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Whole cell currents and mechanical responses of isolated outer hair cells

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Outer hair cells (OHCs) exhibit electrically induced cell movements which are considered to enhance the frequency selectivity and sensitivity of basilar membrane vibration. Using simultaneous whole cell voltage clamp and video analysis, we demonstrate that the mechanical response of OHCs is not altered by agents which alter membrane currents under voltage clamp. Thus the underlying mechanism of OHC movements appears to be dependent upon membrane potential, rather than transmembrane currents.

Outer hair cell; Whole cell voltage clamp; Membrane current; OHC mechanical movements

Introduction

Major differences are known to exist between the two types of hair cells which populate the organ of Corti (see Dallos, 1985a). Whereas in vivo intracellular recordings have clearly shown that both inner and outer hair cells (OHCs) are capable of generating receptor potentials in response to acoustic stimulation (Dallos et al., 1982: Russell and Sellick, 1983), it is becoming increasingly clear that OHCs additionally function as effectors. The electrical activity of the OHCs is thought to modify the mechanical properties of these same cells and in turn influence organ of Corti micromechanics (Mountain, 1980; Siegal and Kim, 1982; Brown et al., 1983; Nuttall, 1985). Thus, an indirect micromechanical interaction between outer and inner hair cells may account for the exquisite tuning characteristics and sensitivity of the eighth nerve fibers, which predominantly innervate inner hair cells. It has been established that OHCs are capable of slow K^+ induced length changes (Zenner et al., 1985), and fast electrically induced, reversible cell movements on the order of micrometers (Brownell, 1983, 1984; Brownell et al., 1985; Ashmore, 1986). Studies using isolated OHCs have shown that cell elongation or contraction begins within 200 μs of an applied voltage clamp stimulus, and that the direction of movement is polarity dependent (Brownell, 1983, 1984; Brownell and Kachar; Ashmore, 1986). Time- and voltage-dependent currents have been identified in OHCs using whole cell voltage clamp recording (Ashmore, 1986, Ashmore and Meech, 1986a, b; Santos-Sacchi and Dilger, 1986, 1988). It has been suggested that transmembrane currents are coupled to the fast mechanical responses of OHCs (Ashmore, 1986; Ashmore and Meech, 1986). We have directly tested this hypothesis by recording OHC whole cell currents under voltage clamp while simultaneously monitoring cell movement. We report here that manipulations which drastically reduce or reverse transmembrane current during voltage clamp, do not interfere with the OHC mechanical response, which remains a nearly linear function of applied voltage over much of its dynamic range.

Methods

OHCs were non-enzymatically isolated from guinea pig cochleas by gentle pipetting of the isolated top two turns of the organ of Corti. The cells were then transferred to a 700 μL perfusion chamber with a cover glass bottom. A Nikon

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Diaphot inverted microscope, with Hoffmann optics, was used to observe the cells during electrical recording. Tissue culture Medium 199 with Hanks salts (GIBCO, NY; major ions: NaCl 137 mM, KCl 5.4 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 0.8 mM, with HEPES or bicarbonate buffer, pH 7.2) was used as the control perfusate. The same ion concentrations were used in test solutions except that NaCl was reduced to maintain osmolarity. Patch electrodes had initial resistances of 3–5 MΩ. Series resistance was typically between 10 and 15 MΩ, and was partially compensated during recording. Residual series resistance was estimated from current transients at the onset of voltage pulses and was corrected for during analysis (Marty and Neher, 1983). Pipette solutions were composed of 140 mM KCl or CsCl, 1 mM or 5 mM EGTA, 2 mM $MgCl_2$, and 5 mM HEPES buffered to pH 7.0–7.2. Both borosilicate and flint glass electrodes were used; flint glass provided better sealing. Giga-ohm seals were obtained at a location slightly above the nuclear level of the OHC membrane prior to whole cell recording.

OHCs were clamped to holding potentials near −60 mV, using a Dagan patch clamp amplifier. Under computer control, hyperpolarizing and depolarizing voltage pulses (50–500 ms), ranging from −100 to +100 mV, were used to elicit membrane currents and mechanical movements. Current records, filtered at 2 kHz with an 8 pole Bessel filter, were digitized and stored on a Data 6000 waveform analyzer, and saved to disk for off-line analysis. Simultaneously, cell movements were taped with a Panasonic AG6300 video recorder for later evaluation. OHC displacement reaches steady state several milliseconds after a voltage clamp step (Ashmore, 1986). Displacements of the cuticular plate region were measured from the video monitor after they had reached steady state. Two methods were employed, either manual measurement from digital still frames using a Toshiba digital VHS (14800 × monitor magnification) or measurement during playback using differential optoresistors (output filtered at 50 Hz) placed across the image of the cuticular plate on the monitor (2800 × monitor magnification). Still frame measurements were repeated at least five times and averaged. Standard deviations of these repeated measurements were about 0.16 μm. The position of the cuticular plate was measured before, during, and after each voltage step. The linearity of the optoresistor method was confirmed by measuring the video taped movement of the tip of a microelectrode driven by a piezoelectric bimorph element. For the optoresistor method, absolute values were determined from manual measurements of cell displacements in response to the largest depolarizing voltage pulse. The movement of the cuticular plate is relative to the stationary insertion point of the electrode, which was typically near the nuclear region. However, upon depolarization, both the apical and basal ends of the OHC move towards the electrode insertion point. Thus, in order to account for total cell length change, the measured displacements of the cuticular region were multiplied by the ratio of total cell length to the electrode-cuticular plate distance, under the assumption that movement is linearly related to length of the cell.

Results

The resting membrane potential of isolated OHCs ranged from −10 to −68 mV with a mean

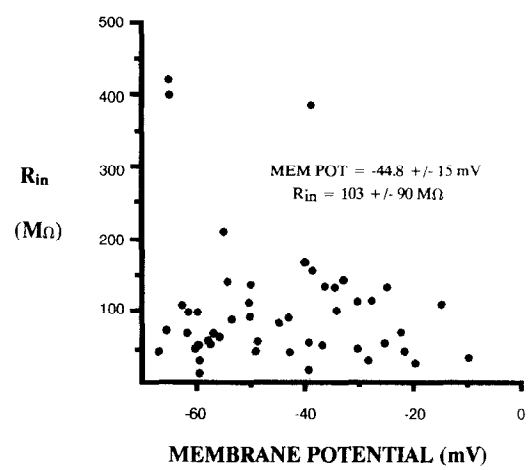

Fig. 1. Input resistance and membrane potential (+/− S.D.) for isolated OHCs whole cell recorded using pipettes containing 140 mM KCl solution. Input resistance was determined at a holding potential near −60 mV using −10 mV voltage steps. Membrane potential was determined by finding zero current level under voltage clamp or alternatively by directly measuring under current clamp. Measures were made a few minutes after the establishment of whole cell recording, i.e., following exchange of pipette and intracellular fluids.

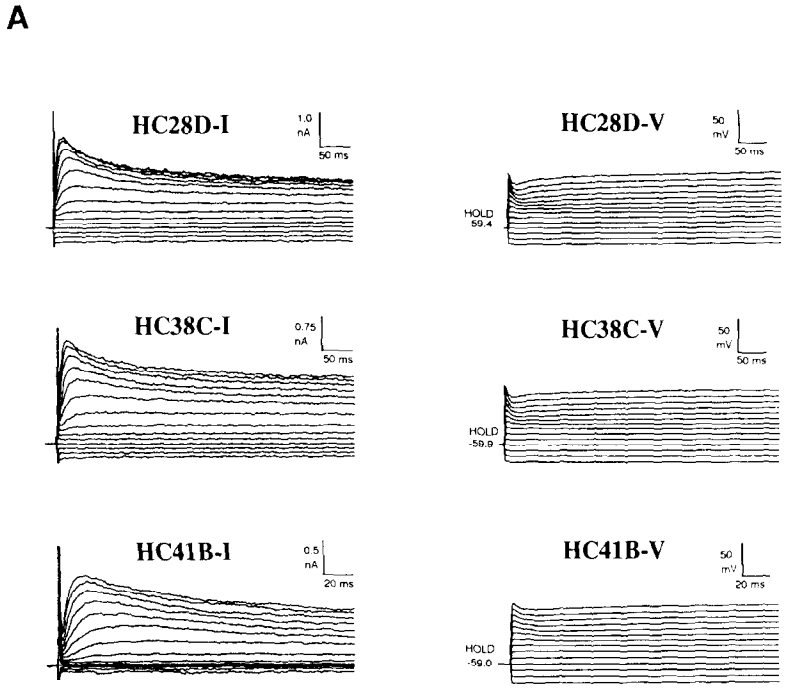

Fig. 2a. Examples of whole cell voltage clamp recordings from three isolated OHCs. Patch electrode contained 140 mM KCl solution. On the left are current traces in response to voltage steps from a holding potential near −60 mV (actual voltage listed in figure). Outward current is upward. On the right are the voltage step magnitudes corrected for estimates of residual series resistance. Depolarization is upward. Upon depolarization above −40 mV, a delayed outward current develops, the magnitude of which is voltage- and time-dependent. Current peaks within 20 ms after the onset of the voltage step, and inactivates slowly over hundreds of milliseconds. Note differences in time and current scales. Membrane potential (determined at zero current level) and input resistance (determined at the holding potential) of each cell: HC28D: −57 mV, 70 mΩ; HC38C: −60 mV, 100 mΩ; HC41C: −38 mV, 380 mΩ.

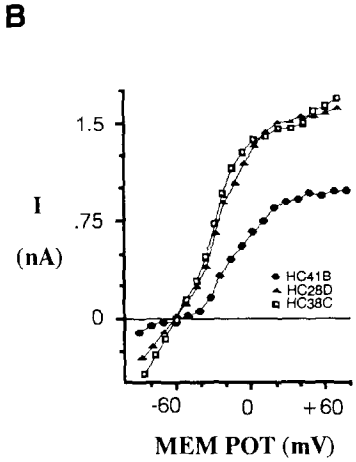

Fig. 2b. I-V plot for the three cells in Fig. 1a. Total transmembrane currents (no leak subtraction) measured at 200 ms. Note the outward rectification upon depolarization from the holding potential. The I-V curves begin to saturate above −10 mV.

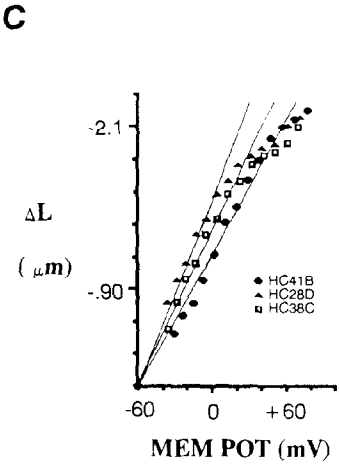

Fig. 2c. Mechanical responses (contractions) of the same three OHCs in response to depolarizing pulses from the holding potential. Mechanical responses from the three cells essentially overlap. Individual cell lengths and percent maximal contraction: HC41C: 64 μm, 3.8%, HC28D: 74 μm, 3.2%, HC38C: 83 μm, 2.7%.

value of −45 mV (Fig. 1). This is similar to the membrane potentials obtained in vivo for apical OHCs (Dallos et al., 1982). Upon establishment of whole cell recordings, membrane potentials often increased over the course of a few minutes. This type of behavior has occasionally been observed from OHCs recorded in vivo using high resistance

electrodes (Dallos, 1985b). With whole cell recording this behavior may arise from the exchange of pipette and cytoplasmic solutions, possibly indicating that isolated OHCs have a reduced internal K^+ concentration. This phenomenon suggests that the membrane potential is dependent upon K^+ conductance. However, we have recorded from OHCs having membrane potentials near −60 mV from the onset of whole cell recording. The conductance of these cells at the holding potential of about −60 mV was also variable (2.4 to 62 ns), having a mean of 9.2 nS.

Under voltage clamp, OHCs exhibit time- and voltage-dependent outward currents upon depolarization from −60 mV (Fig. 2). These currents rise sigmoidally to peak values within 10 to 20 ms (with a voltage-dependent time constant) and partially inactivate on a somewhat longer time scale. Leak-subtracted peak outward currents typically show saturation at pulse potentials above +40 mV, and begin to roll over about +60 mV. Steady state current (no leak subtraction, measured at 200 ms, Fig. 2b) begins to saturate above +10 mV.

OHCs, but not supporting cells, clearly exhibit mechanical movements in response to step changes in holding potential (Fig. 2c). The magnitude of cell concentrations induced by depolarization increases as the size of the voltage step is increased, and in most cells studied the linear relationship between voltage and contraction is lost at high voltage step levels, where the mechanical response falls off. Our largest calculated mechanical response about the −60 mV holding potential was 29 nm/mV. The electrically induced contraction of cells is not limited to any region of the cell; both basal and apical regions contract towards the point of electrode insertion in the supranuclear region. Hyperpolarization causes elongation of the cell, but quantitative measurements of these move-

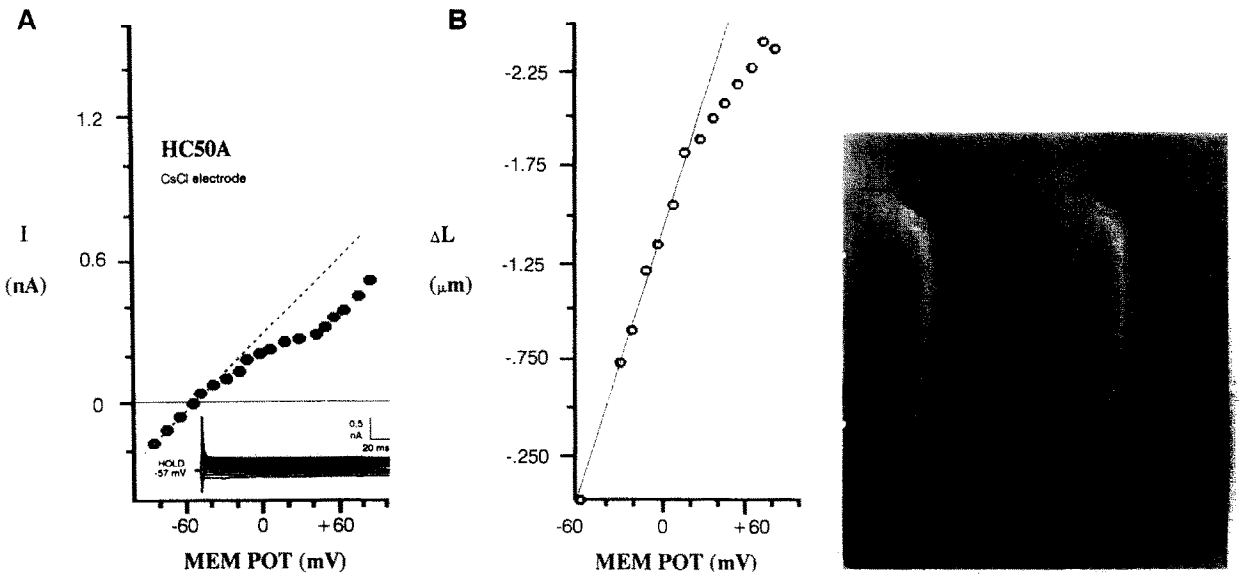

Fig. 3a. I-V curve measured at 200 ms after voltage step for an OHC in which the outward currents were blocked by using a patch pipette containing 140 mM CsCl isolation. Dotted line indicates leakage current. Leakage current subtraction (not shown) revealed inward currents at depolarizations greater than −30 mV, probably indicative of Ca^{2+} influx. Inset shows current traces associated with voltage steps from a holding potential of −57 mV.

Fig. 3b. Mechanical responses of the same OHC in response to step depolarizations from a holding potential of −57 mV. Despite the reduction in outward current the voltage induced mechanical response of the cell is as robust as those exhibiting normal I-V characteristics (see Fig. 1c). In this case, the mechanical response about the holding potential is 23 nm/mV.

Fig. 3c and d. Photographs of video images of same OHC. (c) during the 200 ms pulse of +81 mV, and (d) upon return to the holding potential of −57 mV. Vertical scale: 4.8 μm.

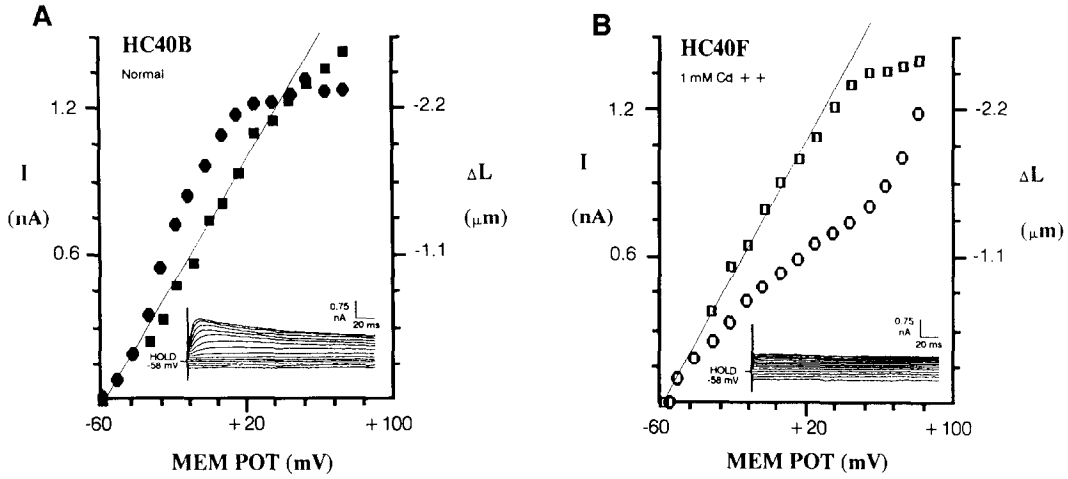

Fig. 4. Steady state current (measured at 200 ms), and mechanical response of an isolated OHC before and after addition of 1 mM $CdCl_2$ to the extracellular perfusate. Pipette contained 140 mM KCl. No leakage current subtraction. Despite reduction of outward current, mechanical responses remain unaltered. (a) Normal extracellular medium. Steady state current: closed circles; mechanical response: closed squares. Mechanical response is 23 nm/mV about the holding potential. Inset: Current traces associated with voltage steps from the holding potential. (b) Normal extracellular medium + 1 mM $CdCl_2$. Steady state current: open circles; mechanical response: open squares. Mechanical response is 25 nm/mV about the holding potential. Inset: current traces associated with voltage steps from the holding potential.

ments were not possible for the small range of hyperpolarizations examined in this study.

Outward currents in OHCs are blocked by various manipulations which are generally considered to suppress K^+ currents in cells. Replacing intracellular K^+ with Cs^+ (Fig. 3a) eliminates most of the outward current. However, mechanical responses are robust despite the reduction of outward current (Fig. 3b). Other treatments which block the outward current including extracellular additions of 25 mM TEA, 10 or 50 mM Ba^{2+}, or 1 mM Cd^{2+} do not interfere with the electrically induced OHC movements. Figs. 4a and b illustrate the effects of 1 mM extracellular Cd^{2+} on OHC currents and mechanical movements. Although the time- and voltage-dependent outward current is reduced by this treatment, the mechanical response is left intact. One mechanism by which Cd^{2+} may suppress outward current is by blocking Ca^{2+} entry through voltage gated Ca^{2+} channels, thus preventing the opening of Ca^{2+}-activated K^+ channels. A voltage-dependent inward current can, in fact, be measured when outward currents are reduced (see Fig. 3). This inward current is Cd^{2+} sensitive and its magnitude is increased when the bath contains 50 mM Ba^{2+} and no Ca^{2+} (Fig. 5). Thus, a link between Ca^{2+}

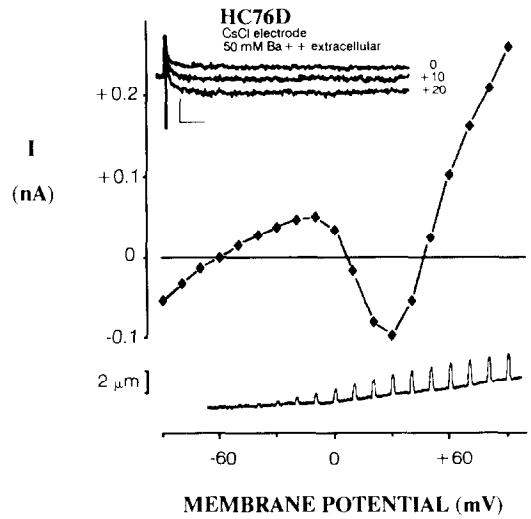

Fig. 5. I-V curve of an isolated OHC, obtained with a pipette solution of 140 mM CsCl, and extracellular solution in which 50 mM Ba^{2+} replaced Ca^{2+}. Note the reversal of net current as the cell is depolarized to levels between +10 and +40 mV. At the top, current traces are depicted at depolarizations of 0, 10, and 20 mV, from a holding potential of −60 mV (insert: horizontal scale 20 ms, vertical scale 0.1 nA). Note the absence of inactivation during the 200 ms pulses. At the bottom, the magnitude of cellular contractions is presented for depolarizing pulses. In this case the responses were recorded with the use of differential optoresistors to measure apical cell movements directly off a video monitor. Note that the cell continues to contract in a graded fashion during net current reversal.

influx and OHC movements can be ruled out. In addition to the inability of extracellular Cd^{2+} or Ba^{2+} to inhibit cell movements, OHC movements persist when the extracellular Ca^{2+} is reduced to zero (either due to omission of Ca^{2+} or chelation with EGTA).

Finally, in the presence of high extracellular Ba^{2+} concentrations there is a net inward current during depolarizing voltage clamp steps to +10 through +40 mV (Fig. 5); yet, the cells still contract rather than elongate, as might have been expected if the direction of movement were dependent upon the direction of current across the membrane.

Discussion

The mechanical response of OHCs has been postulated to be coupled to the magnitude and direction of transmembrane current in the cell (Ashmore, 1986; Ashmore and Meech, 1986). Ashmore (1986) initially reported that replacing intracellular K^+ with Na^+ under whole cell voltage clamp blocked outward K^+ currents and altered OHC mechanical responses, suggesting that outward K^+ current is important for OHC movements. Subsequently, it was postulated that the sensory stimulus to the outer hair cell is directly coupled to the cell mechanical response via these large basolateral outward currents (Ashmore and Meech, 1986). Indeed, this concept prompted others (Gitter et al., 1987) to analyze single K^+ channel kinetics of OHCs in an effort to account for the speed of OHC movements. The data presented here clearly demonstrate that OHC movements are not dependent upon outward K^+ currents, since blocking them with a variety of treatments does not affect movements. Recently, Ashmore (1987) reported that, in fact, replacing intracellular K^+ with Na^+ does not affect OHC movements, and suggested that his initial reports to the contrary were due to the small range of voltages studied and pipette series resistance changes.

While outer hair cells in vivo have resting potentials near −70 mV (Dallos, et al., 1982; Russell et al., 1986), and values of up to −68 mV were observed in this and other studies (Ashmore and Meech, 1986; Zenner et al., 1985), it is clear that potentials in vitro are variable and often poorer than in vivo. Many studies have evaluated mechanical responses of OHCs under current clamp or by means of extracellular current stimulation without simultaneously accounting for changes in cell membrane potential (Brownell et al., 1985; Kachar et al., 1986; Evans, 1988). Under these conditions, the variability of membrane potential and input resistance in vitro may place limitations upon the interpretation of certain aspects of the measured mechanical responses, since we show that mechanical responses are not dependent upon total transmembrane current.

We demonstrate here that OHCs possess a voltage dependent Ca^{2+} current, but that this inward current is not required for mechanical responses. OHCs continue to contract in response to depolarizations when inward Ca^{2+} currents are blocked by Cd^{2+} or when extracellular Ca^{2+} is absent. This result indicates that fast electrically induced OHC movements are not dependent upon an actin-myosin system, as has been suggested for the slow movements of OHCs (Zenner, 1986). This notion is strengthened by the fact that OHCs continue to respond mechanically when intracellular Ca^{2+} is buffered to below 10 nM (Ashmore, 1987), and when metabolic inhibitors are present (Kachar et al., 1986). Furthermore, the frequency response of OHC movements, measured up to 8 kHz, is unlikely to be accounted for by a muscle-like system (Ashmore and Brownell, 1986; Ashmore, 1987).

The average input conductance of OHCs in this study was 9.2 nS (108 mΩ). This contrasts with the range of conductances (25–50 nS; 40–20 MΩ) reported by Ashmore and Meech (1986) for OHCs isolated using trypsin. These authors have suggested that inclusion of the Ca^{2+} chelator BAPTA in the patch pipette solution is necessary to obtain cells with high resistances and membrane potentials. It is difficult to understand how a reduction in internal calcium levels would increase the cell's membrane potential when such measures would be expected to reduce the cell's Ca^{2+}-activated K^+ conductance. In a cell whose membrane potential is dependent upon K^+ conductance, a reduction in the conductance would cause depolarization. We find that with our non-enzymatic isolation technique, cells with high resistances and membrane potentials are obtained with or without Ca^{2+} che-

lators in the pipette solution. Furthermore, the presence of time- and voltage-dependent outward currents was not dependent upon the inclusion of a Ca^{2+} chelator in the pipette solution.

The resting input resistance of OHCs is low compared to hair cells from other systems, where values can be an order of magnitude greater (Ohmori, 1984; Corey and Hudspeth, 1979). This difference is not due to the methods employed in this study since similar measures on isolated Hensen cells (having comparable membrane surface area) demonstrate an average input resistance of 1.1 GΩ (Santos-Sacchi, 1988). The OHC leakage conductance is probably not due to transduction channel activity in the stereocilia since many of the cells studied lacked stereocilia as a result of the isolation procedure. Induced mechanical movements in these cells were indistinguishable from movements of cells with stereocilia. Still, the average input resistance reported here is higher than that determined in vivo (30–50 MΩ, Dallos, et al., 1982; Russell et al., 1986). These in vivo measures probably underestimate true values because they were made with high impedance microelectrodes whose electrode-to-membrane seal resistance is considerably less than that obtained with the patch electrode technique.

Currently, a major effort is being made to elucidate the roles played by inner and outer hair cells in the organ of Corti. The discovery that OHCs can rapidly alter their cell length in response to electrical stimulation (Brownell et al., 1985) fits well with models incorporating active bi-directional transduction processes (mechanical to electrical and vice versa) to account for the high sensitivity and sharp tuning of basilar membrane vibration (Kim, 1986; Geisler, 1986; Mountain, 1986; Jen and Steele, 1987). The elucidation of the mechanism of OHC movements will aid in the understanding of OHC-IHC interactions and pathologies which compromise these interactions. At present, the mechanism of this mechanical response is unknown. The data presented demonstrate that the mechanical response is not dependent upon either the specific ionic currents blocked here or the direction and magnitude of total transmembrane current. Furthermore, studies using the Cl^- channel blocker SITS indicate that a Cl^- current is not involved (Santos-Sacchi, unpublished). While it is possible that movements are dependent upon some unidentified current which is masked by the residual leakage conductance, we believe that a membrane potential dependence is more likely. This may not conflict with Brownell's electro–osmotic theory (Brownell, 1986) of OHC movement if an axial potential gradient is present along the cell. Axial potential gradients are conceivable under physiologic conditions. Under voltage clamp, however, the existence of such a gradient is dependent upon the ability to adequately space clamp the cell. Ashmore has estimated (see comments after Brownell, 1986) that under voltage clamp, the cell is isopotential to within 1%. Of course, this value will depend upon the cable properties of the cell during stimulation, i.e., the magnitude and spatial distribution of basolateral conductances during depolarization. Presumably, in the present study, cells whose basolateral conductances were blocked would have been better space clamped than those under normal conditions. Yet, no differences in mechanical responses were observed. In preliminary experiments (Santos-Sacchi and Brownell, unpublished), voltage clamping OHCs with two patch electrodes, one at the cell apex and one at the cell base, in order to reduce axial potential gradients did not abolish the mechanical movements due to depolarization. Further studies of this sort are necessary.

Whatever underlying mechanism is responsible for the OHC's mechanical response, the results presented here indicate that it is associated with the magnitude of transmembrane potential, and potential differences less than a millivolt produce mechanical responses on the same scale as basilar membrane movements near threshold. It is conceivable that the effects of OHCs on the mechanical properties of the cochlear partition may be influenced by both intracellular and extracellular voltage responses associated with acoustic stimulation.

Acknowledgments

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