils in OHCs and the independence of contractility on an external Ca^{2+} pool argue against a muscle-like contraction mechanism to be the cause of the observed contractile responses. The fact, that colchicine, a microtubule depolymerizing agent, does not influence the contractile response argues against a tubulin-associated mechanism.

External Ca^{2+} was required for relaxation of the contracted OHCs. In other non-muscle cells not very well organized networks of actin filaments with gel like properties are found to undergo Ca^{2+} induced decreases in viscosity [17].

Apart from preliminary reports [2,20], the present study provides direct evidence that mammalian OHCs exhibit a mechanical response by changing their cell length. A plausible connection with cochlear mechanics during an attack of Ménière's disease or noise trauma would be that a change in the physical parameter of hair cell length might alter the resonant properties of the basilar membrane and/or the structures attached to it. Furthermore, the contractile response with a calculated velocity of 1.3 Å/ms might interfere with the postulated active vibrations of 1–4 Å of basilar membrane near threshold [9,10,11,14]. Thus, in addition to endolymphatic hydrops, K^+-evoked mechanical responses of OHCs with subsequent changes of mechanics of a segment of the basilar membrane possibly play a role in pathogenesis of Ménière's disease. The experiments, however, do not yet prove that a K^+-induced contractile process has a physiological role. This proof would require a quantitative analysis in the nanosecond range during the initial phase of the contractile response. Furthermore, a physiological entry of K^+ through the apical OHC membrane has to be demonstrated resulting in a motile response.

Acknowledgement

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