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Tonotopic Variation in the Conductance of the Hair Cell Mechanotransducer Channel

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Summary

Hair cells in the vertebrate cochlea are arranged tonotopically with their characteristic frequency (CF), the sound frequency to which they are most sensitive, changing systematically with position. Single mechanotransducer channels of hair cells were characterized at different locations in the turtle cochlea. In 2.8 mM external Ca^{2+}, the channel's chord conductance was 118 pS (range 80–163 pS), which nearly doubled (range 149–300 pS) on reducing Ca^{2+} to 50 μM. In both Ca^{2+} concentrations, the conductance was positively correlated with hair cell CF. Variation in channel conductance can largely explain the increases in size of the macroscopic transducer current and speed of adaptation with CF. It suggests diversity of transducer channel structure or environment along the cochlea that may be an important element of its tonotopic organization.

Introduction

Transduction in auditory hair cells as in other sensory receptors culminates in the activation of an ion channel in the plasma membrane. The hair cell mechanotransducer channel is located near the tips of the stereocilia (Lumpkin and Hudspeth, 1995; Denk et al., 1995) and when opened by deflection of the stereociliary bundle allows influx of cations to generate a depolarizing receptor potential. At least partly because of the small number of hair cells per cochlea, the molecular structure of the mammalian auditory transducer channel has not yet been identified. Some of its properties, including a high permeability to Ca^{2+} and large unitary conductance, suggest that it is a member of the transient receptor potential (TRP) channel family (Strassmaier and Gillespie, 2002), a classification promoted by the identification of auditory mechanosensitive channels in both fly (Walker et al., 2000; Kim et al., 2003) and most recently fish (Sidi et al., 2003) as members of the TRP family. However, in the absence of a specific molecular taxonomy, there are benefits from documenting the single-channel properties in the intact cell. This is necessary to define the noise and kinetic limitations that the channel imposes on hair cell transduction. It will also provide a norm for comparison with future cloned channels to determine subunit composition as was the case for cyclic nucleotide-gated channels in olfactory receptors (Bönigk et al., 1999). Although there have been several estimates of unitary conductance ranging from 20 to 110 pS (Ohmori, 1985; Holton and Hudspeth, 1986; Crawford et al., 1991; Denk et al., 1995; Géléoc et al. 1997), there have been few direct recordings of single-channel events that permit a systematic investigation of their properties. This paucity of data stems mainly from the difficulty of recording single channels in cell-attached patches (Hamill et al., 1981), a technique that has been successful for delineating the properties of other ion channels (Ashcroft, 2000).

Here we have used whole-cell recording to monitor single mechanotransducer channels in turtle auditory hair cells. These channels had a large unitary conductance that was increased by reducing the extracellular Ca^{2+} concentration. Surprisingly the channel conductance changed systematically along the cochlea in conjunction with the CF, the sound frequency to which the cell was tuned. This parallels variations with CF in the size of the macroscopic transducer current and its rate of adaptation in turtle cochlear hair cells (Ricci and Fettiplace, 1997; Ricci et al., 1998). Such variation may reflect an active process that optimizes the signal-to-noise ratio of transduction in the frequency range encoded by a given hair cell and may be an important ingredient of the cochlea's tonotopic organization (Fettiplace et al., 2001; Fettiplace and Ricci, 2003).

Results

Properties of Mechanotransducer Channels Resemble Macroscopic Current

Because of their large amplitude, single transducer channels were observable in whole-cell recording mode with sufficient signal-to-noise ratio to allow their properties to be quantified. The channels had a predominant size of ~10 pA (range 6.4–13 pA) at a holding potential of −80 mV and 2.8 mM Ca^{2+}, and they were rapidly activated by small deflections of the hair bundle within the physiological range (Figure 1; Crawford et al., 1991). The sensitivity of the averaged single-channel responses to bundle motion and the time course of activation and adaptation for a step displacement were similar to those reported for macroscopic mechanotransducer currents in turtle hair cells (Ricci and Fettiplace, 1997). The probability of opening had a sigmoidal dependence on hair bundle displacement, increasing with motion toward the kinocilium and reaching a maximum for displacements of less than 0.5 μm (Figure 1). Movement of the hair bundle away from the kinocilium closed the channels that were open at rest, indicating that the channel responded to polarized bundle motion and not just cellular deformation. For the two examples in Figure 1, only a single

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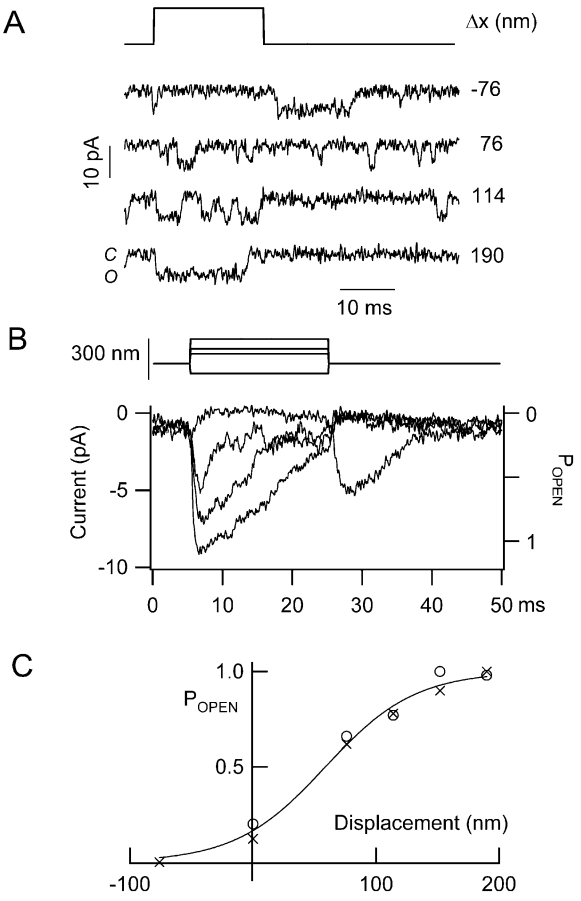

Figure 1. Single-Channel Responses to Hair Bundle Displacement

(A) Examples of single-channel records for hair bundle deflections (Δx) relative to the steady-state position of the bundle. Holding potential, −80 mV. ***C*** and ***O*** in this and subsequent figures denote the closed and open levels of the channel.

(B) Ensemble averages of between 44 and 82 single-channel responses at each stimulus level. The right-hand ordinate gives the probability of opening of the channel, which had an amplitude of 8.2 pA.

(C) Probability of opening, P_{OPEN}, of two different channels as a function of hair bundle displacement, x, relative to the steady-state position (see Experimental Procedures). Smooth curve is given by: $P_{OPEN} = 1/(1 + \exp((x - x_{0.5})/x_e))$, with $x_{0.5} = 59$ nm and $x_e = 36$ nm.

channel was evident even for the largest bundle displacements, but in some other recordings, a second channel appeared for the biggest stimuli. Channel activation occurred on a submillisecond time scale and was followed by fast adaptation (Wu et al., 1999) similar to that observed in the macroscopic current; the mean time constant of adaptation measured in ensemble averages was 2.0 ± 1.1 ms (n = 13; 2.8 mM Ca^{2+}). Such fast adaptation was not seen in previous single-channel measurements (Crawford et al., 1991). For a steady-state open probability of 0.1 ± 0.06 (see Experimental Procedures), the mean open time was 0.58 ± 0.08 ms. The behavior of mechanotransducer channels was sufficiently close to the macroscopic current to indicate that their properties were not seriously altered by the isolation procedure. Assuming a reversal potential of 0 mV (Ohmori, 1985; Crawford et al., 1991; Kros et al., 1992), the channels had a mean chord conductance of 118 ± 31 pS (n = 15; 2.8 mM extracellular Ca^{2+}). As will be shown later, the variation in the channel conductance was linked to hair cell location in the cochlea. In a few recordings (data not shown), a channel with smaller amplitude was also seen as previously reported (Crawford et al., 1991). The ratio of amplitudes of the small to the large principal channel was 0.40 ± 0.026 in five cells with positions along the basilar membrane ranging from 0.2 to 0.7 of the distance from the low-frequency end. The smaller channel event, which may represent a subconductance state (mean = 53 ± 15 pS), also responded to bundle displacements but with a longer mean open time.

Extracellular Calcium Increases Channel Conductance and Slows Adaptation

A characteristic feature of the mechanotransducer current of hair cells is its modulation by calcium. There are two reversible effects of Ca^{2+} on the macroscopic current which reflect block of the channel and promotion of adaptation (Corey and Hudspeth, 1983; Crawford et al., 1991; Ricci and Fettiplace, 1998). Both actions of Ca^{2+} were evident in single-channel responses (Figures 2 and 3). Reducing extracellular Ca^{2+} from 2.8 to 0.05 mM increased the single-channel current amplitude (mean increase = 1.89 ± 0.17; n = 5) to give a chord conductance of 215 ± 65 pS. The decrease in channel amplitude caused by elevating extracellular Ca^{2+} is also manifested in macroscopic currents where the Ca^{2+} dissociation constant for the blocking site has been estimated as 1 mM (Ricci and Fettiplace, 1998). Changing extracellular Ca^{2+} also affected the channel kinetics. Ensemble averages showed that both the time course of activation and adaptation were increased by lowering Ca^{2+} (Figure 2). Since the kinetics were slowed in low Ca^{2+}, an apparent increase in channel amplitude might have occurred because the channel openings were less filtered by the recording system. However, prolonged channel openings in high Ca^{2+} had comparable amplitude to brief ones in the same Ca^{2+} but were clearly smaller than some of the shorter events in low Ca^{2+} (Figure 2A), which is inconsistent with this explanation. Moreover, channel amplitudes at +80 mV remained the same as at −80 mV, which also argues against filtering. Nevertheless, flicker block of the channel by Ca^{2+} may have occurred at a rate that was not measurable by the recording system. If the rate constant for Ca^{2+} to bind to the channel is diffusion limited at 10^9 $M^{-1}s^{-1}$ and the dissociation constant for the blocking site is 1 mM (Ricci and Fettiplace, 1998), then the rate constant for Ca^{2+} unbinding will be 10^6 s^{-1}. This is equivalent to a mean occupancy time of 1 μs. Flickering at this rate would be within the noise and undetectable by our recording system, which at best had a time constant of 50 μs.

The effects of changing Ca^{2+} were entirely reversible (Figure 3A). As the high Ca^{2+} was returned to the bath, channel openings became increasingly blocked, producing fast interruptions in opening and reduced size before attaining the final smaller control amplitude. The mean open time in high Ca^{2+} in the absence of hair bundle stimulation was about a fifth of that in low Ca^{2+} (Figure 3B). Similar reversible channel blocking behavior

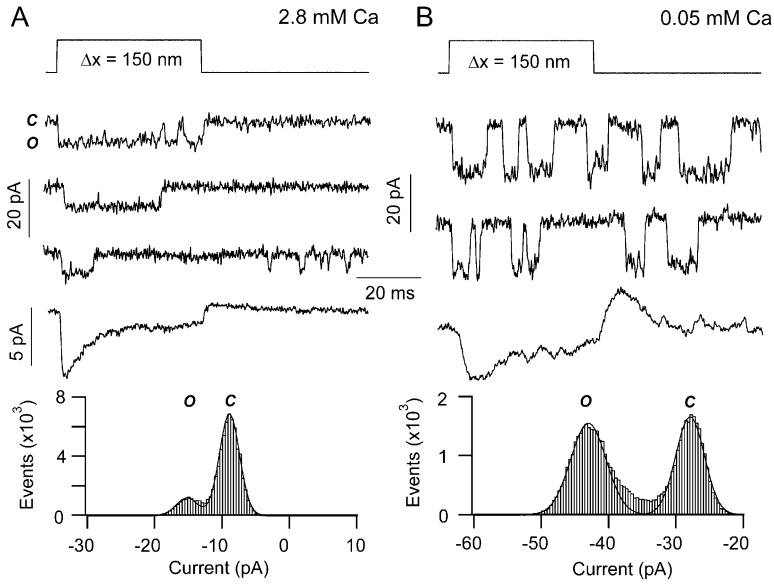

Figure 2. Extracellular Ca^{2+} Modulates Mechanotransducer Channel Amplitude

(A) Three single-channel responses for hair bundle deflections (Δx) of 150 nm in 2.8 mM extracellular Ca^{2+}. Below the individual responses is the ensemble average of 140 channel responses. At the bottom is the amplitude histogram of channel events. ***C*** and ***O*** denote the closed and open levels of the channel.

(B) Two single-channel responses for hair bundle deflections (Δx) of 150 nm in 0.05 mM extracellular Ca^{2+}. Middle is the ensemble average of 250 channel responses, and at the bottom is the amplitude histogram of channel events. Reducing extracellular Ca^{2+} increased the single-channel amplitude from 7 pA to 15 pA and slowed both the activation and adaptation of the ensemble average current. The overshoot in the low-Ca^{2+} average at the end of the displacement step reflects recovery from adaptation. Holding potential, −80 mV.

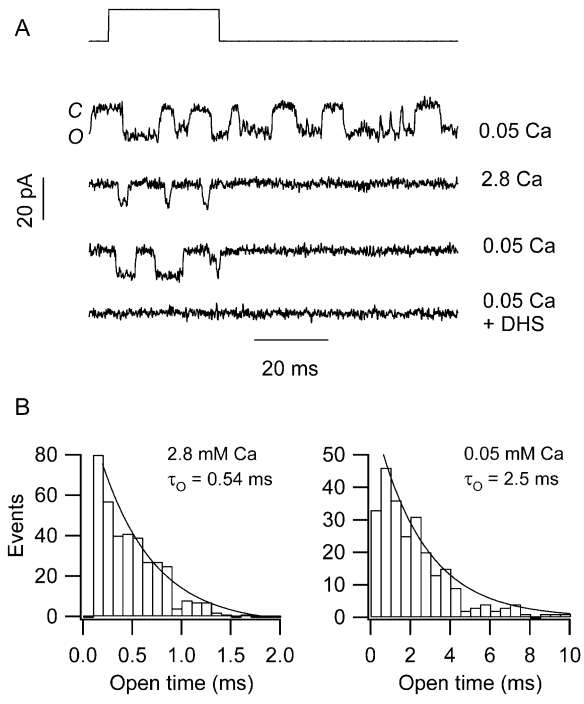

Figure 3. The Effects of Ca^{2+} on Mechanotransducer Channel Kinetics

(A) Examples of single-channel records in 0.05 mM Ca^{2+}, 2.8 mM Ca^{2+}, 0.05 mM Ca^{2+}, and 0.05 mM Ca^{2+} + 0.2 mM dihydrostreptomycin (DHS). There was a small decrease in open probability between traces 1 and 3, probably due to stereociliary loading with Ca^{2+} during the intervening period in high external Ca^{2+}.

(B) Open-time histograms of the mechanotransducer channel in 2.8 mM Ca^{2+} (left) and 0.05 mM Ca^{2+} (right). Smooth curves are single-exponential fits with time constant, τ_o, of 0.54 ms and 2.5 ms. For each histogram, the first 0.1 ms bin has been omitted because of the limited bandwidth of the recording system. Holding potential, −80 mV.

was observed when dihydrostreptomycin (DHS) in low Ca^{2+} saline was perfused over the hair bundle. DHS is known to block the mechanotransducer current in hair cells (Ohmori, 1985; Kroese et al., 1989; Ricci, 2002), and it totally eliminated channel activity. There was no evidence that DHS reduced a standing current, an observation that was previously used to argue that after tip-link destruction the mechanotransducer channels fall open (Meyer et al., 1998). In the recording of Figure 3, although the standing (leak) current increased when the cell was bathed in 0.05 Ca^{2+} (mean current with channel closed = 38.6 ± 0.9 pA), this standing current was unaffected by adding DHS in 0.05 Ca^{2+} (mean = 39.0 ± 1.2 pA). The lack of effect of DHS on the leak current and the large size (15 pA) of the single channels compared with the leak in low Ca^{2+} both argue that the mechanism proposed by Meyer et al. (1998) does not occur in turtle hair cells.

Effects of Holding Potential

If one or both actions of Ca^{2+} on the mechanotransducer channels require influx of the ion across the membrane, they should be susceptible to altering the membrane potential. Changing the holding potential from −80 mV to +80 mV strongly affected adaptation but not channel amplitude (Figure 4). Depolarization, which reduces the electrical driving force on Ca^{2+} entry, was previously shown to abolish adaptation in the macroscopic current (Crawford et al., 1989; Assad et al., 1989). This result, along with the effect of changing the cytoplasmic calcium buffering, was used to argue that Ca^{2+} mediates adaptation by binding to an intracellular site. The present experiments confirm this observation at a single-channel level and demonstrate that at +80 mV the channel can remain open for the entire duration of the stimulus.

In contrast to the results on adaptation, the effects on channel size produced by reducing extracellular Ca^{2+} were not mimicked by depolarization. The ratio of unitary current amplitudes at −80 mV to those at +80 mV was 0.97 ± 0.1 (n = 3; 2.8 mM Ca^{2+}). This is consistent with

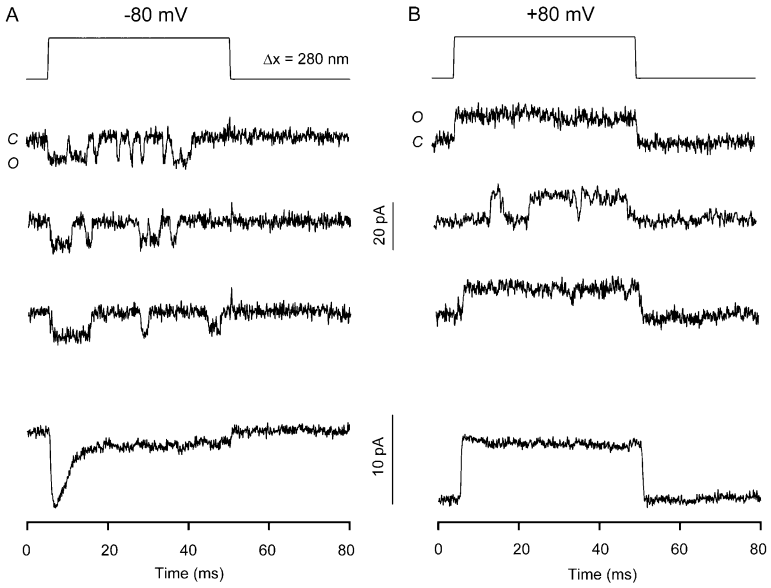

Figure 4. Depolarization Abolishes Adaptation

Single-channel records for hair bundle deflections (Δx) of 280 nm in 2.8 mM extracellular Ca^{2+} at a holding potential of −80 mV (A) and +80 mV (B). Below is the ensemble average of 50 responses showing adaptation of the responses at −80 mV but not at +80 mV. ***C*** and ***O*** denote the closed and open levels of the channel.

a linear current-voltage relationship for the channel with a reversal potential at 0 mV (Ohmori, 1985; Crawford et al., 1991). The effects of Ca^{2+} and voltage on the amplitude of the single channels are consistent with their effects on the macroscopic current, which in low Ca^{2+} increases by the same proportion at all membrane potentials (Crawford et al., 1991).

Tonotopic Variations in Channel Conductance

In the vertebrate cochlea, the hair cells are arranged tonotopically in that the characteristic frequency (CF), the sound frequency to which they are most sensitive, changes systematically with position, with high frequencies located at the base and low frequencies at the apex of the cochlea. The tonotopic map in the turtle (Crawford and Fettiplace, 1980) is associated with cochlear gradients in various cellular properties. These include the size and kinetics of the voltage-dependent currents that underlie electrical tuning (Wu et al., 1995) and the size and adaptation time constant of the mechanotransducer current (Ricci and Fettiplace, 1997). Surprisingly, the amplitude of single mechanotransducer channels, whether measured in normal (2.8 mM) or reduced Ca^{2+}, also varied along the cochlea (Figure 5). In both Ca^{2+} concentrations, the channel amplitude approximately doubled in progressing from 0.2 to 0.7 of the distance along the cochlea from the low-frequency end.

The time constant of adaptation determined from whole-cell macroscopic current also varies with hair cell CF (Ricci and Fettiplace, 1997). A similar though more limited variation in the adaptation time constant was found at the single-channel level (Figure 6). The single-channel records showed a decline in the probability of opening for a maintained deflection of the hair bundle. The measurements were analyzed by constructing ensemble averages of channel activity for nonsaturating stimuli and fitting the adaptive decline in P_{OPEN} with a single exponential. The time constants ranged from 3.3 to 0.9 ms, decreasing inversely with CF. The range of single-channel adaptation time constants is not significantly different from that found with macroscopic transducer currents recorded with an intracellular solution containing 1 mM BAPTA identical to that used in the single-channel recordings (see Figure 4B of Ricci and Fettiplace, 1997).

Discussion

Channel Size and Identity

Single mechanotransducer channels were isolated in whole-cell recording by rapid and brief exposure of the hair bundle to submicromolar Ca^{2+} concentrations (Crawford et al., 1991). The method probably relies on destruction of the tip links (Assad et al., 1991), which removes mechanical input to the majority of channels, causing them to fall closed. Single channels that survived this procedure possessed properties akin to the macroscopic current, including similar kinetics and sensitivity to hair bundle deflection. A drawback of the recording method is the limited bandwidth attributable to the large cell capacitance compared to that of a detached membrane patch (Marty and Neher, 1983). Despite this drawback, the single-channel recordings revealed three attributes of the mechanotransducer channels not deducible from macroscopic currents. First, millimolar extracellular Ca^{2+} blocks the channel by reducing its amplitude. Second, under conditions where Ca^{2+} block is minimized, the channel can have an unexpectedly large unitary conductance of 150–300 pS. Third, in both blocked and unblocked states, the channel conductance changes with the hair cell location along the basilar membrane and hence with CF of the cell. Since comparable tonotopic variation (about 2-fold) in unitary conductance is seen in both high and low Ca^{2+}, it seems likely that it requires a mechanism distinct from variation in Ca^{2+} block.

The unblocked conductance of the auditory mechanotransducer channels, ranging between 150 and 300 pS, is large compared to most types of channel. On the basis of size and properties, there are two channel fami-

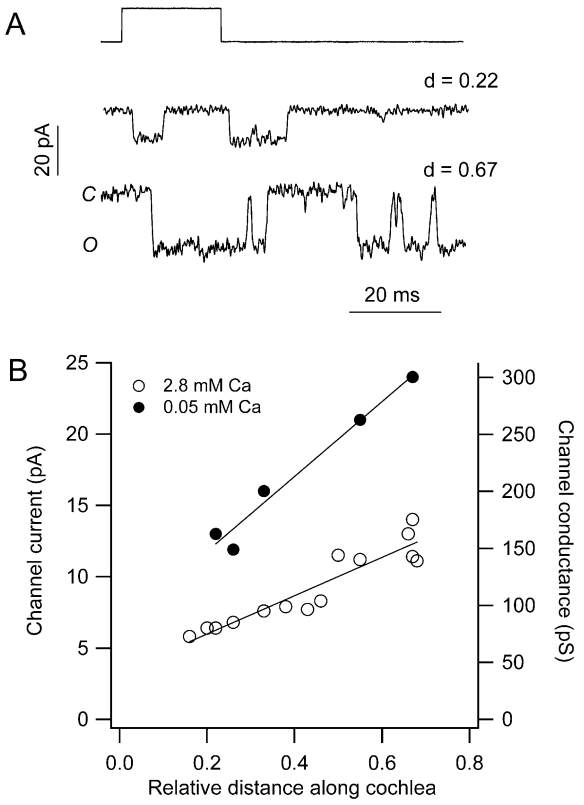

Figure 5. Tonotopic Variations in Mechanotransducer Channel Amplitude

(A) Single-channel records from two hair cells in 0.05 mM Ca^{2+} for hair bundle displacements of 150 nm. The cells were located at fractional distances, d, of 0.22 and 0.67 from the low-frequency end of the cochlea. Holding potential, −80 mV.

(B) Single-channel amplitudes recorded at −80 mV in hair cells at different relative distances along the cochlea from the low-frequency end. Measurements at each location came from different turtles. (o) 2.8 mM Ca^{2+}; (•) 0.05 mM Ca^{2+}. The right-hand ordinate gives the channel conductance assuming a reversal potential of 0 mV. Straight lines are least-squares fits using the Levenberg Marquardt algorithm. Note that the channel conductance increases for hair cells tuned to high frequencies.

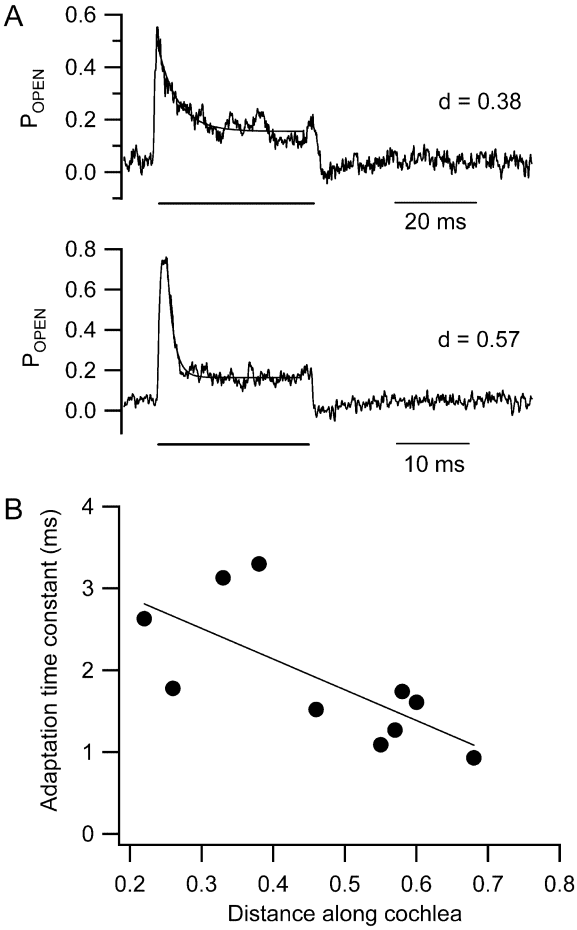

Figure 6. Adaptation of Mechanotransducer Channel Activity

(A) Ensemble average current responses for similar hair bundle deflections for a low-frequency hair cell (top, fractional distance from low-frequency end, d = 0.38; bundle deflection, 0.16 μm) and a high-frequency hair cell (bottom, d = 0.57; bundle deflection, 0.1 μm). The decline in the current was fitted with a single exponential (smooth line) of time constant 3.2 ms (top) and 1.0 ms (bottom). Fifty responses were averaged; holding potential, −80 mV; 2.8 mM extracellular Ca^{2+}. Note different time scales for the two averages. Bars below denote timing of bundle deflection.

(B) Adaptation time constants inferred from fits similar to those in (A) as a function of the fractional distance of the hair cell along cochlea from the low-frequency end. Straight line is least-squares fit.

lies that most resemble the mechanotransducer channel: transient receptor potential (TRP) channels and gap junction channels. The TRP channels, especially the TRPV subtypes, are cation channels with high Ca^{2+} permeability and large unitary conductances of 100 pS or more in the absence of extracellular Ca^{2+} (Minke and Cook, 2002). A single-channel conductance of 172 pS has been reported for TRPV3 (Xu et al., 2002), 310 pS for TRPV4 (Liedtke et al., 2000), and 400 pS for a mechanosensitve TRP channel in yeast (Zhou et al., 2003). Similar to the hair cell transducer channel, TRP channels are also blocked by extracellular Ca^{2+} (Yue et al., 2001). Gap junction channels are not normally regarded as transduction channels, but they can exist as hemi-channels (connexons) in the plasma membrane, with large unitary conductance (up to 300 pS; Verselis and Veenstra, 2000). Furthermore, they too are blocked by external Ca^{2+} at a half blocking concentration of 0.22 mM (DeVries and Schwartz, 1992). Ca^{2+} block of the gap junction channels is thought to occur by a direct effect on channel conformation and pore size (Muller et al., 2002).

Tonotopic Gradients in Channel Properties

In progressing from the low-frequency to high-frequency end of the turtle cochlea, there is an increase in both the amplitude of the mechanotransducer current and the speed of adaptation (Ricci and Fettiplace, 1997; Figure 7). Is it possible to explain these gradients by a single process? The macroscopic measurements (Figure 7) demonstrate an approximately linear increase in total transducer current along the cochlea. Using the line fitted to all points, the total current increases from 334 pA at d = 0.2 to 1169 pA at d = 0.7, where d is the fractional distance of the hair cell along the cochlea

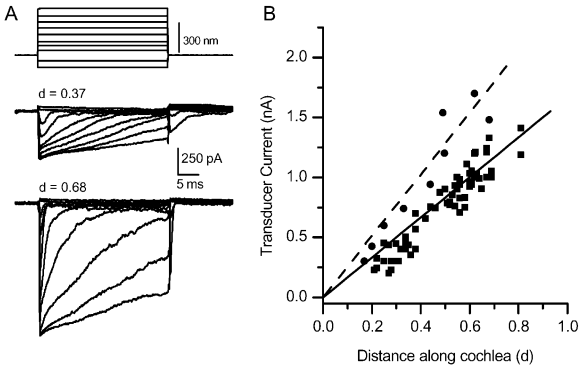

Figure 7. Tonotopic Gradient in Size of Macroscopic Mechanotransducer Current

(A) Averaged transducer currents for a low-frequency hair cell (fractional distance from low-frequency end, d = 0.37) and a high-frequency hair cell (bottom, d = 0.68). Time course of hair bundle displacement is shown at the top. Note that the high-frequency cell has a larger maximum current and exhibits faster adaptation.

(B) Plot of the maximum transducer current for 67 hair cells as a function of their fractional distance along the cochlea (d) from the low-frequency end. Continuous straight line is a least-squares fit to all points (filled squares and circles) with a slope of 1.67 and r = 0.86. The dashed line is a fit to the nine points (filled circles) with the largest currents at each position with a slope of 2.57 and r = 0.93. Because mechanotransducer channels may be lost by damage incurred in the isolation procedure, the dashed line may be more representative of the maximum in vivo current.

from the low-frequency end. There is also a gradient in the number of stereocilia per bundle from ~60 at the low-frequency position to 90 at the high-frequency position (Hackney et al., 1993). Assuming that the mechanotransducer channels are uniformly distributed among the stereocilia, the transducer current per stereocilium is therefore 5.6 pA at the low-frequency position and 13 pA at the high-frequency position. In comparison, the single-channel current increases from 6.4 pA at d = 0.2 to 12.5 pA at d = 0.7 (Figure 5). These numbers suggest that there is only a single mechanotransducer channel per stereocilium (0.88 at d = 0.2 to 1.04 at d = 0.7). However, if the spread of amplitudes at each location reflects varying degrees of damage to the transduction machinery, the largest transducer currents may be most representative of cells least compromised by the isolation procedure. Fitting of the top nine points (Figure 7, dashed line) gave larger values for the transducer current per stereocilium of 8.57 pA at d = 0.2 and 19.9 pA at d = 0.7. After scaling by the single-channel currents (Figure 5), the mean number of channels per stereocilium was calculated as 1.3 at d = 0.2 and 1.6 at d = 0.7. Despite the large size of the transducer currents in Figure 7, all stereocilia may not have been active in those measurements, and therefore even these estimates for the numbers of channels per stereocilium must constitute lower bounds. Each stereocilium may therefore possess between 1 and 2 mechanotransducer channels. Denk et al. (1995) determined the number of active stereocilia contributing to a macroscopic transducer current and concluded that each stereocilium contains two channels, one at either end of the tip link.

Since the rate of fast adaptation is directly proportional to Ca^{2+} influx (Ricci and Fettiplace, 1998), the increase in adaptation rate with hair cell CF may be largely attributable to an increase in channel size: doubling the channel conductance doubles the amount of Ca^{2+} entering and thus halves the adaptation time constant (Figure 6). It was previously argued (Ricci and Fettiplace, 1997; Ricci, 2002) that adaptation would be accelerated by intracellular summation of Ca^{2+}, and it was suggested that the Ca^{2+} originated by influx through multiple channels per stereocilium: more channels produced more Ca^{2+} summation leading to faster adaptation. This hypothesis assumed that the channels had the same conductance in all cells but that they increased in number toward the high-frequency end of the cochlea. The present results demonstrate an increase in single-channel conductance with CF, allowing a greater influx and intracellular summation of Ca^{2+}, which will promote faster adaptation. Additional augmentation of the adaptation rate might be achieved by speeding up the activation and deactivation kinetics of the channel (Ricci, 2002). Changes in the unitary conductance of the channel as shown here and in its kinetics (Ricci, 2002) are most simply explained if different regions of the cochlea contain mechanotransducer channels with differing structure or subunit composition. A precedent exists with the expression of different isoforms of the Ca^{2+}-activated K^+ channel with unique properties to generate variation in the electrical resonant frequency along the turtle cochlea (Jones et al., 1999). However, differences in accessory proteins or lipid environment might also contribute to the diversity in mechanotransducer channel properties. Channel isoforms distinct from those employed in the turtle, with faster kinetics or larger unitary conductance, may also be needed to account for the more rapid adaptation rate seen in mammalian cochlear hair cells (Kennedy et al., 2003).

Experimental Procedures

Preparation and Recording

The preparation and methods of hair cell recording and stimulation in the intact cochlea of the turtle were similar to those previously described (Ricci and Fettiplace, 1997; Fettiplace et al., 2003). A turtle (*Trachemys scripta elegans*) was killed and the cochlear duct extracted using procedures approved by the Animal Care Committees of the University of Wisconsin and Louisiana State University. The cochlea was opened and the tectorial membrane removed following digestion in normal saline of composition NaCl, 125 mM; KCl, 4 mM; $CaCl_2$, 2.8 mM; $MgCl_2$, 2.2 mM; Na pyruvate, 2 mM; Na ascorbate, 2 mM; creatine, 2 mM; glucose 8 mM; NaHEPES, 10 mM (pH 7.6), containing up to 0.06 mg/ml of protease (Type XXIV, Sigma Chemical Company, St. Louis, MO). The preparation was held by strands of dental floss in the bottom of a recording chamber and viewed through a 40× water immersion objective on a Zeiss Axioskop FS microscope. The recording chamber was irrigated with normal saline of composition given above. The upper surface of the hair cell epithelium was independently perfused via a 100 μm pipette with saline of composition NaCl, 130 mM; KCl, 0.5 mM; Na pyruvate, 2 mM; Na ascorbate, 2 mM; creatine, 2 mM; glucose 8 mM; NaHEPES, 10 mM (pH 7.6) containing control (2.8 mM) or reduced (0.05 mM) $CaCl_2$. The lower Ca^{2+} concentration is similar to that measured for turtle endolymph (0.065 mM; Crawford et al., 1991). The time for exchanging the solution around the hair bundle was approximately 2 min. In some experiments, 0.2 mM dihydrostreptomycin (Sigma, St Louis, MO) was applied in 0.05 mM calcium saline to block the mechanotransducer channels (Ohmori, 1985; Kroese et al., 1989). Experiments were performed at 20°C to 22°C. Results are expressed as the mean ± one standard deviation.

Macroscopic and single-channel transducer currents were measured in whole-cell mode with a borosilicate patch electrode attached to an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) with output filter set at 2 or 5 kHz. For single-channel measurements, the time constant of the recording system was between 45 and 217 μs (mean = 117 μs) with series resistances of 4.6 to 13 MΩ. Series resistance compensation was not normally applied, because it introduced excess noise over and above that expected from the increased bandwidth. Patch pipettes were filled with an intracellular solution of composition CsCl, 125 mM; Na_2ATP, 3 mM; $MgCl_2$, 2 mM; Cs_4BAPTA, 1 mM; CsHEPES, 10 mM (pH 7.2). Hair bundles were stimulated with a rigid glass pipette (tip fire-polished to about 1 μm in diameter) waxed to a piezoelectric stack actuator (PA 8/12; Piezosystem Jena, Hopedale, MA) (Fettiplace et al., 2003). The actuator was driven with voltage steps, amplified through a high-voltage driver of 10-fold gain, and filtered with an 8 pole Bessel filter at 10 kHz. Unless otherwise stated, recordings were made at a holding potential of −80 mV. Mechanotransducer currents and voltage steps to the piezoelectric stimulator were digitized with a Power1401 at 100 kHz (CED, Cambridge, UK) and analyzed with CED Signal and IgorPro v4 (Wavemetrics, Lake Oswego, OR) software.

Single Mechanotransducer Channels

Single channels were isolated as described previously (Crawford et al., 1991) by rapid exposure of the hair bundle to a saline of composition NaCl, 130 mM; KCl, 0.5 mM; Na pyruvate, 2 mM; glucose 8 mM; Cs_4BAPTA, 5 mM; $CaCl_2$, 2.5 mM; NaHEPES, 10 mM (pH 7.6), which had a free Ca^{2+} concentration of ~0.2 μM. The submicromolar Ca^{2+} saline was pressure ejected from a patch pipette placed about 20 μm from the bundle with a 2 s "puff" from a Picospritzer (General Valve Corporation, Hollis, NH). A robust macroscopic mechanotransducer response was first recorded for a submaximal mechanical stimulus to the hair bundle. Submicromolar Ca^{2+} saline was then applied, causing transient opening of mechanotransducer channels and the appearance of a large (~1 nA) inward current. The current quickly returned to near zero during the 2 s puff, with loss of the macroscopic transducer response. Fast application of the submicromolar Ca^{2+} solution using the Picospritzer was essential for permanently disabling the majority of the channels (cf. Meyer et al., 1998). In most experiments yielding usable data, the residual standing current was less than 20 pA and included single-channel events whose activity was modulated by hair bundle stimulation. It should be emphasized that only a small proportion of experiments was successful, with 101 recordings from transducing hair cells producing only 19 single-channel events of sufficient stability and signal-to-noise ratio to be analyzable. Failure in the majority of attempts resulted either from an unacceptably large leak current or noise probably due to the persistence of multiple (more than two) channels.

To reveal significant channel activity, it was usually necessary to bias the steady-state position of the hair bundle by displacing it toward the tallest stereociliary rank. The offset may arise because the hair bundle tilts toward its tallest edge when the tip links are severed in low Ca^{2+} (Jaramillo and Hudspeth, 1993; Hackney and Furness, 1995). This adjustment was used to arbitrarily set the open probability at about 0.1 (see Results). Open time distributions of the channel activity were measured at the steady-state position. Opening and closing transitions were detected using half the channel amplitude as a threshold criterion, and the distributions were fitted with a single exponential probability density function. Because of the dead time attributable to filtering by the recording system (Colquhoun and Sigworth, 1983), events shorter than ~100 μs were removed from the histograms. To study single-channel properties in hair cells tuned to different frequencies, data were collected from cells located at fractional distances of between 0.2 and 0.7 along the basilar membrane from the low-frequency end. These would have CFs between 70 Hz and 270 Hz.

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