Ionic Currents and Electromotility in Inner Ear Hair Cells From Humans

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1 Bobby R. Alford Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030; 2 Department of Neurobiology and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02114; 3 Department of Otorhinolaryngology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan; and 4 Texas Ear, Nose, and Throat Consultants, Houston, Texas 77030

OGHALAI, John S., Jeffrey R. Holt, Takashi Nakagawa, Thomas M. Jung, Newton J. Eatock, and William E. Brownell. Ionic currents and electromotility in inner ear hair cells from humans. J. Neurophysiol. 79: 2235–2239, 1998. The upright posture and rich vocalizations of primates place demands on their senses of balance and hearing that differ from those of other animals. There is a wealth of behavioral, psychophysical, and CNS measures characterizing these senses in primates, but no prior recordings from their inner ear sensory receptor cells. We harvested human hair cells from patients undergoing surgical removal of life-threatening brain stem tumors and measured their ionic currents and electromotile responses. The hair cells were either isolated or left in situ in their sensory epithelium and investigated using the tight-seal, whole cell technique. We recorded from both type I and type II vestibular hair cells under voltage clamp and found four voltage-dependent currents, each of which has been reported in hair cells of other animals. Cochlear outer hair cells demonstrated electromotility in response to voltage steps like that seen in rodent animal models. Our results reveal many qualitative similarities to hair cells obtained from other animals and justify continued investigations to explore quantitative differences that may be associated with normal or pathological human sensation.

INTRODUCTION

The inner ear resides within the densest structure of the human body, the otic capsule of the temporal bone. Within its bony labyrinth reside the hair cells of the peripheral vestibular organs and cochlea. The main function of hair cells is to transduce head movements, gravitational forces, and sound vibrations into electrical signals. Transduction occurs as a result of mechanical deflection of the sensory hair bundle, which modulates ion influx and generates a receptor potential. The complement of voltage-dependent conductances within a hair cell shapes its receptor potential and hence regulates synaptic transmission of the sensory stimuli to afferent neurons. In this way, the distinctive electrophysiological signature of a hair cell is related to its specific function. A second function, unique to the cochlear outer hair cell, is its ability to undergo rapid somatic length changes (Brownell et al. 1985) in response to its receptor potential. This electromotile response is thought to amplify the cochlear traveling wave and contribute to the exquisite sensitivity and selectivity of mammalian hearing.

The voltage-dependent conductances within hair cells have been studied in fish, amphibians, reptiles, birds, and rodents (Eatock and Rüscher 1997; Kros 1996; Wu et al. 1995), and electromotility has only been demonstrated in rodent outer hair cells. These data have been used to explain diverse aspects of the senses of balance and hearing in humans. However, there has been no study of living hair cells from humans or other primates. Without such data, we cannot be certain of the relevance of detailed electrophysiological accounts of animal hair cells to human inner ear function. This is of particular concern when attempting to understand human pathophysiology. We investigated the extent to which the physiology of primate hair cells resembles the physiology of nonprimate hair cells.

Because of the inaccessibility of the primate inner ear, harvesting this tissue and keeping it alive is a difficult undertaking. Sacrifice of the human inner ear is occasionally required during surgical removal of tumors of the temporal bone and posterior cranial fossa. These are most often unilateral vestibular schwannomas (incidence 1/100,000/yr) (Tos and Thomsen 1984), where the wide exposure provided by drilling out the inner ear aids tumor dissection. We were able to harvest inner ear organs from this select patient population, in which hearing and vestibular sense cannot be preserved.

METHODS

Harvesting technique

The study protocol was approved by the Baylor College of Medicine Institutional Review Board and informed consent from each patient was obtained after the nature and possible consequences of the surgery were discussed. All 18 patients who required inner ear ablation during a 4-mo period at The Methodist Hospital Neurosensorry Center agreed to donate their inner ear organs and participate in the study. The average patient age was 53 ± 2.5 (SE) yr, and ranged from 35 to 73 yr. Seventeen patients had unilateral vestibular schwannomas, and one patient had a facial neuroma. Fourteen of the patients underwent a translabyrinthine approach (drill out of the semicircular canals and vestibule only) with harvest of the vestibular organs, and four underwent a transfacial approach (drill out of semicircular canals, vestibule, and cochlea) with harvest of both the vestibular organs and the cochlea. The specimens were placed into a vial containing a balanced salt solution (PhysioSol, Abbott, Chicago, IL). This was transported to the laboratory on ice within 5 min. The organs were then transferred into an extracellular...
solution, containing L-15 (GIBCO BRL, Gaithersburg, MD) with 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, Sigma, St. Louis, MO), at room temperature (22–24°C) with a pH of 7.3 and an osmolality of 320 mOsm/kg.

Hair cell preparations

Vestibular hair cells from the semicircular canal organs, utriculus, and sacculus, as well as cochlear outer hair cells from the organ of Corti, were either isolated or studied in situ in their sensory epithelium. To isolate vestibular hair cells, we incubated the semicircular canal organs, utriculus, and sacculus at 37°C for 35 min in extracellular solution containing papain (0.5 mg/ml, Sigma), l-cysteine (0.3 mg/ml, Sigma), and ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA; 1.2 mM, Sigma). The organs were transferred back into normal extracellular solution, and the epithelia were mechanically dissociated with an eyelash. To isolate cochlear outer hair cells, we cut the organ of Corti from the modiolus as one strip using a microscissors. This was placed in a microcentrifuge tube with extracellular solution containing trypsin (0.5 mg/ml, type III, Sigma) and incubated at 37°C for 5 min. Gentle trituration was performed to dissociate the cells. Isolated hair cells were plated onto the glass bottom of a lysine-coated microwell Petri dish (MatTek Corporation, Ashland, MA) and visualized with an inverted microscope with a ×100 oil immersion objective (Axiovert35, Carl Zeiss, Inc., Thornwood, NY). In four specimens, vestibular hair cells were studied in situ in the utricular or saccular epithelium. The organs were laid flat on a coverslip, epithelial surface facing up, and held in place by two thin glass fibers glued to the coverslip at one end. The in situ preparation was visualized under an upright microscope using a ×40 water immersion objective (AxikopFS, Zeiss). All organ study was performed within 5 h of specimen harvest, during which constant perfusion of fresh extracellular solution was maintained.

Recording techniques

Investigations were performed using the tight-seal, whole cell technique. Patch pipettes of 4–6 MΩ were fabricated from borosilicate capillaries using a vertical, two-stage puller (PP-83, Narishige, Tokyo, Japan). The intracellular solution consisted of (in mM) 165 KCl, 0.1 CaCl₂, 10 EGTA, 3.5 MgCl₂, 2.5 Na₂ATP, and 5 HEPES, pH 7.3 and osmolality 295 mOsm/kg. Seals >1 GΩ were formed along the basolateral surface. Currents were recorded with an Axopatch 200A or 200B amplifier and pCLAMP6 software (Axon Instruments, Foster City, CA), and low-pass filtered (4-pole Bessel) with a cutoff frequency of 2 kHz. On-line series resistance compensation varied from 0 to 95%. Residual compensation of steady-state voltages was performed off-line, along with junction potential correction. Images of outer hair cell electromotive force were recorded on Super-VHS videotape, and length measurements were made off-line with National Institutes of Health Image software running on a Power Macintosh 8100/80AV with built-in frame-grabbing capability.

RESULTS

Vestibular hair cells

Isolated vestibular hair cells were classified as type I or type II based on morphological criteria (Fig. 1). Under voltage clamp, we recorded from five type I and five type II hair cells (4 from semicircular canal organs, 4 from utriculi, and 2 from sacculi). The average resting potential of the cells was −57 ± 4.5 mV (mean ± SE, n = 10), and ranged from −38 to −79 mV. We found four voltage-dependent currents, each of which has been reported in hair cells of other animals. Figure 2A shows a family of current tracings representative of a delayed rectifier that was fully activated at the holding potential, −67 mV. We found this current in two type I cells. Its reversal potential was −74 ± 2.0 mV (n = 2), close to the equilibrium potential of K⁺ (−85 mV), indicating that it was mainly carried by K⁺. The activation curve was generated by plotting tail currents (see arrow) versus prepulse potential and scaling to a unity maximum. The voltage range of activation was between −110 and −80 mV (Fig. 2C, ●). To evaluate the kinetic behavior of this current, we fit its deactivation phase with the sum of two exponentials. Both time constants were voltage dependent, ranging from 4.9 and 13.4 ms at −41 ms at 4.9 and 13.4 ms at −4.9 and 13.4 ms at

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\begin{align*}
I_{\text{K,L}} &= \frac{g_{\text{K,L}}}{1 + e^{\frac{V - E_{\text{K,L}}}{\Delta V}}}
\end{align*}
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FIG. 1. Vestibular hair cells from human semicircular canal organs. Many cells had lost their stereocilia. A: type I cells (found only in amniotes) have a constricted neck just below the cuticular plate. B: type II cells (found in all vertebrates) have a more cylindrical shape (Wersäll 1956). Scale bar = 10 μm.
current are consistent with it being a delayed rectifier, similar to that seen in chick cochlear (Fuchs and Evans 1990) and mammalian vestibular (Rennie and Ashmore 1991) hair cells, although we have not ruled out a calcium-dependent component.

Figure 2D illustrates a transient outward current that we found in three type II cells. The peak current amplitude declined in response to a depolarizing voltage step as the holding potential was raised, suggesting that this was an A-type K⁺ current (Connor and Stevens 1971; Neher 1971). The activation curve was generated by measuring the peak current values (from Fig. 2D), calculating the chord conductance assuming K⁺ selectivity, and scaling to a unity maximum. This current demonstrated activation from −60 to +20 mV (Fig. 2E, ●). We fit its activation kinetics with a third-order Hodgkin-Huxley scheme. The activation time constant was voltage dependent, ranging from 4.2 ms at −35 mV to 1.5 ms at +25 mV. The inactivation curve was generated using a prepulse protocol (not shown), in which the initial command stimulus ranged from −85 to +15 mV and was applied for 500 ms before stepping to +35 mV. The peak current values were measured and used as described for the activation curve. This current inactivated between −70 and −20 mV (Fig. 2E, ○). The inactivation phase was fit with a single-order exponential decay, and had a voltage-dependent time constant that ranged from 72 ms at −25 mV to 22 ms at +25 mV. Qualitatively similar transient K⁺ currents have been recorded in bullfrog hair cells (Lewis and Hudspeth 1983). However, although activation occurs in the same voltage range, inactivation occurs at a more negative voltage range (−100 to −70 mV) in the bullfrog. This suggests that, although the transient K⁺ current is inactive at the resting potential in nonmammals (Hudspeth and Lewis 1988; Lang and Correia 1989), it may play a physiological role in humans and other mammals (Saeki 1996). Because of its rapid activation and inactivation kinetics, this conductance may act in vivo to modify the frequency response of the receptor potential.

Figure 2F shows a single current trace in response to a hyperpolarizing voltage step, illustrating a slowly activating, inwardly rectifying current we found in two type II cells. The current was active at potentials negative to −75 mV. Its
activation kinetics were best fit with a first-order Hodgkin-Huxley scheme, with a time constant of 133 ms. This current appeared similar in its voltage range of activation and in its slow kinetics to the hyperpolarization-activated current (I_h) found in frog saccular cells (Holt and Eatock 1995). This is a mixed Na⁺/K⁺ current that tends to counter extended hyperpolarizations. It has also been found in mouse utricle type I and II hair cells (Rüssch and Eatock 1996a; Eatock, unpublished observations).

**Cochlear outer hair cells**

From the cochlea, we isolated outer hair cells (Fig. 3A). In all six outer hair cells in which whole cell recording was attempted, we observed an electromotile response in response to voltage steps. Depolarization led to a decrease in length and an increase in width, whereas the reverse occurred with hyperpolarization. Displacement increased with increasing distance from the point of pipette attachment. We plotted apical displacement versus the applied voltage stimulus to get the electromotility transfer function (Fig. 3B). Motility data were fit with a single-order Boltzmann curve with the function $D = D_{max}/[1 + \exp\{(V_m - V_{1/2})/s\}]$, where $D$ is the displacement at a given membrane potential, $D_{max}$ is the maximum displacement, $V_m$ is the membrane potential, $V_{1/2}$ is the potential at half-maximal displacement, and $s$ describes the voltage dependency of the curve (slope factor). The largest displacement that we observed was 460 nm (1.0% of cell length), with graded responses to smaller voltage stimuli ($V_{1/2} = +4 \text{ mV}$; $s = 21 \text{ mV}$). These properties are consistent with other published data in rodent outer hair cells (Ashmore 1987; Brownell et al. 1985; Dallos et al. 1997; Santos-Sacchi 1989). We fit the region from −25 to +25 mV with a line having a slope of 7.1 nm/mV ($r = 0.98$). This mechanical gain is about one-half to one-third that reported for the guinea pig (15–19.8 nm/mV) (Ashmore 1987; Dallos et al. 1997; Santos-Sacchi 1989). However, the gain we report is probably less than what the cells were capable of because we could only obtain loose (40–100 MΩ) seals in these recordings. Further characterization of the voltage-displacement function under better seal conditions is required.

**DISCUSSION**

We demonstrate that physiologically healthy hair cells can be harvested from the human inner ear. Human vestibular hair cells have similar voltage-dependent conductances to those previously demonstrated in other animals, particularly mammals, suggesting that individual hair cell physiology is essentially conserved between primates and nonprimates. Likewise, our results indicate that outer hair cell electromotility in humans is qualitatively similar to that found in rodents. This suggests that, within humans, the high-frequency sensitivity and selectivity as well as the source of otoacoustic emissions derives from the outer hair cell. Observations from animals on the biophysical and molecular basis of hair cell current and electromotility should be readily transferred to human balance and hearing.

The hair cell is a finely tuned apparatus whose biophysical properties are translated into the overall performance of the organ and sensory system. In the past, we have only been able to obtain from humans psychophysical data along with noninvasive tests of inner ear function, including the vestibuloocular response to rotational testing, the caloric response, posturography testing, pure tone thresholds, the auditory brain stem–evoked response, electrocochleography, and otoacoustic emission testing (Cyr and Harker 1993; Stelmacowich and Gorga 1993). Now, it is conceivable to measure the single hair cell response, the inner ear organ response, and the behavioral response within a single patient. This opens a new approach for the study of human inner ear function—a way to relate cellular function to human perception.

Rapid advances in our understanding of basic hair cell physiology continue to occur with animal models. Our data support the applicability of these single cell data to humans. However, previous reports have shown that the kinetic properties, ionic dependence, and intracellular complement of voltage-dependent channels in nonprimate hair cells are specific to cell type, location, and organ (Eatock and Rüssch 1997; Kros 1996; Wu et al. 1995). In nonmammals, these subtle differences can have a profound impact on hair cell functioning, e.g., the frequency to which a cell is tuned (Wu et al. 1995). Similarly, we have observed quantitative differences among cells in our sample, which may modify the response of the human inner ear to balance and hearing stimuli. So, although animal models have several advantages, they probably will not be able to demonstrate certain properties unique to human sensation and behavior. The technique reported here will be useful in bridging this gap.

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