Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 304-308

FAST MOTILITY OF ISOLATED MAMMALIAN AUDITORY SENSORY CELLS

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Received October 12, 1987

SUMMARY Auditory sensory cells (hair cells) are responsible for sound transduction in the cochlea of the inner ear. In the presence of a longitudinal a.c. field isolated living outer hair cells showed reversible motile responses. They followed the stimulus up to at least 1 kHz. Control experiments in the presence of cytochalasin B, phalloidin and dinitrophenol excluded actomyosin as a molecular basis of the high frequency motility. The results suggest, that outer hair cells might amplify sound-induced oscillations in the inner ear and thus increase sensitivity and frequency selectivity of hearing.

The sensory cells (hair cells) of the cochlea of the inner ear are responsible for transduction of the sound stimulus into bioelectrical and biochemical signals. Using the patch-clamp technique, we and others showed that isolated mammalian hair cells possess predominantly K^+-channels in the outer cell membrane (1,2) thus allowing a signal transfer from the sound stimulus into a receptor potential. In mammals and humans, however, this mechano-electrical transduction cannot explain the high sensitivity of the cochlea near the auditory threshold as well as the high frequency selectivity required for speech discrimination (3). Thus, a biomechanical amplification of the cochlear oscillations has been postulated, whose source was sought in the outer hair cells (4-6). Following an increase of the intracellular calcium concentration outer hair cells were recently shown to produce motile responses (7,8). At the molecular level, we could attribute these active mechanical events to the unusual arrangement of the actomyosin skeleton in outer hair cells (7,9). In addition, electrically induced movements have been described in outer hair cells (10) and further investigated using a photomultiplier technique. They displayed higher frequencies than the slow mechanical events (11). Due to the poor signal-to-noise

0006-291X/87 $1.50

ratio, however, an exact biophysical analysis of the fast motile properties of outer hair cells was not possible. Here, we show that a photodiode technique allowed active vibrations of isolated outer hair cells to be followed up to at least 1 kHz. In addition to the slow actomyosin-dependent movements of the hair cells, these fast movements might influence mechanically the sound-induced vibrations of the basilar membrane possibly cycle-by-cycle, thus constituting a biophysical basis for frequency selectivity and sensitivity of hearing.

MATERIALS AND METHODS Single living outer hair cells were dissected from guinea pig cochleae as described previously (1). They were transferred to standard culture dishes (Falcon) filled with either Hank s solution or electrolyte-free isotonic mannitol or sorbitol solution. Two Ag/AgCl electrodes, made by electrolytically chloriding silver wire, were placed with the aid of micromanipulators (Leitz). The electrodes had a distance of 300 µm and were arranged to provide an electrical field (squarewaves, 3-23 kV/m) parallel to the longitudinal axis of the hair cell. The hair cell between the electrodes was monitored by a video camera and also imaged onto a photodiode (Siemens BPW 34). The photodiode signal was amplified and stored an a FM tape recorder (Racal) with a bandwidth of 1 kHz. For analysis the signal was retrieved from the tape recorder and fed into a spectrum analyser (Ono Sokki). Autocorrelation techniques were used to improve the signal-to-noise ratio. Power density spectra were generated and subsequently plotted on a graphics plotter (Hewlett-Packard).

RESULTS AND DISCUSSION For biochemical and biophysical investigations living single outer hair cells were isolated from the cochleae of guinea pigs. Under whole-cell recording conditions, they produced a negative cell potential of about -70 mV (1). The electrical conductivity of their outer cell membrane is mostly determined by integral K^+-channels e.g. Maxi-K-channels with a conductance of around 200 pS (1,2). In a longitudinal a.c. field, the highly polarized cells contracted and elongated along their longitudinal axis (Fig 1). In order to detect and record possible high frequencies of these movements, which were no longer visible microscopically or by video techniques, we designed a simple position detector system for optical registration. The apical end of an OHC was projected onto a photodiode by means of an inverted microscope (x400). OHCs of the 2nd, 3rd and 4th cochlear turn were investigated. Auto-correlation techniques allowed an increase of the signal-to-noise ratio of the amplified photodiode current. Frequencies were determined by a power density spectrum (Fig 2).

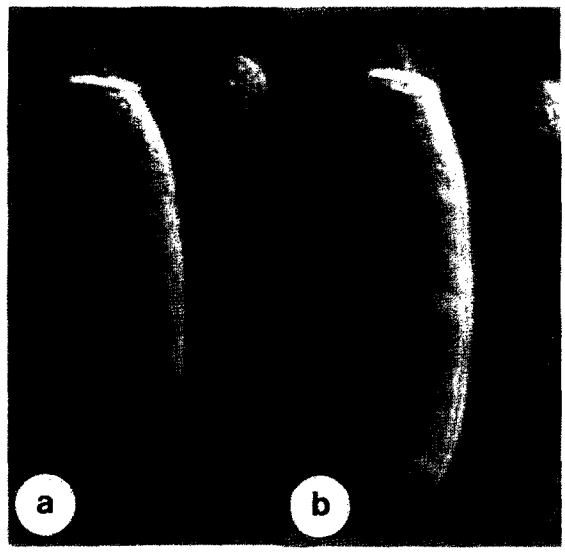

Fig. 1. AN ISOLATED, LIVING OUTER HAIR CELL FROM THE GUINEA PIG COCHLEA. Two phases (a,b) of a motile response cycle in the presence of an alternating 16.7 kV/m a.c. field.

When isolated outer hair cells in Hank's solution (300 mOsm) or in isoosmolar mannitol or sorbitol were exposed under these conditions to an alternating electrical field (typically 16.7 kV/m) along their longitudinal axis, the hair cells followed this field cycle-by-cycle until high frequencies. The power-density spectrum of the photodiode signal did not allow determinations above 1 kHz, so that even higher vibrations of the hair cells could not be excluded. All OHC investigated (n=10) from various cochleae displayed motile responses up to at least 1 kHz. The amplitude of the hair cell movements depended on the voltage of the stimulus. It increased with an increasing electrical field. In the absence of the stimulus or in the presence of the stimulus without a cell, only the basal noise of the photodiode could be detected.

The electrically induced motility could not be suppressed in the presence of 50 µM cytochalasin B, 50 µM phalloidin as well as 1 mM dinitrophenol. The motility, however, was no longer visible when the outer cell membrane was opened with a 0,5 µm microcapillary or was permeabilized by 0,1% Triton X-100. Osmotically rounded hair cells also no longer showed a motile response.

Unusually fast active mechanical processes have been postulated for outer hair cells, since mathematical models of the hearing process required them for frequency selectivity and sen-

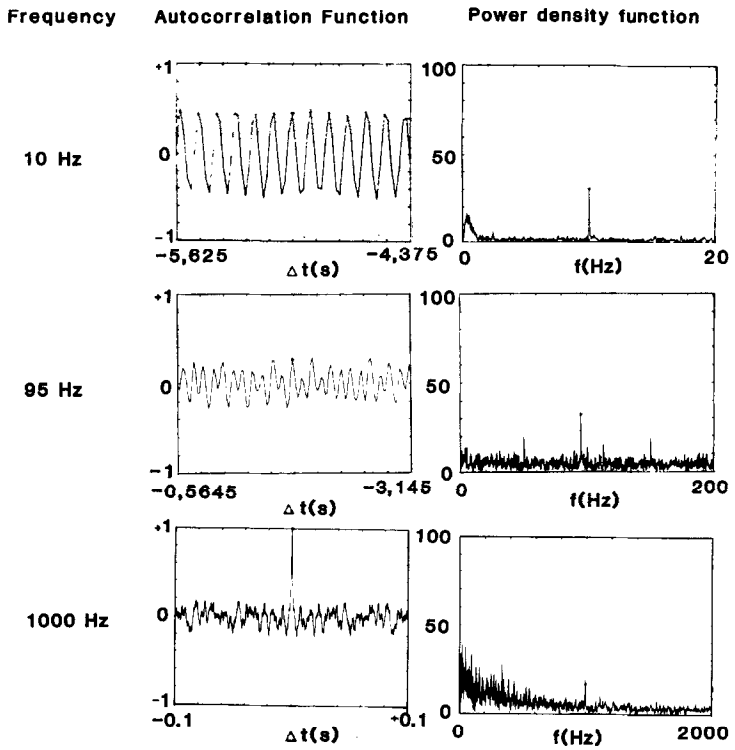

Fig. 2. MOTILE RESPONSES OF AN OUTER HAIR CELL. The cell was prepared from the 4th turn (i.e.: near the low-frequency end of the cochlea). Three different stimulus frequencies were tested. Shown here are the autocorrelation functions and the power density spectra of the measured signals without the corrections of the transfer function of the detector system. The amplitude of the evoked oscillations decreased with higher frequencies by approximately -10 dB/decade. This behaviour is significantly different from simple 1-pol RC-low-pass filters which decrease by -20 dB/decade. The corner frequency, where the signal amplitude dropped to -3 dB, was around 20 Hz.

sitivity near auditory threshold (3,6). The present report shows high-frequency motile responses of OHCs in an a.c. field, which are fast active electromechanical processes. The stimulation procedure used here partly reflects biophysical peculiarities of the inner ear. In contrast to extracochlear cells, the apical ends of outer hair cells project into the extracellular endolymphatic space which posseses a positive resting potential of +85 mV. Thus, a high potential difference of 155 mV is induced across the apical OHC membrane. In vivo, the hair cell potentials and the endolymphatic potential alter with the sound signal cycle-by-cycle and thus in frequencies such as were used here for stimulation of the outer hair cells. Accordingly, outer hair cells might serve as mechanical cochlear amplifiers by fast, active oscilla-

tions. They might thus enhance the amplitude of sound - induced cochlear oscillations (i.e. increase of sensitivity) and sharpen the amplified oscillation in its maximum (i.e. increase of frequency selectivity).

The high frequencies of OHC responses as well as the control experiments in the presence of cytochalasin, phalloidin and dinitrophenol exclude actomyosin as a molecular basis. Furthermore, for actomyosin-dependent movements of outer hair cells we had measured velocities between 3 and 23 nm/msec which cannot explain the fast motility described here. By contrast, our experiments suggest that the underlying biophysical or molecular mechanism is possibly linked to the cylindrical shape as well as intactness of the outer cell membrane of outer hair cells rather than the cytoskeleton.

ACKNOWLEDGMENTS The authors gratefully acknowledge the expert technical assistance of K. Krestel. This work was supported by Leibniz Grant Ze 149/4-1 from the Deutsche Forschungsgemeinschaft.

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