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Acoustic stimulation causes tonotopic alterations in the length of isolated outer hair cells from guinea pig hearing organ

(cell motility)

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ABSTRACT **Isolated outer hair cells from the mammalian cochlea exhibit a motile response to electrical or chemical stimulation. Here we show that isolated outer hair cells can also respond to acoustic stimulation, in the form of a tone burst of 200 Hz, by either shortening or lengthening depending on their cochlear location. Cells from the apical region of the cochlea (long cells) responded by increasing their length, whereas those from more basal regions (short cells) responded by decreasing their length. Cells from intermediate positions showed an equal probability for either elongating or shortening. Both the elongating and shortening response was inhibited by 3 μM poly(L-lysine). It is suggested that this tonotopic and bidirectional acoustic response may be one of the active components underlying the specific phase and frequency displacement of the basilar membrane.**

Current concepts of mammalian auditory physiology include active processes within the organ of Corti that participate in the control of basilar membrane motion (1). Isolated outer hair cells are capable of changing length in response to intracellular electrical stimulation or to alterations in the K^+, Ca^{2+}, or ATP content of the surrounding fluid (2–5). The physiological correlates of these experimentally induced motile events have been suggested to function either to modulate acoustic energy, to improve frequency selectivity, or to relate to the generation of acoustic emissions (1, 6–8). However, the adequate stimulus for outer hair cells is the mechanical energy of sound. The purpose of the present investigation was to determine whether isolated outer hair cells show a motile behavior in response to an acoustic signal.

METHODS

Pigmented guinea pigs (200–500 g) were decapitated, and the cochleae were rapidly removed and opened at the apex and the round window to allow the perfusion of culture medium (Leibovitz's L-15 medium, GIBCO) through the scalae. The major ions of the L-15 culture medium (pH 7.4) were Na^+, 135 mM; K^+, 5.8 mM; Ca^{2+} 1.26 mM; and Cl^-, 147 mM. Outer hair cells were mechanically isolated from segments of the cochlea at a distance of 17, 15, 13, or 11 mm from the round window (corresponding to approximately 0.5, 1.0, 2.0, and 4.0 kHz, respectively) and were placed on a microscope slide in 250 μl of L-15 culture medium at room temperature (20°C). The desired region of the organ of Corti was dissected free and gently pumped in and out of a pipette with a constricted tip. The isolation procedure was complete within 20–30 min, and the majority of cells had a fresh, birefringent appearance when viewed with interference contrast optics in a Reichert inverted microscope at ×630 magnification. A videocamera (Ikegami, Japan) was attached to the microscope and the cells were monitored on a television screen at ×1200 magnification. Cells showing pathological signs such as swelling, displaced nucleus, excessive bending, or Brownian motion were excluded from the study. Distilled water was added to the culture medium intermittently to compensate for evaporation. The cells could be maintained for up to 4 hr after isolation and still appear healthy.

The stimulus was delivered by a minihydrophone ending with a glass pipette filled with culture medium. The minihydrophone had a diameter of 10 ± 2 μm and was connected to a minishaker [Bruel and Kjaer (Denmark) type 4810] driven at 200 Hz. The frequency response of the hydrophone was optimal at 200 Hz. The amplitude of the output was controlled by an attenuator that was graded in 1.5-decibel (dB) steps with a range of 0–88.5 dB (Somedic type TMS 111). The 200-Hz tone burst was 800 ms in duration, had an 80-ms rise/fall time, and was presented at a rate of 20 per minute.

Each selected cell was tested by positioning the stimulus-pipette opening 30–50 μm from and perpendicular to the midportion of the hair-cell wall. Each selected cell was tested by increasing the intensity of the fluid oscillation until a visible motile response could be detected. The intensity from the minihydrophone was then reduced, the minihydrophone was moved 10–15 μm away from the cell, and a threshold for visual detection of movement was determined. An increase in intensity by 3.0 dB resulted in a clearly detectable motion. Threshold values were normalized for the distance between the minihydrophone and the cell that usually varied between 10 and 15 μm.

RESULTS

Fig. 1 *Left* shows an isolated outer hair cell (third row) that was obtained 13 mm from the round window; at rest, the cell length was 76 μm. The minihydrophone is seen 30 μm away from the midportion of the cell. The active movement of the cell began with the onset of the 200-Hz tone burst emitted from the minihydrophone, was maintained for 800 ms, and terminated with the end of the tone burst. The change in hair-cell length (2.3 μm) occurred along the longitudinal axis and was most evident in the cuticular plate region (white horizontal line) and at the base of the cell (bracket arrow). During stimulation, the cuticular plate and the region under the nucleus were observed to move in opposite directions. Fig. 1 *Right* shows an isolated outer hair cell from the 17-mm region corresponding to approximately 0.5 kHz, with a resting length of 82 μm. This cell responds to the tone burst by increasing its length by 1.5 μm and is seen at the cuticular plate region (black horizontal line). In addition, increased organelle movements occurred in these regions during stimulus-induced activity. The location and character of the response were the same when different sites along the lateral wall were stimulated. The same was also true when an assembly of about four outer hair cells was stimulated, or when supporting cells remained attached to the outer hair cell. Further, there was a linear correlation between the

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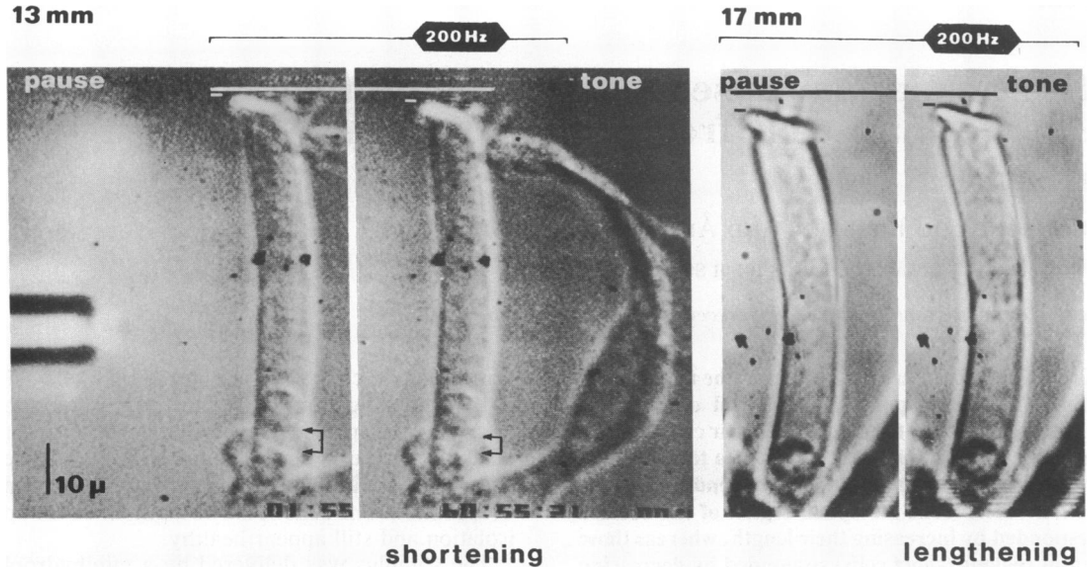

FIG. 1. Effect of acoustic stimulation (200-Hz tone burst) on isolated outer hair cells from the regions 13 mm (*Left*) and 17 mm (*Right*) from the round window. The minihydrophone (inner diameter, 10 μm) is seen to the far left.

intensity of the stimulus and the change in cell length. The maximum increase in cell length for such rather long cells (60–80 μm) was about 4% (2.4–3.2 μm) of the original length. In general, cells obtained from the more basal regions are shorter in length and the observable movement was proportionally smaller.

Eighty percent of the total number (n = 55) of outer hair cells studied showed a motile response. Thirty percent elongated, 50% shortened, and 20% could not be activated. It was found that approximately half of the cells not responding to the stimulus were firmly attached to the bottom of the microscope slide, whereas no apparent reason could be found for the remaining half.

The polarity of the motile event was systematically distributed along the length of the basilar membrane. Fig. 2 illustrates a tonotopic distribution of the motile response of hair cells with respect to position along the cochlear partition and to cell length. Including only the cells showing a positive response, there was a high probability (0.86) of obtaining an elongating response from the 17-mm region; a high probability (1.0) of obtaining a shortening response from the 11-mm region, and an equal chance for either elongating or shortening from the 15- and 13-mm regions. The mean threshold for cells from the 17-mm region was 32.5 ± 4.0 dB of attenuation (n = 10, where 6 elongated, 1 shortened, and 3 were unresponsive). The mean threshold for cells that elongated from the 15- and 13-mm regions was 28.0 ± 3.0 dB of attenuation and for those cells that shortened was 27.0 ± 4.0 of attenuation (n = 28, where 11 elongated, 15 shortened, and 2 were unresponsive). The mean threshold value for the cells from the 11-mm region was 32.5 ± 4.0 dB of attenuation (n = 17, where 0 elongated, 11 shortened, and 6 were unresponsive).

Attempts were made to alter or inhibit the observed motile response. A positively charged molecule, poly(L-lysine) (M_r ≈ 59,000), at a concentration of 3.0 μM, was effective in totally inhibiting the response (elongating and shortening) within 5 min after the application of the solution to the bath. Even at maximum output from the drive unit (35 dB above the visual detection threshold) no motile event could be elicited.

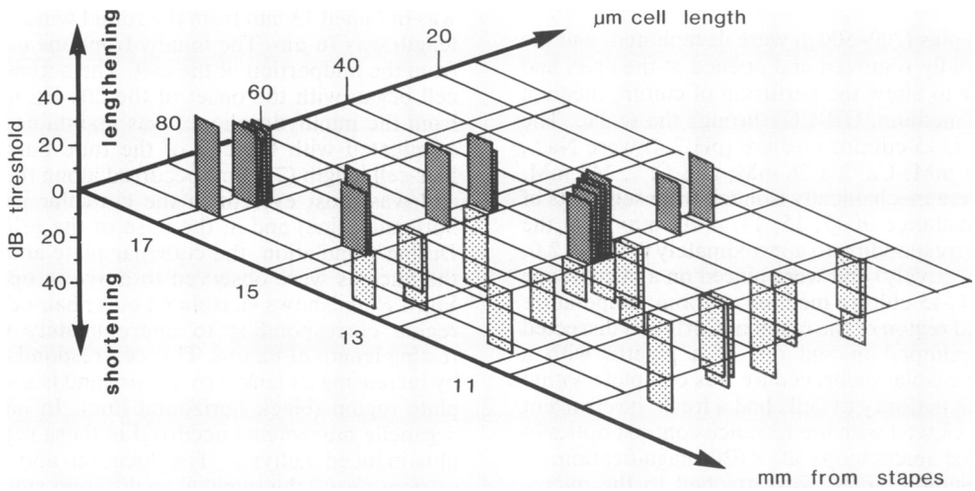

FIG. 2. A tonotopic distribution of the response polarity and threshhold of isolated outer hair cells in relation to their position along the cochlea and their length. Cells that responded to the tone burst by increasing their length are depicted by the dark shaded bars projecting upward, whereas cells that decreased in length are illustrated by the light bars projecting downward. Threshold measurements were not possible for all cells showing a positive response.

The effect of poly(L-lysine) on the motility of outer hair cells suggests a possible charge-dependent event. When inner hair cells were stimulated by the 200-Hz tone burst, an alteration in cell length was never observed. Neither did supporting cells, damaged outer hair cells, or cells fixed in 2.5% glutaraldehyde show an active motile response. Furthermore, 2.0 mM NaCN failed to cause any alteration in the response of the outer hair cells. Attempts were also made to alter the sensitivity of hair cells by overstimulation *in vitro*. Two cells were stimulated at 6 dB over their threshold value for 5 or 30 min, respectively. Despite the cell performing 100 or 600 repetitive cycles of motility, there was no change in the direction, degree, threshold, or quality of the response.

Occasionally, nerve endings remain attached to the base of the isolated outer hair cells. To test whether or not synaptic activity was influencing the motile response, a 20 μM atropine or 50 μM tetrodotoxin was applied to the bath. Immediately after the application of these inhibitors, the cell was continuously stimulated at an intensity 6 dB above threshold for 30 min. During this period there was no obvious change in either the character or the threshold of the hair-cell motion.

DISCUSSION

The results reported here show that (*i*) an acoustic stimulus can elicit a motile response in solitary outer hair cells, (*ii*) inner hair cells do not respond to similar acoustic stimuli, (*iii*) an acoustic alternating signal induces a sustained unidirectional response, (*iv*) outer hair cells can elongate as well as shorten, and (*v*) the polarity of the response is correlated with cochlear location.

The fact that a 200-Hz tone burst can give rise to a motile response in hair cells brings this phenomenon one step closer to the adequate stimulus for the organ of Corti, which is acoustic energy rather than electrical or pharmacological excitation. Also, the sound stimulus, which is of alternating nature, produces a sustained change in length that appears to relate to the intensity of the envelope of the tone. Preliminary results show that this is also true at higher frequencies. Possibly this mechanism provides frequency analysis overriding the low-pass filtering of the receptor current that occurs due to membrane capacitance in outer hair cells (9). The existence of biasing in the position of the basilar membrane during high-frequency tones has been demonstrated in the intact organ of Corti, implying that the length changes reported here for solitary hair cells may have an "*in vivo*" correlate (10). It remains to be seen whether the acoustically induced tonotopic alterations in the length of outer hair cells reported here relate to a number of intriguing observations concerning frequency-specific phase transitions of basilar membrane motion and the excitation of cochlear afferent nerve fibers (10–13).

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