Electrical Resonance of Isolated Hair Cells Does Not Account for Acoustic Tuning in the Free-standing Region of the Alligator Lizard's Cochlea

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The cochlea of the alligator lizard is divided into two morphologically and physiologically distinct regions. In the “tectorial region,” hair bundles of hair cells are draped by a tectorial membrane, whereas in the “free-standing region,” hair bundles are said to be free-standing because there are no overlying tectorial structures. The acoustic tuning of the free-standing region depends at least in part on mechanical resonances of the hair bundles. In the turtle cochlea, in contrast, acoustic tuning depends in large part upon the electrical properties of the hair cells. We have investigated the electrical properties of hair cells isolated from the free-standing region of the alligator lizard's cochlea. When injected with steps of depolarizing current, these “free-standing hair cells” exhibited electrical resonances that were comparable in frequency and quality to electrical resonances in cochlear hair cells from turtles, chicks, and alligators, and in saccular hair cells from frogs and fish. In the lizard's free-standing hair cells, however, the electrical resonance frequencies (<300 Hz) were a decade below the cells' acoustic characteristic frequencies (between 1 and 4 kHz), showing that the electrical resonance is not likely to contribute to acoustic tuning. The electrical resonances were not apparent at rest. The cells' resting potentials were significantly more negative than the activation voltage (~ -40 mV) of the Ca^{2+} -dependent K^+ current upon which the electrical resonance has been shown to depend in other hair cells. At potentials more negative than -50 mV, an inwardly rectifying K^+ conductance dominated.

Because we observed no electrical tuning above 300 Hz, our results indirectly support a mechanical origin for acoustic tuning in the free-standing region of the alligator lizard cochlea. These results further show that acoustic tuning cannot be inferred solely from the electrical resonances of isolated hair cells.

[Key words: hair cells, tuning, frequency selectivity, electrical resonance, cochlea, voltage-dependent currents, voltage oscillations]

A salient feature of stimulus processing by auditory hair cells is sharp selectivity (tuning) for stimulus frequency. Such selectivity may arise at different stages in the stimulus processing cascade. In the cochleas of mammals and of the alligator lizard, evidence exists for frequency tuning that precedes the gating of the mechanoelectrical transduction channels in the hair bundles. In the mammalian cochlea, the motion of the cochlear partition is sharply tuned (Khanna and Leonard, 1982; Sellick et al., 1982; Robles et al., 1986), and the frequency to which it is tuned is systematically related to position along the cochlea. In the alligator lizard, the motion of the cochlear partition is neither sharply tuned nor tonotopically organized (Peake and Ling, 1980). It has been proposed that, instead, the acoustic tuning of hair cells in the high-frequency region of this organ depends primarily on mechanical resonances of the hair bundles (Weiss et al., 1978b).

The cochlea of the alligator lizard (Fig. 1a) is clearly divided into two regions with distinct morphological and physiological attributes. In the *tectorial region*, hair bundles are draped by a tectorial membrane, whereas in the *free-standing region* they contact endolymph alone. The cochlear nerve fibers from both regions have tuning curves whose quality (sharpness) and sensitivity (thresholds) are comparable to those of mammals (Weiss et al., 1976). The acoustic characteristic frequency (CF_a), the sound frequency to which an auditory cell is most sensitive, varies between 50 and 800 Hz in cochlear fibers receiving input from the tectorial region. In the free-standing region, CF_a progresses smoothly from ~ 1 kHz immediately adjacent to the tectorial region to ~ 4 kHz at the other end (Weiss et al., 1976; Holton and Weiss, 1983b). Correlated with the progression of CF_a is a gradual decrease in hair bundle height, from about 38 μm at the 1 kHz end to about 7 μm at the 4 kHz end (Mulroy, 1974; Mulroy and Williams, 1987). This correlation stimulated the development of a micromechanical model of acoustic tuning in the free-standing region (Weiss and Leong, 1985a; Freeman and Weiss, 1990a–d). In the model, the hair bundles resonate at frequencies between 1 and 4 kHz, and gradation in hair bundle height accounts for the graded change in CF_a with position along the free-standing region.

The model is supported by experiments on excised preparations of the alligator lizard cochlea, which showed tuned and tonotopically organized hair bundle motion in the free-standing region. Holton and Hudspeth (1983) measured the amplitude of acoustically driven hair bundle deflection and showed that (1) a 1 kHz stimulus deflects the tall hair bundles more than the short hair bundles, and (2) the tall hair bundles are deflected more by a 1 kHz stimulus than by a 4 kHz stimulus. The con-

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Figure 1. The cochlea of the alligator lizard. *A*, Scanning electron micrograph of the cochlea, viewed from scala vestibuli after removal of the vestibular membrane. The hair cells sit in a strip of tissue (oriented left to right in the micrograph) in a valley created by ridges of connective tissue. In the tectorial region (to the left) the hair bundles are covered by a delicate tectorial membrane that is attached to the connective tissue ridge. In the larger free-standing region the hair bundles have no overlying structure. *B*, Hair cell isolated from the tectorial region. *C*, Hair cell isolated from the free-standing region. Comparison of the height of this hair bundle with maps of variation in hair bundle height along the cochlear epithelium (Mulroy and Williams, 1987) suggests that the cell comes from the region between 50 and 150 μm from the basal end (at the right in *A*) of the papilla. A map of the variation in acoustic characteristic frequency (CF_a) of nerve fibers innervating the epithelium shows that CF_a values in this region are about 3 kHz (Holton and Weiss, 1983b). Scale bars: *A*, 100 μm ; *B* and *C*, 10 μm .

verse is true for a 4 kHz stimulus. Frishkopf and DeRosier (1983) measured the phase angle of motion between the tip and base of the hair bundle. They showed that the hair bundles resonate and that the resonant frequency changes with position along the free-standing region in a manner closely approximating the gradation in CF_a . The observed resonance was too broad to account for the quality of acoustic tuning of the receptor

potential at low sound pressure levels in free-standing hair cells (Holton and Weiss, 1983b). Possible explanations for the discrepancy include (1) the relatively high stimulus levels required to evoke detectable hair bundle displacements in the excised preparations, given that tuning of the receptor potential in free-standing hair cells is sharpest at low sound pressure levels (Holton and Weiss, 1983a,b); (2) damage to the excised preparations; and (3) the existence of additional tuning mechanisms *in vivo*. Therefore, it is not yet established whether mechanical resonances of the hair bundles together with broad tuning of the basilar membrane fully account for the tuning of the receptor potential in free-standing hair cells.

A potential source of tuning that had not previously been explored in lizard hair cells is the electrical properties of the basolateral membrane. Fettiplace and Crawford found that in the turtle cochlea, current steps injected into hair cells evoked oscillatory voltage responses with frequencies that matched the cells' CF_a values (Fettiplace and Crawford, 1978, 1980; Crawford and Fettiplace, 1981). They proposed that in the turtle cochlea a broadly tuned transduction current activates voltage-dependent conductances that confer sharp tuning upon the receptor potential. Sharp electrical resonance has also been demonstrated in hair cells from the saccules of frogs (Ashmore, 1983; Lewis and Hudspeth, 1983b), goldfish (Sugihara and Furukawa, 1989), and toadfish (Steinacker and Romero, 1992), from the amphibian papilla of frogs (Pitchford and Ashmore, 1987), and from the cochleas of chicks (Fuchs et al., 1988) and alligators (Fuchs and Evans, 1988). In each case, electrical resonance has occurred at frequencies roughly consistent with expected values of acoustic or vibration characteristic frequency. In addition, the temperature dependence of acoustic tuning in some nonmammals (Smolders and Klinke, 1977, 1984; Eatock and Manley, 1981; Schermuly and Klinke, 1985; van Dijk et al., 1990) has been put forth as indirect support for nonmechanical, possibly electrical, mechanisms of acoustic tuning.

Together, the observations of sharp electrical resonance have led to a view that such resonance constitutes an important tuning mechanism in the auditory and vibration-sensitive organs of nonmammalian vertebrates. The mechanical tuning of these organs, however, is poorly understood. Although electrical tuning can occur in the absence of mechanical tuning—that is, in isolated hair cells lacking hair bundles (Lewis and Hudspeth, 1983b)—it is possible that *in vivo*, electrical tuning operates in conjunction with sharp mechanical resonances of accessory structures or hair bundles, or even that the two kinds of resonance are coupled (Weiss, 1982; Crawford and Fettiplace, 1985). Moreover, given that electrical resonances occur in other electrically excitable cells such as the squid giant axon (Hodgkin and Huxley, 1952; Mauro et al., 1970; Fishman, 1975), it is possible that in some cases electrical resonances in hair cells are not closely related to their acoustic tuning.

We investigated whether the electrical properties of hair cells might contribute to acoustic tuning in the free-standing region of the alligator lizard's cochlea. We report here that many of the cells showed sharp electrical resonances comparable to those described in other hair cells, but at frequencies far below the acoustic characteristic frequencies of the cells *in vivo*. We also describe some basic features of the cells' major voltage-dependent currents, an inwardly rectifying K^+ current and a Ca^{2+} -dependent K^+ current.

Some of these data have been presented in preliminary form (Eatock and Saeki, 1991; Eatock et al., 1992).

Materials and Methods

Preparation of cells

Alligator lizards (*Gerrhonotus multicarinatus*, 8–40 gm) from southern California were anesthetized with sodium pentobarbital (Nembutal; 50–65 mg/kg, i.p.) and then decapitated. In the first series of experiments, removal of the cochleas was performed in chilled, oxygenated 3.3 Ca²⁺ HBSS: modified Hanks' balanced salt solution (Sigma), containing (in mM) 148 Na⁺, 5.4 K⁺, 0.8 Mg²⁺, 3.3 Ca²⁺, 156 Cl[−], and 5.6 D-glucose, and buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) to pH 7.3. The osmolality was estimated as ~300 mmol/kg, using a vapor pressure osmometer (model 5500, Wescor, Logan, UT). Enzymatic and mechanical dissociation of the cells was accomplished in 0.1 Ca²⁺ HBSS: similar to the 3.3 Ca²⁺ HBSS but with 1.3 mM Ca²⁺ (the standard concentration in HBSS) and 1.2 mM ethylene glycol-bis(β -aminoethylether) *N,N,N',N'*-tetraacetic acid (EGTA), producing a calculated Ca²⁺ concentration of 100 μ M (calculated using EQCAL software, Biosoft, Miltown, NJ). The hair cells were mechanically isolated following (1) two treatments in 0.1 Ca²⁺ HBSS containing 50 μ g/ml of Sigma's protease XXVII, (2) one treatment in 0.1 Ca²⁺ HBSS containing 26 μ g/ml of hyaluronidase (Sigma type V), (3) four to six treatments in 0.1 Ca²⁺ HBSS containing L-cysteine (2.5 mM) and crude papain (Sigma; 500 μ g/ml), and (4) one or two treatments of bovine serum albumen (500 μ g/ml). All enzyme treatments were for 10 min in 4 ml of solution, at 30°C. In later experiments, L-15 medium (Leibovitz' L-15, GIBCO) was substituted for HBSS in all of the above solutions, increasing the osmolality to ~330 mmol/kg. We found that the cells lasted longer in the L-15 medium than in HBSS. Examples of isolated hair cells are shown in Figure 1, *b* and *c*.

Cell identification

During recording, the isolated cells were viewed at 400 $\times$ with Hoffman modulation optics on an inverted microscope (Olympus IMT-2, Olympus Corporation, Lake Success, NY). In the most recent series of experiments, only the free-standing region was dissociated, so that all hair cells were known to be from that region. In earlier experiments, in which we dissociated the entire sensory epithelium, we were able to identify some hair cells as "free-standing" (from the free-standing region) by virtue of their distinctive hair bundles (compare Fig. 1*b,c*). Hair bundles from the free-standing region vary in height from about 7 to about 38 μ m, whereas those from the tectorial region are shorter than 10 μ m (Mulroy and Williams, 1987). Even when of comparable size (7–10 μ m), hair bundles from the two regions have a different appearance because the stereocilia of tectorial hair cells are of finer diameter than those of the free-standing hair cells (Mulroy and Williams, 1987). Those hair cells with partial or missing hair bundles, however, might have been from either the free-standing or the tectorial region; we refer to these simply as "cochlear." Even without hair bundles, hair cells were readily distinguished from supporting cells by their appearance: shape, cuticular plate, and optical properties. Hair cells with partial or no hair bundles often were healthy by other measures, including the stability and amplitude of currents and zero-current potentials in whole-cell mode and the appearance of the somata. Others have also reported that the electrical properties of the basolateral membranes of hair cells are not necessarily compromised by loss or damage to hair bundles (Lewis and Hudspeth, 1983b).

Recording

The isolated cells were studied using either the conventional breakthrough method (Hamill et al., 1981) or perforated-patch method (Horn and Marty, 1988) of tight-seal whole-cell recording. All experiments were done at temperatures between 23°C and 26°C. The control external solution was either 3.3 Ca²⁺ HBSS or 3.3 Ca²⁺ L-15. For experiments on Ca²⁺ currents, external solutions were designed with similar ionic components to L-15, but lacking any phosphates or sulfates. This was necessary because a precipitate formed in L-15 when we increased Ca²⁺ or used Ba²⁺. These solutions are defined in the Figure 11 and 12 captions. The external solution was oxygenated and continuously refreshed using a perfusion system.

The pipette solution used for conventional whole-cell recording was usually a KCl saline containing (in mM) 142, 152, or 162 K⁺; 2 Mg²⁺; 0.1 Ca²⁺; 120, 130, or 140 Cl[−]; 2 ATP; 8 or 11 EGTA; and 10 HEPES (pH 7.3; osmolality 260, 280, or 300 mmol/kg). For perforated-patch experiments, the pipette solution comprised, in mM, 145 K⁺, 2 Mg²⁺,

1 Ca²⁺, 72 Cl[−], 70 aspartate[−], 10 EGTA, 5 HEPES, and 50–75 μ g/ml nystatin (pH 7.3, 260 mmol/kg). To record Ca²⁺ channel currents, we used the conventional breakthrough method and an internal solution that contained Cs⁺ to block K⁺ currents and creatine phosphate and creatine phosphokinase to slow Ca²⁺ current rundown (Forscher and Oxford, 1985). This solution is defined in the Figure 11 caption.

The patch-clamp amplifier (L/M EPC7, Adams and List Associates, Ltd., Great Neck, NY) was used in either the voltage-clamp or current-clamp mode. Stimuli were generated and data were sampled with 12 bit digital/analog and analog/digital converters (Scientific Associates, Inc., Rochester, NY), controlled by data acquisition software (DATAQ, JPM Programming, Rochester, NY). Sampling intervals were as low as 10 μ sec, but usually 40 μ sec. Electrode resistances were usually between 5 and 7 M Ω . In voltage-clamp mode, membrane currents were low-pass filtered using the patch-clamp amplifier's internal filters (three-pole Bessel, with cutoff frequencies of 10 kHz or 3 kHz). A holding potential of −60 mV was used unless the cell's zero-current potential was more negative, in which case the holding potential was set to −65 or −70 mV. Capacity transients during 10 or 20 mV test steps were compensated using the amplifier's compensation circuitry. Cell capacitance and series resistance were usually taken to be the readings provided by the amplifier's compensation dials. We have found such readings to agree within 10% with estimates obtained by fitting monoexponential functions to capacity transients. Series resistance was usually compensated between 50% and 75%. After compensation, residual series resistance (R_s) varied from 1.3 to 50 M Ω , with a mean value of 13 ± 10.4 M Ω ($\pm$ standard deviation, SD; $n = 92$) and a median value of 11 M Ω . This median value together with the mean capacitance of the cells (10.4 ± 0.40 pF, $\pm$ standard error of the mean, SEM; $n = 83$) gives a clamp time constant of 114 μ sec. In steady-state current–voltage (I/V) relations, membrane voltages have been corrected for errors resulting from uncompensated series resistance and for liquid junction potentials that exceeded 2 mV. Leakage subtraction and averaging were generally not necessary, excepting those occasions on which we recorded the relatively small calcium channel currents.

Electrical resonance was investigated in current-clamp mode by applying current steps from zero holding current. Voltage errors resulting from uncompensated series resistance were less than 10 mV and have been corrected. Averaging was not performed. Filtering in current-clamp mode was examined empirically using our setup without any cell: a standard patch pipette filled with KCl internal solution (7 M Ω) and immersed in external medium (3.3 Ca²⁺ HBSS), and our standard data acquisition protocols (sampling interval, 40 μ sec). For a constant-amplitude swept-frequency sinusoidal input to the amplifier, the gain of the system was flat below 500 Hz, had a small (1 dB) resonant peak at about 2 kHz, a half-power (−3 dB) frequency of 3.7 kHz and a high-frequency slope of −40 dB per decade. This frequency response was adequate, given that the half-power frequency exceeds the acoustic characteristic frequencies of nearly all of the primary afferents innervating the free-standing region, as shown in Figure 5*B*.

Analysis

Electrical resonance. Electrical resonance was analyzed from voltage records to current steps. For voltage records with repeated oscillations, the frequency of the electrical resonance (f_e) was taken to be the reciprocal of the mean interval between successive peaks. The quality factor, Q_e , was obtained from Equation 1 after estimating τ , the time constant of decay of the voltage oscillations, by fitting an exponential function to the peaks of the voltage record (Hudspeth and Lewis, 1988b):

$$Q_e = \sqrt{(\pi f_e \tau)^2 + 0.25} \quad (1)$$

An example of this fitting procedure is shown in Figure 2*A*. This method (method 1) was used to analyze electrical resonance in 37 of 42 cells.

For the remaining five cells in the sample, the voltage records did not show multiple oscillations. In these cases, f_e and Q_e were estimated by fitting the voltage waveform with an equation for a resonant electrical circuit:

$$V(t) = IR \left[1 + \pi f_e \tau e^{-t/\tau} \sin \left(2\pi f_e t - \tan^{-1} \frac{1}{\pi f_e \tau} + \phi \right) \right] + IR' \quad (2)$$

(adapted from Crawford and Fettiplace, 1981, Eq. 14). I is the applied current step, t is time relative to the onset of the current step, $V(t)$ is membrane voltage at time t , ϕ is a parameter that accounts for the delay in the onset of voltage oscillations, and R' is a constant resistance needed

Figure 2. Methods used to estimate the electrical resonant frequency, f_e , and quality, Q_e , in a free-standing cell showing repeated voltage oscillations in response to a current step (high- Q_e response) (A), and a cochlear cell with a more damped (lower- Q_e) response (B). In A, f_e (172 Hz) is taken to be the reciprocal of the average interval between peaks, and Q_e (18.8) is estimated from the fit of an exponential function (thick line) to the time course of decay of the oscillations and from Equation 1. Current step, +260 pA. Steady-state voltage (V_{ss} , broken line), -19 mV. Cell C, 92.01.22. In B, f_e (31 Hz) and Q_e (0.9) are obtained from the fit of Equation 2 to the response waveform and from Equation 1. The fit is shown in as a thick line superimposed on the data (thin line). Current step, +125 pA. V_{ss} , -27 mV. Cell B, 91.05.03.

to match the steady-state membrane potential (the voltage to which the oscillations converge). Q_e was then calculated from the values of f_e and τ provided by the fit and Equation 1. Figure 2B illustrates this fitting procedure for one of the five cells (method 2). For both methods 1 and 2, curve fitting was done with SIGMAPLOT software (Jandel Scientific, Corte Madera, CA), which uses the Marquardt-Levenberg algorithm to minimize the sum of squares of differences between the dependent variable and the observations.

As an additional check, we used both methods to estimate f_e and Q_e in seven voltage records with multiple oscillations. The mean ratios of the parameters estimated by the two methods (method 2/method 1) were, for the f_e ratio, 0.978 ± 0.04 ($\pm$ SD), and for the Q_e ratio, 1.1 ± 0.56 ($\pm$ SD).

Boltzmann fits to conductance-voltage relations. For the inwardly rectifying current, we derived chord conductance-voltage (G/V) relations by dividing current by driving force, the difference between the membrane voltage and the reversal potential of the current. We derived the instantaneous conductance from the tail currents at the offset of voltage

Figure 3. Examples of electrical resonance in four free-standing cells. Voltage records are in response to current steps of different amplitude (not shown) that begin and end at the times indicated by the inverted triangles. (In the bottom traces in A–C, no current steps were applied.) Voltage records have been offset vertically with respect to each other for clarity. No holding current was applied. A, High- Q_e resonance, recorded by the conventional (breakthrough) method of whole-cell recording. Internal solution, KCl (11 mM EGTA, 280 mmol/kg); external solution, 3.3 Ca^{2+} L-15. Current steps, from bottom to top: 0, +80,

+140, and +320 pA. This cell had a zero-current potential (V_z) of -68 mV, close to the mean V_z recorded from free-standing hair cells. Resonance was first evident in response to a current step of +60 pA (not shown), and the highest-quality resonance occurred in response to the step at +140 pA. The resonant frequency increased with increasing current amplitude; it was 141 Hz during the +140 pA step. Cell C, 92.01.22. B, High- Q_e resonance, recorded with the perforated patch method. Internal solution, K-aspartate/KCl (75 $\mu\text{g}/\text{ml}$ nystatin); external solution, 3.3 Ca^{2+} HBSS. Current steps: 0, +75, +125, and +225 pA. V_{ss} , -54 mV. The minimum current required to evoke the resonance within the 35 msec current step was between +25 and +50 pA (not shown). The highest-quality resonance was observed in response to a +125 pA step (third record from the bottom), at which its frequency was 148 Hz. Cell A, 91.04.22. C, Resonance with a Q_e value near the median in our sample. Perforated patch method. Internal and external solutions were as in B, except that the nystatin concentration was 50 $\mu\text{g}/\text{ml}$. Current steps: 0, +75, +225, and +375 pA. V_{ss} , -65 mV. Resonance was first evident in response to the +75 pA current step. The quality (sharpness) of the resonance in response to the +225 pA step equals the median quality for the free-standing cells. Cell A, 91.04.09. D, Regenerative, spike-like behavior evoked by current steps (middle two records). Internal and external solutions as in A. V_{ss} , -65 mV. Current steps: +75, +120, +150, and +200 pA. Regenerative behavior was first apparent in the response to +135 pA (not shown), at a frequency of 16 Hz. For large current steps (top record), the oscillations in membrane potential become more sinusoidal. Cell C, 92.03.09.

Figure 4. Dependence of f_e (top panels) and Q_e (bottom panels) on V_{ss} (steady-state voltage, or the voltage to which the oscillations converge; see Fig. 2). A–D are the relations for the corresponding data in Figure 3. The solid symbols show the highest Q_e and the corresponding f_e , which is designated the characteristic electrical resonant frequency (CF_e). The solid lines in the upper panels are monoexponential fits to the data.

steps. Tail current amplitude at step offset was estimated by fitting a single exponential function to the tail current and extrapolating back to the time at which the step turned off. The G/V curves were fit with a Boltzmann equation:

$$G = \frac{G_{\max}}{1 + e^{(V - V_{1/2})/s}}, \quad (3)$$

where G is chord conductance, $G_{\max}$ is the maximum conductance, V is membrane potential, $V_{1/2}$ is the membrane potential corresponding to $G_{\max}/2$, and s is the voltage range corresponding to an e -fold increase in G . Again, SIGMAPLOT's curve fitter was used to fit the Boltzmann relation. Fits of monoexponential functions to tail currents or to activation or deactivation of inward rectifier currents were performed with software that uses the Simplex algorithm (JPM Programming, Rochester, NY).

Results are presented as mean $\pm$ SEM, unless otherwise noted.

Results

The data presented here are from 53 cells, 31 of which were known to be from the free-standing region; the remainder are identified simply as "cochlear" (see Cell identification in Materials and Methods). Electrical resonance and ionic currents were examined in 25 free-standing hair cells and 17 cochlear hair cells. Ionic currents alone were recorded from the remaining six free-standing hair cells and five cochlear cells. Because the free-standing region contains more hair cells (~ 100) than the tectorial region (~ 50) and dissociated much more readily with the protocol that we used, it is likely that most of the "cochlear" hair cells were from the free-standing region. Consistent with this view, the data from cochlear hair cells did not differ systematically from those known to be free-standing. We first describe electrical resonance observed in current-clamp mode, and then ionic currents obtained in voltage-clamp mode.

Electrical resonance

For all of the cells from which we obtained electrical resonance data, the mean zero-current potential (V_z , potential in the absence of injected current) was -66 ± 1.0 mV ($n = 42$; range, -45 to -77 mV). For those cells that were known to be free-standing, the mean zero-current potential was -67 ± 1.2 mV ($n = 25$; range, -54 to -77 mV).

Figure 3 shows current-clamp data from four free-standing hair cells. Electrical resonance was never observed in the absence

of a current stimulus (bottom traces in Fig. 3A–C), at the offset of current steps, or during hyperpolarizing current steps. Small depolarizing current steps also usually failed to evoke any electrical resonance, as is illustrated by the voltage response to a $+75$ pA step in the bottom record in Figure 3D. However, depolarizing current steps above a given amplitude evoked repeated voltage oscillations in most cells (Fig. 3A–D, top three records in each panel). The minimal current that evoked the resonance ranged from 40 to 200 pA, with a mean of 112 ± 7.2 pA for all cells ($n = 42$), and 98 ± 7.5 pA ($n = 25$) for free-standing cells.

The data in Figure 3, A and D, were obtained with the conventional breakthrough method of whole-cell recording, using a KCl internal solution. The data in Figure 3, B and C, were obtained with the perforated-patch method, which is considered to interfere less with intracellular function than does the conventional breakthrough method (Korn and Horn, 1989; Lucero and Pappone, 1990). We used the perforated patch method to examine electrical resonance in a sample of four cells. The properties of the electrical resonance and ionic currents measured in this sample were representative of the entire population (see Fig. 14). This suggests that such properties were not disturbed by any changes in divalent ion or other second messenger concentrations that may have ensued during recordings with the conventional breakthrough method.

In the cell in Figure 3D and in one other free-standing cell (data not shown), moderate depolarizing current steps evoked relatively low-frequency, repetitive activity that was spike-like in waveform (note the prepeak shoulder and the postpeak trough) and that did not show appreciable damping. As more current was injected the spikes increased in frequency, eventually (top record in Fig. 3D) becoming quasisinusoidal and showing damping.

The resonant frequency (f_e) and the quality (Q_e) of the oscillations were measured from records such as those in Figure 3: f_e from the period of the oscillations and Q_e from the decay of the oscillations, as described in Materials and Methods. Q_e is a conventional measure of the sharpness of tuning in resonant electrical circuits. Large values of Q_e , which correspond to long decay times for the oscillations, imply sharp tuning. As illustrated qualitatively in Figure 3 and graphically in Figure 4, both

Figure 5. Distributions of electrical and acoustic characteristic frequency in the cochlea of the alligator lizard. Bins are from 0 to 99 Hz, 100 to 199 Hz, etc. *A*, The CF_e distribution from our sample of isolated cochlear hair cells. The outline of the distribution shows all CF_e values; the subset of identified free-standing hair cells is shown as solid bars. *B*, The CF_a distribution from an *in vivo* study (Weiss et al., 1976) in the alligator lizard of the tuning of 219 cochlear afferent fibers to pure-tone sound stimuli. A bimodal distribution is seen; mapping studies have shown that the fibers with CF_a values below 800 Hz (hatched bars) innervate the tectorial region, while those with higher CF_a values (solid bars) innervate the free-standing region (Weiss et al., 1976). The tuning of hair cells in the free-standing region has been shown to correspond closely to that of afferent fibers in the same region (Holton and Weiss, 1983b). The data in *A* and *B* were collected at about the same temperatures (22–26°C).

Q_e and f_e varied with steady-state membrane voltage (V_{ss}). The data in Figure 4 are from the cells of Figure 3. In Figure 4*A–C*, as reported in turtle cochlear and bullfrog saccular hair cells (Art et al., 1984; Hudspeth and Lewis, 1988b), f_e was an exponential function of V_{ss} , and Q_e peaked at a voltage several millivolts positive to the most negative V_{ss} at which oscillations were observed.

For the cell of Figures 3*D* and 4*D*, f_e was an approximately linear function of V_{ss} over the range examined. The Q_e/V_{ss} plot also differed for this cell and the other “spiking” cell (data not shown), in that peak Q_e values were extremely high and occurred at the lowest V_{ss} at which any spiking was seen (Fig. 4*D*). Such differences suggest that the mechanisms underlying the regenerative behavior in these two low-frequency cells may differ from the resonant mechanisms in other cells by more than just speed.

Following the example of Crawford and Fettiplace (1981), we have defined a cell’s characteristic electrical resonant frequency, CF_e, to be that f_e value corresponding to the peak value of Q_e . Peak Q_e values varied from 0.9 to 480 (median 5.8) for all cells. If the two “spiking” cells (Fig. 3*D*) are excluded, the highest Q_e was 94. CF_e varied from 16 to 230 Hz (mean $\pm$ SD = 108 $\pm$ 59.1; n = 42). CF_e and Q_e values for hair cells known to be from the free-standing region did not differ significantly from those

for the entire sample. CF_e ranged from 16 to 175 Hz (88 $\pm$ 49.8, mean $\pm$ SD; n = 25) and Q_e ranged from 1.7 to 480 (median 6.3); when the spiking cells are excluded, the highest Q_e value was 66.

The CF_e range in the alligator lizard’s hair cells is similar to previously reported ranges of CF_e for hair cells from other organs (see Table 1, below), but is well below the CF_a range (1–4 kHz) of the free-standing region. Figure 5 contrasts the distribution of CF_e values in our sample with the distribution of CF_a values in a large sample of nerve fibers innervating the alligator lizard’s cochlea, from a study by Weiss et al. (1976). Fourier transformation of the oscillatory voltage responses from isolated free-standing hair cells revealed no significant power above 500 Hz. As described in Materials and Methods, we determined that this was not a consequence of low-pass filtering by the recording system, which had a half-power frequency above the CF_a values of >99% of primary afferents innervating this cochlea (Fig. 5*B*).

Ionic currents

With standard internal and external solutions, voltage steps to potentials more negative than –80 mV evoked an inward current that increased with hyperpolarization. This current was found to be an inwardly rectifying current (I_{IR}). Steps to potentials more positive than –40 mV evoked an outwardly rectifying current (I_o). The examples in Figure 6 show the range of currents expressed by these cells. I_{IR} was generally large, as in Figure 6, *B* and *C*, and I_o varied from large (>5 nA; Fig. 6*A*) to small (<1 nA; Fig. 6*C*) in an approximately continuous manner (see Fig. 14). The steady-state amplitude of I_o in Figure 6*B* approximates the mean steady-state amplitude of I_o in the free-standing cells. I_{IR} and I_o will be considered in turn.

Inwardly rectifying current

Together, the following observations, described more fully in subsequent paragraphs, suggested that an inwardly rectifying current was on at the cells’ zero-current potentials. Voltage steps from the holding potential (between –60 and –70 mV) to potentials below –80 mV evoked instantaneous inward currents that further activated with a monoexponential time course that was voltage dependent. The mean steady-state current at –90 mV was –1.2 $\pm$ 0.16 nA (n = 36; range, 0–5 nA). The offset of such hyperpolarizing voltage steps evoked outward tail currents, which declined with a monoexponential time course. Depolarizing voltage steps from the holding potential also evoked instantaneous currents, which in this case were outward and subsequently deactivated with a monoexponential time course. The maximum steady-state amplitudes (about 100 pA) of these outward currents occurred between –60 and –50 mV (mean –54 $\pm$ 1.0 mV, n = 11). [Above –40 mV, an outwardly rectifying current (I_o) also was observed, but the two currents were well separated in time, with the instantaneous outward current significantly deactivated before the outwardly rectifying current became appreciable.] Of 43 cells, only one lacked discernible I_{IR} .

Dependence of inward rectification on time. Both activation and deactivation of I_{IR} following steps to new potentials had time courses that appeared, from visual inspection, to be well fit by monoexponential functions with voltage-dependent time constants (Fig. 7*A*). In four cells tested, the time constants showed a bell-shaped dependence on voltage, peaking between –80 and –85 mV (i.e., near the half-maximal voltage, $V_{1/2}$; see below) at

Figure 6. Exemplar whole-cell currents (*top panels*) and corresponding steady-state I/V relations (*bottom panels*) from three free-standing hair cells. The current, time, and voltage axes in *A* apply to all panels. Superimposed in the *top panels* are the currents evoked by a series of voltage steps (not shown) between about -120 mV and $+90$ mV. Steady-state I/V relations show the currents near the ends of the iterated voltage steps. External solution, 3.3 Ca^{2+} L-15. In this and all subsequent figures, whole-cell voltage clamp was achieved by conventional breakthrough, not perforated patch, and no averaging or leak subtraction was performed unless otherwise stated. *A*, Holding potential (V_h), -60 mV; V_z , -54 to -73 mV; residual series resistance after compensation (R_s), $5.3 \text{ M}\Omega$. Internal solution, KCl (260 mmol/kg , 8 mM EGTA). Cell A, 91.12.12. *B*, V_h , -70 mV; V_z , -74 mV; R_s , $11 \text{ M}\Omega$. Internal solution, KCl (280 mmol/kg , 11 mM EGTA). Cell G, 92.02.06. *C*, V_h , -70 mV; V_z , -70 mV; R_s , $7.7 \text{ M}\Omega$. Internal solution, KCl (300 mmol/kg , 11 mM EGTA). In *C*, the iterated voltage step was terminated by a step to -50 mV, not back to the holding potential of -70 mV. Hence, the outward tail currents following the iterated voltage steps were larger than those in *A* and *B*. Cell K, 92.04.06.

5–10 msec (data not shown). The time-dependent increase in the rectification of I_{IR} can be appreciated by comparing a cell's steady-state current-voltage (I/V) relation with its onset I/V relation, obtained by fitting an exponential function to the current during the step and extrapolating to obtain its value at the onset of the step (Fig. 7*A,B*). I_{IR} decayed with time during large hyperpolarizing steps, perhaps as a result of block by Na^+ , as observed in inwardly rectifying currents from other cell types (Ohmori, 1978; Silver and DeCoursey, 1990). This time-dependent block caused the curvature of the steady-state I/V relation below -120 mV in Figure 7*B*.

K^+ selectivity. Both onset and steady-state relations in Figure 7*B* reverse near the estimated equilibrium potential for K^+ (-81.7 mV), far from that for Cl^- (-7 mV). I_{IR} was blocked by external Ba^{2+} (4 mM) (see Fig. 11) or by external Cs^+ (5 mM) (data not shown), providing further evidence that the channels are K^+ selective.

Dependence of the inwardly rectifying conductance on voltage. In Figure 8, the instantaneous chord conductance (G) is plotted as a function of voltage over the range of activation of I_{IR} . Figures 7 and 8 show that there was little overlap in the voltage ranges of activation of I_{IR} and I_o . This allowed us to fit a Boltzmann equation (Eq. 3, Materials and Methods) to conductance-voltage curves such as that in Figure 8. From the conductance-voltage relations of five free-standing cells, the mean s (voltage corresponding to an e -fold change in G) was 14 ± 0.7 mV, the mean maximum instantaneous conductance, G_{max} , was 32 ± 6.3 nS, and the voltage corresponding to half-maximal conductance, $V_{1/2}$, varied from -79.6 to -88.1 mV, with a mean of -84 ± 1.5 mV.

Outwardly rectifying current

Ca^{2+} dependence. In most cells, depolarizing steps from a holding potential of -60 to -65 mV activated prominent outward currents with a distinctive voltage dependence (Fig. 6*A,B*). The currents became appreciable above about -40 mV and increased with further depolarization up to between 0 and $+50$ mV, above which they decreased. This N-shaped I/V relation is considered a hallmark of Ca^{2+} -dependent currents (Meech, 1978). The decline in the Ca^{2+} -dependent current at high positive potentials reflects the decreased entry of Ca^{2+} into the cell as the driving force diminishes. We found that for the lizard's hair cells also, the N-shape of the I/V relation resulted from the Ca^{2+} dependence of a large fraction of I_o . In the example shown in Figure 9, reduction of external Ca^{2+} from 3.3 mM (Fig. 9*A*) to $100 \mu\text{M}$ (Fig. 9*B*) nearly eliminated I_o but had no effect on I_{IR} . The effect on I_o was completely reversible (Fig. 9*C*) and was repeated several times with the same cell. Figure 9*D* shows the difference current obtained by subtracting the data in $100 \mu\text{M}$ Ca^{2+} from the data in 3.3 mM Ca^{2+} . In Figure 9*E*, the I/V relation of the difference current is compared with that in 3.3 mM Ca^{2+} . The comparison shows that between -30 and $+50$ mV, most of the voltage-dependent current was Ca^{2+} dependent, and outside that voltage range, almost none of the current was affected by the removal of most of the external Ca^{2+} . The small outward current between -30 and -80 mV was I_{IR} .

K^+ selectivity. At holding potentials of -60 to -65 mV, the offset of depolarizing voltage steps evoked outward tail currents, suggesting that I_o was selective for K^+ over other ions. Its susceptibility to K channel blockers provided further evidence for

Figure 7. Time dependence of inward rectification. *A*, Monoexponential fits (solid lines) of the activation and deactivation of I_{ir} following voltage steps from a holding potential of -80 mV. Open circles are data points; every third point is shown. The command potentials and the corresponding τ values of the monoexponential fits were -150 mV, 719 μ sec; -130 mV, 1.67 msec; -110 mV, 3.14 msec; -90 mV, 5.42 msec; and -30 mV, 336 μ sec. V_{ss} , -79 mV; R_s , 9.5 M Ω ; cell capacitance, 7.5 pF; clamp time constant ($R_s C_m$), 71 μ sec. Internal solution, KCl (280 mmol/kg, 11 mM EGTA); external solution, 3.3 Ca $^{2+}$ L-15. Free-standing cell B, 92.03.09. *B*, Comparison of onset (∇) and steady-state ($\bullet$) I - V relations for the data in *A*. The onset current values were obtained by extrapolating the monoexponential fits to the onset of the voltage step. Steady-state current values were obtained between 14 and 15 msec after the onset of the 15 msec step.

its K $^{+}$ selectivity. Replacement of K $^{+}$ with Cs $^{+}$ in the internal solution blocked most of the outward current; the residual, which was probably Cs $^{+}$ current through K $^{+}$ channels (Hille, 1992), was blocked by replacement of external Ca $^{2+}$ with Ba $^{2+}$ or by inclusion of 30 mM tetraethylammonium (TEA) in the external medium (see Figs. 11, 12). We will refer to the Ca $^{2+}$ -dependent component of I_o as $I_{K(Ca)}$.

Other properties of the outward current. The voltage at which I_o became appreciable was estimated by scanning steady-state I/V relations. The estimated values ranged from -28 to -47 mV, with a mean of -37 ± 1.0 mV ($n = 36$).

The maximum steady-state I_o (peak in the I/V relation) had a mean value of 3.4 ± 2.81 nA ($\pm$ SD, $n = 52$), ranging from 600 pA to 9.5 nA. Such variability might exist *in vivo* or arise as a result of the isolation or recording procedures. Perforated-patch experiments showed that peak I_o was variable even when the cells controlled their own Ca $^{2+}$ levels. In five cells for which

Figure 8. Voltage dependence of inwardly rectifying conductance. $\bullet$, Instantaneous chord conductance as a function of prepulse voltage, calculated by dividing the driving force into the peak tail current at -30 mV following 8 msec prepulses to a range of potentials between -130 and -15 mV. Driving force was estimated by subtracting the reversal potential of the steady-state current (-76.5 mV in this case) from the membrane voltage. The peak tail current was estimated by extrapolating a monoexponential fit of the tail current back to the offset of the prepulse. The data have been fit with a Boltzmann equation (Eq. 3), with $G_{max} = 38$ nS, $V_{1/2} = -86.2$ mV, and $s = 15.1$ mV. Internal solution, KCl (300 mmol/kg, 11 mM EGTA); external solution, 3.3 Ca $^{2+}$ L-15. V_{ss} , -70 mV; $V_{1/2}$, -76.5 mV; R_s , 7.7 M Ω . Free-standing cell K, 92.04.06.

ionic current data were recorded in perforated-patch mode, peak I_o was 0.06 , 0.45 , 3.3 , 7.3 , and 9 nA. Even with conventional recording, some cells with small I_o had stable electrical properties, low leak, and healthy appearances for up to an hour after the onset of recording, arguing that the small I_o was not a consequence of cellular deterioration. Rundown of the Ca $^{2+}$ -dependent component generally occurred slowly (over tens of minutes) if at all, possibly because the internal solutions contained ATP (Forscher and Oxford, 1985) and GTP. In several cells, $I_{K(Ca)}$ increased substantially (e.g., more than twofold) in the first 10 min following breakthrough.

Effects of series resistance. The apparent voltage at which I_o peaked ranged from 0 to 50 mV, with a mean value of 17 ± 2.1 mV ($n = 49$). The more positive voltages in this range may reflect inadequate estimation of series resistance, which would render the true membrane potential less positive than the intended voltage and would maximally affect the estimation of voltages corresponding to peak currents. Series resistance also may have distorted the time course of I_o in those cells with large $I_{K(Ca)}$ (Fig. 6A). At large positive potentials, activation of I_o was increasingly delayed until a potential was reached at which it did not activate at all within the time window of the voltage step (up to 50 msec). Such delayed or slow activation in response to large depolarizations has previously been reported for Ca $^{2+}$ -dependent currents (Lux, 1980; Lux and Hofmeier, 1982). This may occur when series resistance is comparable to the cell's slope resistance, such that the cell is not voltage clamped (Marty and Neher, 1985). In lizard cochlear cells with large outward currents, slope resistances measured at high positive potentials from steady-state I/V relations were typically between 5 and 10

Figure 9. Ca^{2+} dependence of the outwardly rectifying current. External solution, L-15 with either 3.3 mM Ca^{2+} (A, C) or 100 μM Ca^{2+} (B). Records in A–C were recorded consecutively, about 2 min apart. Solution changes were made with a local solution changer, in which different lines were fed into a pulled glass pipette (50 μm tip diameter) positioned within 100 μm of the cell. The records in D were made by subtracting the records in B from those of C. E, Isochronal I/V relations, showing current values between 40 and 45 msec after the start of the step, for the control currents in C and the difference currents in D. Internal solution, KCl (260 mmol/kg, 8 mM EGTA). V_h , –60 mV; V_z , –75 mV; R_s , 11 M Ω . Cochlear cell B, 91.12.12.

M Ω , while the residual series resistance after any compensation ranged from 1.3 to 29 M Ω .

Effects of prepulses and holding potential. I_o inactivated little if at all ($\leq 10\%$) during 50 msec depolarizing voltage steps from holding potentials between –60 and –70 mV. Thus, for holding potentials corresponding to the mean zero-current potential, there was little indication of a fast-inactivating component, or A-current, in I_o .

In several free-standing hair cells, we examined the effects of 100 msec prepulses to different potentials on the outward current at +30 or +35 mV. Figure 10 illustrates the results for two cells with peak outward currents of about 3 nA. As prepulses were made increasingly positive between –115 and 0 mV, the steady-state current at +35 mV fell 22%. Two-thirds of the drop occurred below –70 mV, that is, in a voltage range negative to the mean zero-current potential and the presumed physiological range. [Because the free-standing cells do not receive efferent innervation (Weiss et al., 1978b), large inhibitory postsynaptic potentials such as those seen in turtle cochlear hair cells (Art et al., 1984) will not occur. Small hyperpolarizations may occur in response to the phases of acoustic stimuli that close transduction channels, but these hyperpolarizations will be attenuated at the stimulus frequencies to which the cells are most sensitive (≥ 900 Hz) as a result of low-pass filtering by the hair cell membrane (Holton and Weiss, 1983a).]

The effects of more tonic changes in membrane potential were examined by testing the sensitivity of I_o to holding potential (held for > 1 min) in two free-standing cells. The currents evoked by stepping to voltages between –60 and +60 mV were recorded while holding potential was varied. Over the range of voltages likely to include the cells' natural resting potentials (–70 to –40 mV), there was no consistent effect of holding potential on voltage-dependent outward current.

In summary, these experiments show that voltage-dependent inactivation is minor within the likely range of resting potentials for these cells. This suggests that any differences between *in vitro*

and *in vivo* resting potentials would not have a major impact on the cells' voltage-dependent currents.

Calcium channel current

Block of all K $^{+}$ currents with internal Cs $^{+}$ (83 mM) and external Ba $^{2+}$ (4 mM) revealed a small inward current that was activated by depolarization above –50 mV and peaked at about –10 mV (Fig. 11). This current did not inactivate rapidly (not shown), suggesting that it was not through voltage-dependent Na $^{+}$ channels and therefore was likely to be Ba $^{2+}$ current through voltage-dependent Ca $^{2+}$ channels. To examine whether this putative Ca $^{2+}$ -channel current inactivates, we measured it with Ca $^{2+}$ as the charge carrier, because Ba $^{2+}$ does not support the Ca $^{2+}$ -dependent inactivation that is characteristic of some Ca $^{2+}$ cur-

Figure 10. Effects of 100 msec prepulses to different voltages on steady-state outward current at +35 mV, in two free-standing cells. Currents were normalized by dividing by the maximum. Δ , cell C, 92.03.30; V_z , –72 mV; V_h , –65 mV; R_s , 10 M Ω . $\bullet$, cell D, 92.03.30; V_z , –65 mV; V_h , –65 mV; R_s , 10 M Ω . Internal solution, KCl (300 mmol/kg, 11 mM EGTA); external solution, 3.3 Ca^{2+} L-15.

Figure 11. Inward current revealed by block of all K⁺ currents with time following breakthrough. Internal solution (in mM): 83 Cs⁺, 5 Mg²⁺, 1 Ca²⁺, 0.12 Li⁺, 24 Cl⁻, 24 F⁻, 96 D-glucose, 20 di-Tris creatine phosphate (Sigma; Tris is Tris[hydroxymethyl]aminomethane), 10 EGTA, 10 HEPES, plus 50 U/ml creatine phosphokinase (pH 7.3, 290 mmol/kg). External solution (in mM): 163.5 Na⁺, 5.8 K⁺, 4.0 divalent cations, 176 Cl⁻, 5.6 D-glucose, 5 HEPES (pH 7.3, 330 mmol/kg). The external divalent composition was initially 3.3 Ca²⁺/0.8 Mg²⁺ (●), and subsequently 4 Ba²⁺ (▽). *A*, Steady-state *I/V* relations (●) within 1 min of breakthrough, with Ca²⁺ and Mg²⁺ as the external divalent cations and 5 min later (▽), with Ba²⁺ as the sole external divalent cation. *B*, The same *I/V* relations as in *A*, with expanded scales. The change to Ba²⁺ externally and the dialysis of Cs⁺ internally blocked *I_{IR}* and *I_{K(Ca)}*, revealing an inward current that was likely to be Ba²⁺ current through Ca²⁺ channels. *V_h*, -60 mV; *R_s*: 14 MΩ (●), 43 MΩ (▽). Free-standing hair cell B, 92.01.17.

rents (e.g., Fenwick et al., 1982). Figure 12 shows an example in which external Ca²⁺ was raised to 20 mM to enhance *I_{Ca}* and K⁺ currents were blocked by internal and external Cs⁺ and external TEA. Depolarizing voltage steps activated a small inward current that did not inactivate within 50 msec. It reversed at about +25 mV, far below the expected reversal potential for Ca²⁺ (+190 mV). This may be a consequence of Cs⁺ efflux through Ca²⁺ channels (Fenwick et al., 1982; Lee and Tsien, 1982).

Dependence of resonance on external Ca²⁺

Reducing external Ca²⁺ reduced the amplitude of *I_{K(Ca)}* in parallel with the quality of the resonance. Figure 13 illustrates an example in which external Ca²⁺ was reduced from 3.3 mM to 500

Figure 12. Noninactivating inward current activated by depolarization, likely to be Ca²⁺ current. *A*, Current records. Linear currents ("leak") were subtracted with a *P*, -*P*/4 protocol (Armstrong and Bezanilla, 1974). Average of 10 records. *B*, Steady-state *I/V* relation for the data in *A*. Internal solution was as in Figure 12; external solution (in mM): 105 Na⁺, 20 Ca²⁺, 5.8 K⁺, 5 Cs⁺, 0.8 Mg²⁺, 162 Cl⁻, 30 TEA, 5.6 D-glucose, 5 HEPES (pH 7.3, 330 mmol/kg). *V_h*, -60 mV; *R_s*, 20 MΩ. Free-standing cell D, 92.04.01.

μM, reducing peak *I_o* from 5.9 to 2.3 nA and the peak *Q_e* value from 66 to 6. (In this cell, reducing external Ca²⁺ to 100 μM eliminated >90% of *I_o*; no current-clamp data were obtained at 100 μM Ca²⁺.) In another free-standing cell, reduction of external Ca²⁺ from 3.3 to 1.3 mM reduced the peak *I_o* from 2.4 to 1.75 nA and *Q_e* from 6.3 to 3.7. In other hair cells that show sharp electrical resonance, that resonance has been shown to depend on *I_{Ca}* and *I_{K(Ca)}* (Lewis and Hudspeth, 1983b; Art and Fettiplace, 1987; Hudspeth and Lewis, 1988a,b; Fuchs and Evans, 1990). Reducing external Ca²⁺ affects both, so our experiments do not distinguish the separate contributions of the two currents to the resonance.

Does *Q_e* vary with the amplitude of *I_{K(Ca)}* across cells? Although we do not have direct measures of *I_{K(Ca)}* in most cells, experiments in low Ca²⁺ have shown that most of *I_o* at the peak of N-shaped *I/V* relations is Ca²⁺ dependent (Fig. 9). If one excludes the two low-frequency cells with exceptionally high *Q_e* values, *Q_e* is correlated with the peak amplitude of *I_o* (Fig. 14A). This is also the case for CF_e (Fig. 14B). The plots of Figure 14 are useful mostly in that they show that (1) the spiking cells fall outside the trends displayed by the other cells, (2) free-standing and cochlear cells show similar trends, and (3) data from the

Figure 13. Parallel effects of reduced external Ca^{2+} on the outwardly rectifying current and on the quality of the electrical resonance. External solution, L-15 with 3.3 mM Ca^{2+} (A, B) or 0.5 mM Ca^{2+} (C, D). A and C, Current-clamp records of membrane voltage in response to steps to -20 , $+60$, $+140$, $+220$, and $+300$ pA, from zero holding current. B and D, Voltage-clamp records of membrane current in response to steps from a holding potential of -70 mV to between -120 and $+60$ mV, in 20 mV increments. The greater noise in D is a consequence of reduced temporal resolution (125 μsec vs 40 μsec in B). In the current responses to voltage steps to -60 or above, the early outward transients are through inwardly rectifying channels that were open at the holding potential and that deactivated at the more positive potentials. Internal solution, KCl (280 mmol/kg, 11 mM EGTA). R_{in} , 7.2 M Ω . Free-standing cell C, 92.01.22.

four cells recorded with the perforated-patch method (solid symbols) fall within the overall trends and cover most of the range, suggesting that any changes in internal second messengers and steady-state Ca^{2+} concentrations that occurred during conventional breakthrough recordings did not greatly disturb the resonant behavior.

Discussion

Ionic currents

Inward rectifier. An inwardly rectifying K-selective current was the dominant voltage-dependent current at potentials more negative than -40 mV. As in inwardly rectifying currents from some other cell types (Hagiwara et al., 1976; Ohmori, 1978; Kurachi, 1985; Silver and DeCoursey, 1990), (1) rectification seen with voltage steps away from the zero-current potential had both instantaneous and slow components, (2) the slow component developed with first-order kinetics that were voltage dependent, and (3) the current was maximally activated at potentials 40 mV below the equilibrium potential for K^+ (see Fig. 8). The steepness (s) of the voltage dependence of activation of the current was less than that of some other K^+ -selective inward rectifiers (Hagiwara et al., 1976; Silver and DeCoursey, 1990).

The large I_{IR} may contribute to the relatively negative zero-current potentials of many of the lizard's hair cells. Inward rectifiers are correlated with relatively negative resting potentials in other hair cells. In the chick cochlea (Fuchs et al., 1988), apical hair cells were found to have a large I_{IR} and a mean zero-current potential of -78 mV. Basal hair cells, with smaller I_{IR} , had a mean zero-current potential of -61 mV. A similar correlation appears to hold in the alligator cochlea (Fuchs and Evans, 1988). In the goldfish saccule, the hair cells with the most negative resting potentials had the largest I_{IR} (Sugihara and Furukawa, 1989). Hair cells isolated from the bullfrog saccule have on average smaller I_{IR} than do the lizard's free-standing cells (J. R. Holt and R. A. Eatock, unpublished observations), and also have more positive zero-current potentials, between -50 and -60 mV (Hudspeth and Lewis, 1988a).

Ca^{2+} -dependent K^+ current. The N-shaped I/V relations of most cells together with experiments such as that illustrated in

Figure 9 suggest that, in most cells, much of I_o was Ca^{2+} dependent. $I_{\text{K(Ca)}}$ was noisy relative to I_{IR} (see Fig. 9), suggesting that it may be carried by large-conductance ("maxi") channels, as described in other hair cells (Fuchs and Evans, 1988, 1990;

Figure 14. Dependence of Q_e (A) and CF_e (B) on the peak amplitude of the outwardly rectifying current. A is a semilogarithmic plot. Squares, free-standing cells; triangles, cochlear cells. Open symbols, recordings made with the conventional breakthrough method of whole-cell recording; solid symbols, recordings made with the perforated-patch method. X, the two spiking cells, both free-standing and recorded with the conventional breakthrough method. Straight lines, linear regressions through the points, excluding the values from spiking cells. The regression slopes, y-intercepts, and correlation coefficients are, for A, 0.15 nA^{-1} , 0.33, and 0.73; and for B, 16.7 Hz/nA, 56.2 Hz, and 0.74.

Fuchs et al., 1988; Hudspeth and Lewis, 1988a; Steinacker and Romero, 1991). The activation voltage for $I_{K(Ca)}$ (-28 to -47 mV) was more positive than in other hair cells, where it ranges from -60 to -50 mV.

It has been argued that the remarkable sharpness of the electrical tuning of some hair cells (Q_e values of ≥ 5) arises from the delayed kinetics and steep apparent voltage dependence of activation that Ca^{2+} dependence confers upon their major outward K^+ current ($I_{K(Ca)}$) (Ashmore and Attwell, 1985). This proposal receives support from comparative observations. In hair cells that exhibit sharp electrical resonance, outward currents are dominated by $I_{K(Ca)}$ (Lewis and Hudspeth, 1983b; Art and Fettiplace, 1987; Fuchs and Evans, 1988; Fuchs et al., 1988), whereas in hair cells with heavily damped electrical resonance, a substantial fraction of the outward current is through voltage-dependent K^+ channels, such as delayed-rectifier and A-current channels (Correia et al., 1989; Lang and Correia, 1989; Kros and Crawford, 1990; Rennie and Ashmore, 1991; Eatock and Hutzler, 1992).

Our results fit qualitatively with this picture. Reducing external Ca^{2+} reduced both $I_{K(Ca)}$ and the quality of the resonance. Both the frequency and the quality of the resonance were correlated with the peak amplitude of the outward current, which reflected in large part the amplitude of $I_{K(Ca)}$. The voltage dependence of $I_{K(Ca)}$, which presumably depends heavily on that of I_{Ca} , is likely to be responsible for the lack of resonance at potentials more negative than -40 mV.

Ca^{2+} current. Other studies have shown that $I_{K(Ca)}$ in hair cells is activated by voltage-dependent Ca^{2+} current (I_{Ca}) (Lewis and Hudspeth, 1983b; Art and Fettiplace, 1987; Fuchs et al., 1988; Hudspeth and Lewis, 1988a; Roberts et al., 1990). The same current may mediate synaptic transmission, as at other chemical synapses. We observed small Ca^{2+} currents that activated rapidly and did not inactivate within 50 msec, consistent with most other reports of I_{Ca} in hair cells (Lewis and Hudspeth, 1983a; Ohmori, 1984; Art and Fettiplace, 1987; Fuchs and Evans, 1988; Fuchs et al., 1988, 1990; Sugihara and Furukawa, 1989; Steinacker and Romero, 1991).

The small size of the I_{Ca} that we observed in the isolated free-standing hair cells may be a factor in determining the relatively positive activation voltage of $I_{K(Ca)}$ in these cells. In frog saccular hair cells (Lewis and Hudspeth, 1983a) and turtle cochlear hair cells (Art and Fettiplace, 1987), I_{Ca} is larger and $I_{K(Ca)}$ activates at more negative voltages than in the lizard's cells. If the I_{Ca} of free-standing cells is larger *in vivo* than in the isolated cells, then smaller inward transduction currents might evoke the electrical resonance. As discussed in subsequent sections, however, *in vivo* evidence from the alligator lizard's free-standing region argues against electrical resonance at rest or in response to low sound pressure levels.

Spiking cells

Several observations create the impression that the two free-standing cells that showed low-frequency, undamped, spike-like oscillations differed qualitatively from the rest of the sample. Their Q_e values were exceptionally high and, when plotted against I_o , were far removed from the trend for other Q_e values (Fig. 14A). The zero-current potentials of both cells were about -75 mV, significantly more negative than average. The outwardly rectifying portions of their steady-state I/V relations were not N-shaped, suggesting that any Ca^{2+} -dependent component of I_o was less prominent than it was in hair cells that showed quasi-

sinusoidal voltage oscillations. These two cells may belong to a class of hair cells that is widespread; spiking cells have been described in the cochleas of alligators (Fuchs and Evans, 1988) and chicks (Fuchs et al., 1988), and in the saccules of goldfish (Sugihara and Furukawa, 1989) and toadfish (Steinacker and Romero, 1992). Spiking cells originate in the apical (low-frequency) parts of the chick and alligator cochleas and in the caudal part of the goldfish saccule, but are not regionally segregated in the toadfish saccule. We have not attempted to localize cells within the small free-standing region of the lizard cochlea.

The physiological role of the spikes is not known. Fuchs and Evans (1988) suggested that such spiking might serve to enhance the speed of transmission of low-frequency signals, presumably by releasing a large, fast bolus of transmitter with each spike. The tradeoff would be, as with electrical resonance, poor reproduction of the temporal properties of the stimulus.

Electrical resonance

Table 1 compares the ranges of CF_e and Q_e in this study to those reported for other preparations in which sharp electrical resonance occurs. These preparations include auditory and seismic organs of amphibians, reptiles, and birds; hair cells from the mammalian cochlea show only very low-quality electrical resonance if any at all (Kros and Crawford, 1990; Housley and Ashmore, 1992). All of the hair cell data have been obtained at room temperature (between 20°C and 26°C in the various studies). Table 1 also provides, where available, the range of acoustic or seismic characteristic frequencies recorded from primary afferent neurons; we will refer to these collectively as CF_a values. Most of these studies were performed at unspecified, and therefore probably room, temperature. The study of cochlear afferents in the caiman was performed at 27.1°C (Klinke and Pause, 1980).

Table 1 shows that the electrical resonances in the alligator lizard are comparable in frequency and quality to those reported in other hair cells. For most of the examples in Table 1, however, the CF_e range is within an octave of the CF_a range, and the CF_a range is below 1 kHz at room temperature. In these respects, the free-standing region of the alligator lizard is exceptional. In another difference (not shown in Table 1), electrical resonance occurs at or near the resting potential in many preparations, but not in the alligator lizard. In the remainder of the Discussion, we consider these differences between our results and others' and review the evidence that has led to a view that electrical resonance in hair cells, when it occurs, underlies acoustic or seismic tuning.

Discrepancy between electrical and acoustic CF values in free-standing hair cells

Is it possible that isolation of the lizard's hair cells shifted electrical resonant frequencies down by a decade? The CF_e values of turtle cochlear hair cells do drop by an octave upon isolation of the cells (Art and Fettiplace, 1987), and the hair cells of a terrestrial vertebrate such as the lizard may be significantly more sensitive to surgical manipulations that could produce transient hypoxia. We would have to postulate, however, that the free-standing hair cells were substantially more sensitive to such insults than were hair cells isolated from the adjacent tectorial region of the same organ. In preliminary results from the latter, we have found sharp electrical resonances at frequencies be-

Table 1. Comparison of acoustic or seismic characteristic frequencies (CF_a), electrical characteristic frequencies (CF_e), and electrical quality factors (Q_e) in nonmammalian inner ear organs

Preparation	CF_a range, Hz (1° afferents)	CF_e range, Hz	Q_e range
Alligator lizard cochlea			
All cells	50–4000 ¹	16–230 (42) ²	0.9–94 (40) ²
Free-standing only	900–4000 ¹	16–175 (25) ²	1.7–66 (23) ²
Turtle cochlea			
Half-head	30–700 (142) ³	108–440 (8) ⁴	1.36–11 (8) ³
Excised epithelium		31–171 (10) ⁵	
Isolated hair cells		9–350 (35) ⁶	≤31 ⁵
Frog sacculus			
Bullfrog	20–150 ⁷	80–160 (20) ⁸	≤12.6 ⁸
Leopard frog		11–85 (16) ⁹	0.7–3 (16) ⁹
Frog amphibian papilla			
Rostral plus mid-region	≤540 (18) ¹⁰	160–250 (5) ¹¹	
Rostral	140–300 (11) ¹⁰	60–240 (19) ¹²	1–10 (30) ¹²
Mid-region	340–540 (7) ¹⁰	210–330 (12) ¹²	1–10 (30) ¹²
Crocodilian cochlea			
Caiman cochlea	50–2800 (390) ¹³		
Alligator apical cochlea		4–10 (4) ¹⁴	
Alligator mid-region		50–157 (10) ¹⁴	≤4 ¹⁴
Chick cochlea			
Apical		5–20 (8) ¹⁵	
Mid-region to basal		100–250 (8) ¹⁵	
Toadfish sacculus	≤90 (106) ¹⁶	107–175 (28) ¹⁷	≤6 ¹⁷
Goldfish sacculus			
Rostral (S1 fibers)	250–400 ¹⁸	40–200 (16) ¹⁹	≤4 ¹⁹
Caudal (S2 fibers)	700–800 ¹⁸	30–180 (32) ¹⁹	≤4 ¹⁹

Numbers of nerve fibers or hair cells are given in parentheses after the ranges. Sources: ¹Weiss et al., 1976; ²present study; ³Crawford and Fettiplace, 1980; ⁴Crawford and Fettiplace, 1981; ⁵Crawford and Fettiplace, 1985; ⁶Art and Fettiplace, 1987; ⁷Koyama et al., 1982; ⁸Lewis and Hudspeth, 1983b; ⁹Ashmore, 1983; ¹⁰Lewis et al., 1982; ¹¹Roberts et al., 1986; ¹²Pitchford and Ashmore, 1987; ¹³Klinke and Pause, 1980; ¹⁴Fuchs and Evans, 1988; ¹⁵Fuchs et al., 1988; ¹⁶Fine, 1981; ¹⁷Steinacker and Romero, 1992; ¹⁸Furukawa and Ishii, 1967; ¹⁹Sugihara and Furukawa, 1989.

tween 94 and 285 Hz ($n = 9$), well within the CF_a range of the tectorial region (50–800 Hz; Fig. 5B).

The best evidence against the possibility that the free-standing hair cells normally have CF_e values that match their CF_a values comes from *in vivo* experiments in which receptor potentials were recorded from free-standing hair cells in response to acoustic clicks and tones (Baden-Kristensen and Weiss, 1983; Holton and Weiss, 1983a). The *in vivo* data are generally consistent with a cascade model of stimulus processing (Weiss and Leong, 1985a,b; Weiss et al., 1985) that places tuning before transduction, implying mechanical tuning. In particular, in the CF_a range (1–4 kHz) the fundamental component of the receptor potential falls off at about 20 dB per decade relative to the DC component (cutoff frequency ≤ 800 Hz) (Holton and Weiss, 1983a), indicating a low-pass filter mechanism rather than a resonance. This is true for acoustic stimuli large enough to saturate the receptor potential as well as for smaller stimuli. Thus, even for the largest receptor potentials (which come closest to the large depolarizations that activate the low-frequency electrical resonance in the isolated cells), no electrical resonance in the frequency range of the CF_a values is detected *in vivo*.

Another possibility is that cell isolation or whole-cell recording caused expression of a high-quality resonance that does not normally occur. Two known differences between the circumstances of the free-standing cells *in vivo* and in our experiments

might promote high-quality electrical resonance in the isolated cells. First, most of our measurements have been made in 3.3 mM Ca^{2+} , 1–2 mM higher than its probable value in perilymph (Peterson et al., 1978). Nevertheless, we find that when external Ca^{2+} is between 0.5 and 1.3 mM, the quality of the resonance is lower but still appreciable (Fig. 13). Second, in the free-standing region, hair cells are coupled by gap junctions to supporting cells (Nadol et al., 1976). The strength of electrical coupling is not known, but intracellular recordings showing substantial sound-evoked potentials in dye-labeled supporting cells (Weiss et al., 1974) suggest that the coupling is significant. Such coupling might dampen the electrical resonance in the hair cells *in vivo*.

In summary, our results are in accord with *in vivo* data that suggest that no posttransduction resonances occur at acoustic characteristic frequencies. It remains possible that the cell isolation procedure uncovered electrical resonance that does not normally occur. If so, our use of standard procedures suggests that such a problem may apply to other studies on isolated hair cells. The following points argue that we have been careful within the limitations of these procedures and that our data have been obtained in conditions comparable to others'. (1) Our standard internal solutions closely resemble those used in experiments on chick (Fuchs et al., 1988) and alligator (Fuchs and Evans, 1988) cochlear hair cells. (2) In frog saccular hair cells,

we routinely observe high-quality electrical resonance (R. A. Eatock, M. Saeki, and D. Feingold, unpublished results) at similar frequencies to those reported by other investigators in the same cells (Lewis and Hudspeth, 1983b; Roberts et al., 1986; Hudspeth and Lewis, 1988b). For these experiments we use a KCl/K-aspartate internal solution. When we use a similar internal solution (but higher osmolarity) on the lizard hair cells, we observe electrical resonance similar to that obtained with our standard KCl internal solutions. (3) Use of the perforated-patch technique did not affect the results in the lizard cochlear cells (Figs. 3, 4, 14). By allowing the exchange of only small monovalent ions between the cell and the pipette, the perforated-patch method preserves a more normal intracellular environment.

The lack of electrical resonance at zero-current potential

In hair cells from some other preparations (Crawford and Fettiplace, 1981; Lewis and Hudspeth, 1983b; Pitchford and Ashmore, 1987; Fuchs et al., 1988), electrical resonance occurs at the resting potential or in response to small (<50 pA) depolarizing currents. This electrical tuning is therefore positioned to affect the responses to low-level stimuli. In the alligator lizard's hair cells, on the other hand, the current required to evoke the resonance varied from 40 to 200 pA. Although transduction currents have not been measured in the alligator lizard, the largest receptor potentials recorded *in vivo* from the free-standing hair cells are about 10 mV (Holton and Weiss, 1983a). These depolarizations would not suffice to activate $I_{K(Ca)}$ from the resting potential, assuming that the zero-current potentials of the isolated cells are not substantially more negative than resting potentials *in vivo*. This assumption may not be justified; because the transduction current in hair cells is 5–25% activated at rest (Corey and Hudspeth, 1983; Ohmori, 1987; Crawford et al., 1989), some hyperpolarization is expected if the transduction process is damaged by the isolation procedure, eliminating the standing inward current through the transduction channels. Some of our hair cells lacked hair bundles and therefore transduction current. On the other hand, one cannot automatically conclude that the resting potentials of such cells were more negative than they would be *in vivo*. Other factors may act *in vivo*, but not in isolated cells, to hold the resting potential relatively negative. In particular, hair cells in the free-standing region are electrically coupled to supporting cells (Weiss et al., 1974), which have considerably more negative resting potentials (Weiss et al., 1978a). Resting potentials recorded *in vivo* from alligator lizard cochlear hair cells, using microelectrodes, were in fact more negative (-73 ± 8 mV, $n = 5$; Weiss et al., 1978a) than the zero-current potentials in our experiments.

An indirect approach to estimating the true resting potential is to consider the activation voltage range of the calcium current. The most negative potential at which I_{Ca} is apparent is between -60 and -50 mV in many hair cells (e.g., Art and Fettiplace, 1987; Hudspeth and Lewis, 1988a; Fuchs et al., 1990). Given that I_{Ca} is likely to mediate synaptic transmission and that there is background discharge in primary afferents innervating the free-standing region, one might expect the hair cells' *in vivo* resting potentials to be more positive than the resting potentials that we measured. It is premature to draw this conclusion, for the following reasons. First, we used nonphysiological concentrations of divalent cations (4 mM Ba^{2+} or 20 mM Ca^{2+}) to study I_{Ca} . Because external divalent concentrations affect the apparent voltage dependence of currents, we do not know the voltage

dependence of I_{Ca} *in vivo*. Second, the relationships between I_{Ca} , transmitter release, and postsynaptic discharge are not yet described for any hair cells. Small amounts of Ca^{2+} influx at potentials negative to that at which I_{Ca} rises above the experimental noise may suffice to mediate spontaneous levels of transmitter release. Thus, it is not yet possible to infer hair cell resting potentials from discharge rates in the afferent neurons.

Our data showing no electrical resonance at the resting potentials of the isolated hair cells are consistent with *in vivo* measurements showing no CF_e -related oscillations in the spontaneous activity of free-standing hair cells and afferents. Such oscillations occur in hair cells from the turtle cochlea (Crawford and Fettiplace, 1985) and from the frog sacculus (Ashmore, 1983; Lewis and Hudspeth, 1983a) and amphibian papilla (Pitchford and Ashmore, 1987). We also have observed such spontaneous oscillations in frog saccular hair cells and in some hair cells isolated from the tectorial region of the alligator lizard's cochlea (R. A. Eatock and M. J. Hutzler, unpublished observations). These oscillations are a manifestation of the electrical resonance at resting potential. Intracellular voltage recordings from the free-standing hair cells *in vivo* revealed no spontaneous voltage oscillations (T. F. Weiss, personal communication). Furthermore, nerve fibers innervating either cochlear region in the alligator lizard show no spontaneous periodicities (W. A. Frezza, T. Holton, and T. F. Weiss, unpublished observations). In the turtle half-head preparation, the background discharges of cochlear afferents have preferred intervals at multiples of about $1/CF_e$ (Crawford and Fettiplace, 1980), as would be expected if the afferents innervate hair cells that undergo CF_e -related voltage oscillations at the resting potential. Such CF_e -related spontaneous activity has also been described in cochlear fibers with CF_e values below 1 kHz from starling (Manley, 1979), gecko (Eatock et al., 1981), and lacertid lizard (Manley, 1990).

One might expect that if the electrical resonance that we have observed *in vitro* did occur *in vivo*, it would be evident in acoustic tuning curves generated from the AC components of the receptor potential in free-standing hair cells. Most of the available acoustic tuning curves, however, are isoresponse curves with low criterion responses (≤ 3 mV) (Holton and Weiss, 1983a,b). On the basis of our *in vitro* data, we would not expect such small receptor potentials to activate the electrical resonance.

In summary, *in vivo* data from the alligator lizard cochlea are consistent with the relatively negative resting potentials that we have recorded from isolated hair cells, and with there being no electrical resonance at or near those resting potentials.

Evidence from other preparations on the relationship between hair cell electrical resonance and acoustic tuning

Because our results do not fit neatly with a view that electrical resonance in hair cells is important in acoustic tuning, it is relevant to review whether the evidence from other preparations (summarized in Table 1) justifies that view.

It has clearly been established that in the turtle cochlea electrical resonance is an important acoustic tuning mechanism. CF_e and CF_a were shown to be identical in individual cochlear hair cells in a semi-intact preparation, the "half-head," of the red-eared turtle (Crawford and Fettiplace, 1981). The acoustic tuning in this preparation is consistent with two filters: that due to the electrical resonance, and a rather broad band-pass filter that was attributed to mechanical tuning. CF_e values in the turtle cochlear nerve fibers extend up to 700 Hz (Crawford and Fet-

tiplace, 1980). The discrepancy between this upper limit and that observed in the hair cells (300–440 Hz) (Crawford and Fettiplace, 1981, 1985; Art and Fettiplace, 1987) (Table 1) was attributed to (1) in the experiments on the semi-intact preparation, a lack of access to the high-frequency hair cells; and (2) in isolated cells, an effect of the isolation or whole-cell recording procedures. Even in the turtle cochlea, however, it is possible that mechanical tuning of hair bundles contributes to acoustic tuning; Art and Fettiplace (1987) noted that CF_e (and therefore CF_a) was inversely correlated with hair bundle height.

Electrical resonance data from the cochleas of alligators and chicks are relatively similar to each other and are more complex than the turtle results (Fuchs and Evans, 1988; Fuchs et al., 1988). Hair cells from apical, low- CF_a regions of the alligator and chick cochleas tend to spike at low frequency (<20 Hz) in response to depolarizing current injections. Hair cells from mid-regions of the cochleas (higher- CF_a cells) are more likely to show “ringing” (quasisinusoidal voltage oscillations); the oscillation frequencies are higher than the spike frequencies of the apical cells. The highest CF_a values in the caiman, a close relative of the alligator, are 2800 Hz at 27°C (Klinke and Pause, 1980). It is not known whether hair cells from the high- CF_a (basal) region of the alligator show electrical resonances at such high frequencies, because hair cells were isolated only from the apical and mid-regions. The narrow range of CF_e values observed (50–157 Hz), however, does not warrant extrapolation to 2800 Hz, particularly since electrical resonances above 500 Hz have not been reported in hair cells.

The range of CF_e values of resonant hair cells from the mid-region of the chick cochlea (100–250 Hz) was not very different from the range in the alligator, but Fuchs et al. (1988) estimated that at chick body temperature, CF_e values would be about two octaves higher and consistent then with a frequency map of basilar membrane motion that was generated by von Békésy (1960). Three hair cells from adjacent positions in the mid-region of the chick cochlea had CF_e values that progressed from 100 to 185 Hz, raising the possibility of a tonotopic map of CF_e within the mid-region. As for the alligator cochlea, however, the only tonotopy of which one can be confident is that manifested by the low spiking frequencies of the apical cells and the higher resonant frequencies of the basal cells (Fuchs and Evans, 1990).

In hair cells in excised amphibian papillae of frogs (*Rana temporaria*) (Pitchford and Ashmore, 1987), electrical resonant frequencies depended on the location of the hair cell in a manner reasonably consistent with the expected acoustic tuning of the location. Hair cells from the rostral region had electrical resonant frequencies between 60 and 240 Hz, whereas those from the mid-region fell between 210 and 330 Hz. In a study by Lewis et al. (1982), CF_e values of afferent fibers to the rostral and mid-regions of the amphibian papilla of a different frog, the bullfrog, fell between 140 and 300 Hz and between 340 and 540 Hz, respectively. In the bullfrog sacculus, electrical resonances occur both in isolated hair cells and in an epithelial preparation from which otolithic structures have been removed (Lewis and Hudspeth, 1983b). The CF_e values of isolated hair cells (80–160 Hz) fall within the range of *in vivo* measures of the seismic best frequencies of the primary afferents (20–150 Hz; Koyama et al., 1982). Other parameters of electrical and seismic tuning in these cells differ, however. The quality of seismic tuning in the primary afferents is generally rather low (quality factors < 2) and phase angle versus frequency plots do not show the sudden

changes in phase angle at the seismic best frequencies that are characteristic of linear filters like the electrical resonance (Lewis, 1988). Lewis (1988) points out that the significance of the electrical resonance in isolated cells is not clear, particularly if *in vivo* there is bidirectional coupling between mechanical and electrical elements.

The CF_e values of hair cells isolated from the toadfish sacculus (between 100 and 200 Hz; Steinacker and Romero, 1992) are within a factor of 2–3 of *in vivo* measures of acoustic tuning (Fish and Offutt, 1972; Fine, 1981), but do not vary with region of origin. This is also the case for hair cells isolated from the goldfish sacculus (Sugihara and Furukawa, 1989), which is known to be tonotopically organized along the rostrocaudal axis (Furukawa, 1978; Sento and Furukawa, 1987). Thus, while it remains possible that electrical resonance contributes to acoustic tuning in toadfish, its role in the goldfish is unclear.

In summary, it has been directly demonstrated that in the turtle cochlea, the electrical tuning of individual cells contributes importantly to their tuning to natural stimuli. For other hair cell organs, such direct demonstrations are lacking and in most cases the narrow ranges of CF_e values reported make it difficult to be confident that they are tonotopically distributed. Furthermore, the CF_e ranges resemble the range in hair cells isolated from the free-standing region of the alligator lizard, where CF_e values and CF_a values do not match, and from the goldfish sacculus, where CF_e values do not vary systematically relative to the dimension along which CF_a values are organized. A preliminary report on hair cells isolated from the pigeon cochlea also found, in 19 cells, no correlation between CF_e and position within the cochlea (Correia et al., 1991). Therefore, despite expectations based on the turtle study, demonstrations in isolated hair cells of electrical resonances that are in about the right frequency range do not imply a role for those resonances in acoustic or vibration tuning. An alternative interpretation of the available data is that sub-kilohertz electrical resonances occur frequently in hair cells isolated from auditory or vibration-sensitive organs, but only in some cases serve acoustic or vibration tuning. In other cases, the resonances may simply be an epiphenomenon. This would be most obvious in hair cells such as the free-standing cells, which come from cochlear regions with CF_a values well above the frequency range at which electrical resonances commonly occur. Electrical resonance at higher frequencies may be precluded by limits on the speed and voltage dependence of the participating ion channels (Roberts et al., 1988).

Concluding remarks

The electrical tuning of isolated free-standing hair cells is not coincident with the acoustic tuning of these cells *in vivo*. Therefore, our results indirectly support the proposal that in the free-standing region, sharp acoustic tuning above 1 kHz depends principally on the mechanical properties of the hair bundles (Weiss et al., 1978b; Frishkopf and DeRosier, 1983; Holton and Hudspeth, 1983; Weiss and Leong, 1985a; Freeman and Weiss, 1990a–d).

The high-quality, low-frequency electrical resonance in the free-standing hair cells would seem to be a liability for an orderly place code of frequency. In general, hair cell electrical resonances will interfere with representation of the temporal waveform of the acoustic or seismic stimulus (Lewis, 1987). One possible role for the relatively large I_{IR} in the free-standing hair cells is

to make the resting potential more negative than the voltages at which $I_{K(Ca)}$ and the electrical resonance become significant.

The frequency range and quality of electrical resonance in the alligator lizard's free-standing cells are consistent with reports from other hair cells. Thus, the results from the lizard suggest that electrical tuning measured in hair cells *in vitro* must be interpreted with caution. At present, congruence of electrical and acoustic tuning has been explicitly demonstrated in only one cochlea, that of the red-eared turtle.

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