Two Components of Transducer Adaptation in Auditory Hair Cells

YUH-CHERNG WU, A. J. RICCI, AND R. FETTIPLACE
Department of Physiology, University of Wisconsin Medical School, Madison, Wisconsin 53706

Wu, Yuh-Cherng, A. J. Ricci, and R. Fettiplace. Two components of transducer adaptation in auditory hair cells. J. Neurophysiol. 82: 2171–2181, 1999. Mechanoelectrical transducer currents in turtle auditory hair cells adapted to maintained stimuli via a Ca\(^{2+}\)-dependent mechanism characterized by two time constants of \(\sim 1\) and 15 ms. The time course of adaptation slowed as the stimulus intensity was raised because of an increased prominence of the second component. The fast component of adaptation had a similar time constant for both positive and negative displacements and was unaffected by the myosin ATPase inhibitors, vanadate and butanedione monoxime. Adaptation was modeled by a scheme in which Ca\(^{2+}\) ions, entering through open transducer channels, bind at two intracellular sites to trigger independent processes leading to channel closure. It was assumed that the second site activates a modulator with 10-fold slower kinetics than the first site. The model was implemented by computing Ca\(^{2+}\) diffusion within a single stereocilium, incorporating intracellular calcium buffers and extrusion via a plasma membrane Ca\(^{2+}\)ATPase. Theoretical results reproduced several features of the experimental responses, including sensitivity to the concentration of external Ca\(^{2+}\) and intracellular calcium buffer and a dependence on the onset speed of the stimulus. The model also generated damped oscillatory transducer responses at a frequency dependent on the rate constant for the fast adaptive process. The properties of fast adaptation make it unlikely to be mediated by a myosin motor, and we suggest that it may result from Ca\(^{2+}\) binding to the transducer channel or a nearby cytoskeletal element.

INTRODUCTION

Hair cells of the internal ear detect mechanical stimuli by gating of mechanosensitive ion channels located in their stereociliary bundles. The common view of transduction is that force is delivered to the mechanically sensitive channels by extracellular tip links connecting the top of one stereocilium with the side wall of its taller neighbor (Pickles et al. 1984). Deflection of the bundle toward its taller edge transmits force via the tip links to open transducer channels attached at either end of the link (Denk et al. 1995). Hair cells, like other sensory receptors, possess an adaptation mechanism to reduce their sensitivity in the face of a sustained stimulus (Crawford et al. 1989; Eatock et al. 1987). Adaptation shifts the transducer activation curve, changing the range of displacements to which the channel is sensitive without diminishing the maximum response.

Transducer adaptation is regulated by changes in stereociliary Ca\(^{2+}\) concentration that reset the range of bundle displacements detected by the channel (Assad et al. 1989; Crawford et al. 1989; Ricci and Fettiplace 1997, 1998). One proposed mechanism for resetting sensitivity entails a force generator that adjusts the tension in the tip link by translating the tip link’s attachment point along the side of the stereocilium (Howard and Hudspeth 1987). The force generator may be myosin I\(\beta\) linking the transducer channel with the internal actin cytoskeleton (Hudspeth and Gillespie 1994). Ca\(^{2+}\) influx through open transducer channels is posited to inhibit the actomyosin interaction, causing the channel to slip down the stereocilium and relieve the stimulus to the channel. A difficulty with this mechanism is that adaptation can occur on a submillisecond time scale (Ricci and Fettiplace 1997), too fast for the kinetics of the full actomyosin cycle. It is conceivable that fast adaptation relies on another mechanism with kinetics swifter than achievable with actomyosin interactions.

To assess this hypothesis, we have characterized the time course of transducer adaptation in turtle auditory hair cells to look for fast and slow components identifiable with different mechanisms. To support experimental observations, we devised a model for adaptation in which Ca\(^{2+}\) entering through open transducer channels binds at two intracellular sites to trigger separate processes leading to channel closure. The model, incorporating diffusion of Ca\(^{2+}\) within the stereocilium in the presence of intracellular Ca\(^{2+}\) buffers, employs computational techniques introduced in a previous model of hair-cell calcium dynamics (Wu et al. 1996). Our model differs from previous theoretical schemes (Assad and Corey 1992; Lumpkin and Hudspeth 1998) in providing an explicit formulation of the role of stereociliary Ca\(^{2+}\) in transducer channel regulation. It takes for its background prior measurements of the channel’s Ca\(^{2+}\) permeability (Ricci and Fettiplace 1998), and experimental data on the effects of extracellular Ca\(^{2+}\) and intracellular calcium buffers on adaptation in turtle hair cells (Ricci and Fettiplace 1997, 1998; Ricci et al. 1998). Both experimental and theoretical manipulations provide further information about the properties of fast adaptation.

METHODS

The preparation and techniques for hair-cell recording and stimulation in the intact basilar papilla were similar to those previously documented (Crawford and Fettiplace 1985; Ricci and Fettiplace 1997). Turtles (Trachemys scripta elegans, carapace length 100–125 mm) were decapitated, and the cochlear duct was dissected out and opened. After digestion in saline [composed of (in mM) 125 NaCl, 4 KCl, 2.8 CaCl\(_2\), 2.2 MgCl\(_2\), 2 Na pyruvate, 8 glucose, and 10 Na-HEPES, pH 7.6] containing up to 0.1 mg/ml of protease (Sigma type XXIV), the hair bundles were exposed by removal of the tectorial membrane. The preparation was mounted, hair bundles uppermost, in a silicone elastomer (Sylgard) well of a recording chamber mounted on the stage of a Zeiss Axioskop FS microscope. The preparation was perfused with saline containing (in mM) 128 NaCl, 0.5 KCl, 2.8 CaCl\(_2\), 2.2 MgCl\(_2\), 2 Na pyruvate, 8 glucose, and 10 Na-HEPES, pH 7.6. The upper surface of the hair-cell epithelium facing the endolym-
phatic compartment was separately and continuously perfused by a large pipette with an internal diameter of 100 µm introduced into the cochlear duct. Hair bundles were stimulated with a rigid glass pipette, fire-polished to a tip diameter of ~1 µm and cemented to a piezoelectric bimorph (Crawford et al. 1989). The bimorph was driven differentially with voltage steps, filtered with an eight-pole Bessel at 3 kHz and amplified through a high-voltage driver of 20-fold gain, to yield a fast stimulator with a 10–90% rise time of ~100 µs.

Whole cell currents were measured with a List EPC-7 amplifier attached to a borosilicate patch electrode. Patch electrodes were filled with an internal solution of composition (in mM) 125 CsCl, 3 Na,ATP, 2 MgCl₂, and 10 CsHEPES, pH 7.2 to which various amounts of the calcium buffers bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA; Molecular Probes, Eugene, OR) or EGTA (Fluka, NY) were added. Buffer concentrations of 0.1, 1, and 10 mM were used, and with the highest concentration, the CsCl was reduced to keep the osmolarity constant. After application of ~50% series-resistance compensation, the electrode access resistance was 3–10 MΩ, which gave a recording time constant of 45–150 µs. Transducer currents were measured at a holding potential that, after correction for the junction potential, was ~90 mV. To inhibit intracellular myosin ATPases, sodium metavannate (Aldrich Chemical Company, Milwaukee, WI) was added to the patch electrode solution and butanedione monoxime (Sigma Chemical, St. Louis, MO) was dissolved in the extracellular solution.

**RESULTS**

**Fast and slow components of adaptation**

Transduction in auditory hair cells, evoked by step deflections of the hair bundle, is characterized by rapid opening of the mechanically gated channels closely followed by an adaptation that, despite maintenance of the stimulus, causes the channels to shut again. Figure 1A shows a family of transducer currents measured experimentally in response to a range of bundle displacements. Over the entire dynamic range, the adaptive decline in the transducer current could be well described by two exponential components, one with a time constant of ~1 ms and the other an order of magnitude slower (Fig. 1B). The fast time constant, \( \tau_f \), dominated for small displacements, but the slower time constant, \( \tau_s \), became more conspicuous with increasing stimulus amplitude. The double-exponential fits in Fig. 1A were derived by determining the value of the \( \tau_f \) from small responses and then holding this value fixed while allowing the contribution of \( \tau_s \) to vary for larger stimulus amplitudes (Fig. 1). These fits showed that over much of the dynamic range, \( \tau_s \) had a constant value of 11 ms, but for the largest displacements, it increased to 70 ms.

For stimuli that elicit less than half-maximal responses, adaptation is dominated by \( \tau_f \), which we have used previously to assay the calcium sensitivity of the underlying process. Over a range of ionic conditions, the rate of adaptation (\( 1/\tau_f \)) was proportional to \( \text{Ca}^{2+} \) influx (Ricci and Fettiplace 1998) and inversely proportional to the concentration of intracellular calcium buffer, BAPTA (Ricci and Fettiplace 1997). Application of two-exponential fits to transducer currents recorded with different concentrations of BAPTA showed that the buffer concentration had parallel effects on the limiting values of both \( \tau_f \) and \( \tau_s \). Mean values for \( \tau_f \) were 0.74 ± 0.14 (SD) ms \((n = 7)\) in 0.1 mM BAPTA, 1.32 ± 0.11 ms \((n = 9)\) in 1 mM BAPTA, and 1.68 ± 0.13 ms \((n = 9)\) in 10 mM BAPTA. The corresponding values for \( \tau_s \) in 0.1, 1, and 10 mM BAPTA respectively were 9.3 ± 1.4 ms \((n = 7)\), 14.9 ± 2.6 ms \((n = 9)\), and 19.5 ± 4.4 ms \((n = 9)\). All these measurements were obtained with 2.8 mM external \( \text{Ca}^{2+} \). Thus increasing the BAPTA concentration from 0.1 to 10 mM roughly doubled the values of both fast and slow time constants. The buffer effects may be due to a reduction in amplitude and a slowing of the intracellular \( \text{Ca}^{2+} \) transient after opening of the transducer channel. The buffer results indicate that the mechanism underlying \( \tau_s \) also must be \( \text{Ca}^{2+} \) dependent.

Other features of the transducer responses, linked to adaptation, also depend on the stereociliary \( \text{Ca}^{2+} \) dynamics (Ricci and Fettiplace 1997). Increasing the concentration of intracellular calcium buffer diminished the extent of adaptation, defined as the reduction in current in the steady state relative to the initial peak (Fig. 2). Thus in 0.1 mM BAPTA, there was no steady-state response for small stimuli; this is equivalent to 100% adaptation. However, with 10 mM BAPTA, the extent of adaptation never exceeded 50%. The fraction of transducer current turned on at the hair bundle’s resting position also varied with the concentration of intracellular calcium buffer (Fig. 2). This difference reflects a translation of the transducer’s activation relationship along the displacement axis (Fig. 2).
2). Previous experiments have indicated that two manifestations of adaptation, the fast adaptation time constant and the fraction of current activated at rest, are differentially sensitive to various experimental manipulations. These include changing the nature of the intracellular calcium buffer (Ricci et al. 1998) or treatment with cyclic AMP (Ricci and Fettiplace 1997). Such observations support the notion that different aspects of adaptation may be associated with distinct Ca$^{2+}$-binding sites.

### Effects of myosin ATPase inhibitors on fast adaptation

Previous experimental analysis of adaptation in turtle hair cells has focused on the fast component that dominates the responses (Ricci and Fettiplace 1997). The prevailing theory for the mechanism of adaptation involves operation of a myosin ATPase motor that adjusts the force delivered by the tip links to the transducer channel (reviewed in Hudspeth and Gillespie 1994). In support of this mechanism in frog saccular hair cells, agents that block the ATPase also inhibit adaptation (Yamoah and Gillespie 1996). We examined the effects on adaptation of two potential inhibitors of the myosin ATPase: the phosphate analogue, vanadate, and the membrane-permeable butanedione monoxime (BDM), an inhibitor of myosin II and myosin V ATPases (Cramer and Mitchison 1995). Vanadate (1 mM), introduced via the patch electrode solution, or 10 mM BDM perfused extracellularly had similar effects on the transducer currents (Fig. 3). Both agents shifted the current-displacement relationship to the right and decreased its slope. The positive shifts in the current-displacement relationship were $203 \pm 32$ nm ($n = 5$, vanadate) and $154 \pm 52$ nm ($n = 3$, BDM). Similar shifts produced by other ATPase inhibitors have been previously reported (Yamoah and Gillespie 1996). However, neither agent significantly diminished the fast component of adaptation (Fig. 3). The fast time constant, $\tau_{\text{fast}}$, had mean values of $1.54 \pm 0.12$ ms ($n = 4$, control), $1.45 \pm 0.14$ ms ($n = 3$, BDM) and $1.65 \pm 0.14$ ms ($n = 5$, vanadate), all with 1 mM internal BAPTA. The time constant of the slow component, $\tau_{\text{slow}}$, measured in the same cells was $7.5 \pm 2.0$ ms (control), $12.8 \pm 1.6$ ms (BDM), and $9.5 \pm 1.3$ ms (vanadate). Because vanadate was delivered via the patch electrode solution, it was not possible to obtain a good control in the same cell due to the “wash-in” of vanadate occurring over a similar time course to that of BAPTA. The controls therefore represent measurement on other cells. It should be noted that the effects of the ATPase inhibitors resemble qualitatively those produced by application of 8-bromo cyclic AMP (Ricci and Fettiplace 1997).

Interpretation of the effects of ATPase inhibitors is complicated by the fact that they also block the Ca$^{2+}$ extrusion from hair cells (Tucker and Fettiplace 1995). Consistent with those results, both vanadate and BDM produced prolonged tail currents at the offset of depolarizing current steps due to sustained activation of the small-conductance Ca$^{2+}$-activated K$^+$ (SK) channels (see Fig. 6 of Tucker and Fettiplace 1995). Effects on the transducer current-displacement relationship therefore could be a combination of an elevation of stereociliary Ca$^{2+}$ concentration and block of the slow component of adaptation. However, the lack of any significant effect on $\tau_{\text{fast}}$ argues that fast adaptation is unlikely to be mediated by a myosin ATPase.

The fast process of adaptation showed linear behavior for small displacement steps about the resting position of the bundle (Fig. 4). The linearity was most evident under conditions where the resting probability had been raised by lowering the concentration of external Ca$^{2+}$ or by increasing the amount of intracellular calcium buffer. Thus in Fig. 4 recorded in 0.35 mM Ca$^{2+}$, the responses for small positive and negative steps are mirror images of one another, and $\tau_{\text{fast}}$ has a similar value for stimuli in either direction. However, the same linearity held under other conditions provided the amplitude of the negative stimulus was sufficiently small not to turn off the transducer current during the initial peak of the response. Collected measurements of $\tau_{\text{fast}}$ for small positive and negative stimuli, obtained under a range of conditions, are plotted in Fig. 4B, which shows a good correlation between the adaptation time
constants measured with the two stimulus polarities. This linearity implies that the reaction involved in generating the fast component of adaptation is a reversible one and contrasts with the behavior expected for a myosin-based motor in which the adaptation rate for positive stimuli is faster than for negative stimuli (Assad and Corey 1992). The fast positive rate was attributed to “slipping” of myosin’s attachment to the actin cytoskeleton, whereas the slower negative rate was limited by myosin ascending on the actin core of the stereocilium.

Outline of the two-site model

The central tenet of the model is that the transducer channels respond to the difference \((x - x')\) between an external stimulus, \(x\), and an internal “set point” \(x'\). As the channels open, \(\text{Ca}^{2+}\) ions enter the stereocilium and bind to an intracellular site triggering a change in the set point that opposes the external stimulus. \(\text{Ca}^{2+}\) is thus part of a negative feedback loop. The sequence of events ensuing from an increase in the external stimulus \(x \rightarrow x'\) can be summarized as follows: the channels open in response to the new stimulus \((x' - x)\) promoting \(\text{Ca}^{2+}\) influx and binding to an intracellular site \(S\); the proportion of \(S\) bound catalyzes a change in the set point, \((X_a - X_a')\), causing the channels to adjust their probability of opening in response to the new stimulus \((x' - X_a')\). To implement the \(\text{Ca}^{2+}\) feedback, a three-dimensional model of the stereocilium was constructed to simulate the diffusion of free \(\text{Ca}^{2+}\) ions and mobile \(\text{Ca}^{2+}\) buffers within the cytoplasm (see APPENDIX). Major components of the model are as follows: each turtle hair-cell stereocilium contains a small number of mechanoelectrical transducer channels (Ricci and Fettiplace 1997) represented as a diffuse \(\text{Ca}^{2+}\) source, 10 nm radius, located at the stereociliary tip (Jaramillo and Hudspeth 1992). \(\text{Ca}^{2+}\) influx was estimated from the transducer current per stereocilium and the proportion of the current carried by \(\text{Ca}^{2+}\) (Ricci and Fettiplace 1998). The time course of internal \(\text{Ca}^{2+}\) is determined by diffusion and binding to calcium buffers and by extrusion via a plasma membrane \(\text{CaATPase}\) known to occur in turtle hair cells (Tucker and Fettiplace 1995).

\(\text{Ca}^{2+}\) is assumed to interact with two classes of intracellular binding site, \(S_1\) and \(S_2\), associated with the fast and slow adaptation processes, respectively

\[
K_D = \frac{\text{Ca}^{2+} + S_i}{\text{Ca}S_i},
\]

where \(K_D\) is the \(\text{Ca}^{2+}\) dissociation constant for the two sites, and the subscript, \(i = 1\) or 2, corresponds to the fast and slow sites respectively. Both sites are diffusely distributed over cylindrical regions of radius 1.5 nm and of longitudinal extent 20–50 nm from the channel for \(S_1\) and either 50–100 nm for \(S_2\). \(S_1\) was positioned to encompass the “crossing points” of the \(\text{Ca}^{2+}\) gradients in different \(\text{BAPTA}\) concentrations (see Fig. 10 of Ricci et al. 1998). Because \(S_2\) is associated with the slower process, it initially was located further from the channel than \(S_1\); the effects of varying the position of \(S_2\) will be described in the following text. Owing to
the steep Ca$^{2+}$ gradient within the stereocilium dictated by the concentration of diffusible buffer, S1 must have a Ca$^{2+}$ dissociation constant ($K_{D1}$ = 20 μM) higher than that of S2 ($K_{D2}$ = 0.5 μM). Previous studies (Ricci et al. 1998) indicate that the Ca$^{2+}$ concentration may decline from several hundred micromolar near the channel to a few micromolar at a distance of 100 nm, so the dissociation constants were chosen appropriately for the location of the two sites within the gradient. The dissociation constants are within the range of values reported for calmodulin (Linse et al. 1991).

The fraction, $f_{SCa}$, of each calcium-binding site occupied catalyzes a change in $X_a$ that takes place in two stages. First, a conformational transition is assumed to occur in a modulator molecule converting it from an inactive form $M$ to an active form $M^*$:

$$M \xrightarrow{k_{u}} f_{SCa} \xrightarrow{k_{n}} M^*$$  \hspace{1cm} (2)

Each binding site has its own modulator ($M_1$ and $M_2$) with distinct kinetics, the rate constants for the second site being 10 times slower than those for the first site.

Second, $X_a$ is scaled linearly according to the concentration of the active form of each type of modulator

$$X_a = \sum \lambda_i \cdot M_i^*$$  \hspace{1cm} (3)

in which $\lambda_i$ and $\kappa_i$ are constants and the modulator concentration, $M_i^*$, is integrated over the regions specified for each binding site. The effects of the modulators are assumed to sum independently to control the set point $X_a$. Because the modulator concentration, $M_i$, takes values between 0 and 1, the constants $\lambda_i$ and $\kappa_i$ determine the dynamic range of the feedback. A restricted dynamic range is consistent with the limited extent of adaptation reported by Shepherd and Corey (1994).

Model transducer responses

The theoretical responses for three different intracellular BAPTA concentrations are given in Fig. 5. The simulations expressed as the probability of opening of the transducer channel have been inverted for easier comparison with the inward currents recorded experimentally. Comparison of the model with the experimental records in Fig. 2 shows a number of similarities in terms of the overall shape of the response and their sensitivity to BAPTA. Thus the extent of adaptation was comparable in the different conditions and was reduced with an increase in BAPTA concentration. The steady-state responses for small displacements in 0.1 mM BAPTA are all more closely grouped compared with 10 mM BAPTA, reflecting nearly a 100% adaptation in the low buffer concentration. Only a single external Ca$^{2+}$ concentration (1 mM) is illustrated in Fig. 5, but model responses at other Ca$^{2+}$ concentrations from 0.07 to 2.8 mM showed comparable agreement with the experimental transducer currents.

As expected, the model responses exhibited two components of adaptive decay, one component, $\tau_{fast}$, with time constant of 1 ms, and a second component, $\tau_{slow}$, of 14 ms. Fitting of the decays with double exponentials indicated that the slow component became more pronounced with an increase in stimulus amplitude. Both $\tau_{fast}$ and $\tau_{slow}$ increased with intracellular concentration.

![Fig. 5. Effects of intracellular BAPTA concentration on the model transducer responses. Theoretical families of transducer channel open probabilities in response to 50-ms stimulus steps, for 3 BAPTA concentrations in 1 mM external Ca$^{2+}$. To compare with experimental responses (Fig. 2), increasing probability of opening is plotted downward. Bundle displacements (in μm) are: −0.5, −0.2, −0.05, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, and 1.2. Transducer activation curves for the 3 BAPTA concentrations are given in the bottom right, the peak probability of opening for each trace being plotted against bundle displacement. More points are plotted than shown in the theoretical responses. Increasing the intracellular BAPTA concentration shifts the relationship to the left as with the experimental results in Fig. 2.](http://jn.physiology.org/ by 10.220.32.246 on May 11, 2017)
BAPTA concentration in a similar manner to the experimental measurements. The mean values of $\tau_{\text{fast}}$ are plotted in Fig. 6, and the values for $\tau_{\text{slow}}$ were 11, 14, and 70 ms in 0.1, 1, and 10 mM BAPTA, respectively. It should be noted that the magnitude of $\tau_{\text{slow}}$ in the model responses remained approximately constant over the dynamic range in contrast to the experimental results where $\tau_{\text{slow}}$ increased at large displacements. This discrepancy indicates a nonlinearity in the slow process. The fraction of current activated at rest in the model, as in the experiments, also increased with buffer concentration due to a shift in the current-displacement relationship.

To complement the calcium buffer results, the effects of varying the external Ca$^{2+}$ concentration also were examined. This was implemented by altering the fraction of current carried by Ca$^{2+}$ in line with the values determined experimentally (Ricci and Fettiplace 1998). Reducing the external Ca$^{2+}$ increased the fraction of current turned on at rest and slowed the adaptation time constant (Fig. 6), effects that agree qualitatively with the experimental observations (see Ricci et al. 1998). However, the Ca$^{2+}$ sensitivity of the parameters, especially the adaptation time constant, was weaker in the model than in the experimental results. This defect might be corrected by making the sites bind multiple Ca$^{2+}$ ions in a cooperative fashion as occurs with calmodulin-based receptors.

![Figure 6: Effects of external Ca$^{2+}$ concentration on the parameters of adaptation for the theoretical responses.](http://jn.physiology.org/)

**Properties of the second Ca$^{2+}$-binding site**

The model was useful for distinguishing the relative contributions of the two sites, a manipulation that is difficult to perform experimentally. The simulations were repeated in the absence of one or other site by setting the scaling constants, $\lambda$ and $\kappa$, for that site to 0. The responses are shown in Fig. 7 for the case of 1 mM internal BAPTA and should be compared with the equivalent simulations with both sites present in Fig. 5. Removing site 1 produced responses with slow adaptation with a time constant of ~14 ms that was independent of level. The removal of site 2 gave responses, characterized over most of the range solely by a fast time constant of 1 ms similar to that seen in the two-site model. Neither set of responses in Fig. 7 for a single Ca$^{2+}$-binding site provided as good a match to the experimental results as did the two-site model.

In the majority of simulations, S2 was placed further from the transducer channel (150–200 nm) than the Ca$^{2+}$-binding site for the fast process (20–50 nm). However, if S2 was moved closer to the channels, similar responses could be achieved provided that the Ca$^{2+}$-dissociation constant for the site ($K_{D2}$) was increased. With S2 at 50–100 nm from the channel, it was necessary to raise the Ca$^{2+}$-dissociation constant, $K_{D2}$, from 0.5 $\mu$M (the standard value) to 3 $\mu$M. In contrast, it was not possible to alter significantly the range for S1 and still retain fast adaptation.

![Figure 7: Contribution of the fast and slow components of adaptation to the theoretical transducer responses.](http://jn.physiology.org/)
Damped oscillatory responses

A consistent feature of the model responses for the lower BAPTA concentrations was an under-damped oscillatory approach to the steady state (Fig. 5), a manifestation of resonance stemming from negative feedback control of the transducer channels. Such resonance theoretically could produce frequency tuning for sinusoidal stimuli (Crawford and Fettiplace 1981), with the transducer current being maximal at the resonant frequency. An expanded version of the smallest theoretical responses in 1 mM BAPTA from Fig. 5 are shown in Fig. 8A, where the oscillations are clearly evident at the onset and termination of the step. This type of resonance has been observed experimentally at frequencies ranging from 58 to 230 Hz (Ricci et al. 1998). Figure 8A includes an example of such experimental transducer currents recorded with 1 mM intracellular BAPTA. These currents exhibit damped oscillations at a similar frequency, 180 Hz, to the model responses.

The main parameter controlling the resonant frequency in the model responses was the speed of the fast adaptive process. Figure 8B shows the results of altering the rate constants for the modulator transition. A threefold increase in the rate constants from the standard value elevated the resonant frequency from 180 to 270 Hz. Conversely, a threefold decrease in the rate constants slowed the adaptation to the point where the resonance was not visible. Two conclusions may be drawn from these results: first, the resonant behavior stems from the operation of the fast adaptation process; second, some of the variability in the appearance of the oscillations may be caused by differences among cells in the kinetics of the fast adaptive feedback.

Effects of speed of stimulus onset on adaptation

An important experimental variable influencing the appearance of the fast component is the rate of onset of the displacement step. In the present experiments, the driving voltage to the piezoelectric stimulator was filtered with an eight-pole Bessel at 3 kHz. This yielded a 10–90% rise-time in the stimulating probe of ~0.1 ms, which is comparable with or less than the rise time of the transducer current (Crawford et al. 1989). When the driving voltage was filtered at 100 Hz, equivalent to a rise time of 3 ms, both the onset and adaptation time constants were slowed (Fig. 9). The example illustrated shows that the fast adaptation time constant, $\tau_{\text{fast}}$, increased from 1.3 to 4.6 ms. The additional filtering also desensitized transduction (Fig. 9A), such that a larger stimulus was required to produce the same peak current amplitude. As a consequence, the current-displacement relationship for the stimulus filtered at 100 Hz was shifted to more positive displacements relative to that for the 3-kHz filtered stimulus.

In the computed responses, the stimulus onset was normally instantaneous, but filtering of the stimulus with a single pole filter of time constant 0.1 ms had no effect on $\tau_{\text{fast}}$. However, when the filter time constant was raised to 2 ms, $\tau_{\text{fast}}$ increased from 0.7 ms to 5.6 ms (Fig. 9C). For theoretical as with the experimental responses, it was necessary to increase the stimulus amplitude with the more heavily filtered step to produce the same magnitude of response. An explanation for these changes is that with slower stimulus onsets, the rate of change and extent of the Ca$^{2+}$ excursion at the first site are both reduced, which slows and diminishes the magnitude of fast adaptation. Both experimental and theoretical observations em...
phasize the importance of using a stimulus with a rapid attack to reveal the fast adaptive process.

DISCUSSION

Two components of adaptation

Characterization of the time course of mechanoelectrical transducer currents showed that adaptation in turtle auditory hair cells proceeds with at least two time constants differing by an order of magnitude. To account for this observation, and other evidence summarized in Ricci et al. (1998), we constructed a model of adaptation of the transducer channels that involved two processes with different kinetics, each governed by stereociliary Ca$^{2+}$ levels. The model reproduced several features of the experimental responses, including the sensitivity to the concentrations of external Ca$^{2+}$ and intracellular calcium buffer, BAPTA, and a dependence on the onset speed of the stimulus. The model also mimicked the behavior of the turtle hair cell’s transducer in its capacity to generate damped oscillatory responses. The resonant behavior depended on the kinetics of the mechanism responsible for the fast component of adaptation.

Models of hair-cell transducer adaptation assume that intracellular Ca$^{2+}$ controls the range of bundle displacements detected by the mechanoelectrical transducer channel. This assumption is expressed in our model by the notion of the channel’s “set point.” One mechanism by which the set point might be altered invokes a myosin motor connected to both the transducer channels on the stereocilium’s side wall and the internal actin cytoskeleton (reviewed in Hudspeth and Gillespie 1994). The speed of a myosin motor will be limited by the kinetics of myosin ATPase, which for fast skeletal muscle has a cycle time on the order of 50–100 ms at room temperature (Hibberd and Trentham 1986; Pollard et al. 1991). Although the most precise kinetic information is available for the skeletal muscle myosin II, the adaptation motor may depend on an unconventional myosin-I known to be present in hair-cell stereocilia (Hasson et al. 1997). The cycle time of myosin-I also may approach 50 ms (Pollard et al. 1991).

The properties of the fast component of hair-cell adaptation, its submillisecond kinetics, its symmetry for small positive and negative displacements, and its insensitivity to the ATPase-inhibitors vanadate and BDM, all argue that it does not rely on a conventional myosin-based motor. An alternative hypothesis is that fast adaptation is mediated by conformational rearrangements in the channel protein itself (Crawford et al. 1989) or in molecules directly connecting it to the cytoskeleton. A specific mechanism would be that the Ca$^{2+}$-dependent modulator, $M_1$
(Eq. 2), is an auxiliary subunit of the transducer channel, the activation of which alters the gating kinetics of the channel stabilizing it in its closed configuration (Fettiplace et al. 1992). Activation of $M_1$ would result from association with Ca$^{2+}$ bound to S1, which itself may be a separate Ca$^{2+}$-binding protein like calmodulin or may be an integral part of $M_1$.

Location of the Ca$^{2+}$-binding sites

Arrangement of the two Ca$^{2+}$-binding sites along the stereociliary axis is convenient for a model constructed in cylindrical coordinates but may be physically unrealistic particularly with respect to the more distant second site. S1, positioned at 20–50 nm from the center of the transducer channel complex, is of dimensions only slightly greater than ion channels (~10 nm diam), which may be arranged in a cluster. Furthermore S1 does not need to be located directly on the axis, and its placement anywhere within a hemispherical shell centered on the channel complex would yield similar theoretical results. The spatial extent of S1 might represent the local cytoplasmic distribution of a Ca$^{2+}$-binding protein like calmodulin, which has been suggested to mediate calcium’s role in adaptation (Walker and Hudspeth 1996). The transducer channels were assumed to be entirely located at the apex of the stereocilium but channels may be present at both ends of the tip links (Denk et al. 1995). In the current model, channels placed on the side wall of the stereocilium were neglected due to the added geometric complexity incurred, which would have removed the radial symmetry and considerably lengthened the computations. Because S1 is located close to the channels, our model will still provide an adequate description of fast adaptation for channels on the side wall.

The location of S2 is more problematic because its distance from the transducer channels (150–200 nm in most calculations) was large relative to the size of the channel. However, we found that provided that the Ca$^{2+}$-affinity of the site was adjusted, similar theoretical responses could be achieved with S2 positioned 50–100 nm from the transducer channels. Such distances are within the dimensions of the electron-dense plaques, representing cytoskeletal linking proteins or arrays myosin head groups, into which the tip links insert (Hudspeth and Gillespie 1994). Nevertheless, we do not feel it is possible from our results to derive a precise location for S2, and thus the coordinates for S2 in the model may not impose major limitations on its physical realization. In particular, the results neither establish nor eliminate a myosin motor as the mechanism of the slow component of adaptation.

**APPENDIX**

**Geometric considerations of the model stereocilia**

A single cylindrical stereocilium, radius, $a = 0.2 \mu m$ and length, $L = 6 \mu m$, was compartmentalized in cylindrical coordinates ($r$, $\theta$, $z$). Ca$^{2+}$ influx occurred via transducer channels located in the center of the top of the stereocilium. It was assumed that the whole cytoplasmic volume was available for diffusion, though in reality some fraction will be occupied by actin filaments and therefore will be inaccessible.

Cytosplasmic diffusion of Ca$^{2+}$ ions, and both free and Ca$^{2+}$-bound mobile buffers, BAPTA and ATP, was represented as follows:

$$\frac{\partial u}{\partial t}_{\text{diffusion}} = D_r \cdot \nabla^2 u = D_r \left( \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial u}{\partial r} \right) + \frac{\partial^2 u}{\partial z^2} \right)$$ (A1)

where $u$ is the concentration of the appropriate species and $D_r$ its diffusion coefficient.

**Mechanoelectrical transducer channel**

The kinetics of the mechanoelectrical transducer channel in turtle hair cells can be fit by a scheme (Crawford et al. 1989) involving two closed states ($C_1$ and $C_2$) and one open state ($O_n$):

$$\begin{align*}
    k_i & C_i \equiv C_i = O_n \\
    k_o & \equiv k_o
\end{align*}$$ (A2)

with state probabilities given by

$$\begin{align*}
    \frac{d p_{C_i}}{d t} &= k_i \cdot p_{C_i} - k_o \cdot p_{C_i} \\
    \frac{d p_{O_n}}{d t} &= k_o \cdot p_{C_i} - k_i \cdot p_{O_n} \\
    \frac{d p_{C_1}}{d t} &= k_1 \cdot p_{C_1} + \alpha_n \cdot p_{O_n} - (k_1 + \beta_n) \cdot p_{C_1} \\
    \frac{d p_{C_2}}{d t} &= k_2 \cdot p_{C_1} + \beta_n \cdot p_{C_2} - (k_2 + \alpha_n) \cdot p_{O_n}
\end{align*}$$ (A3)

in which $p_{O_n} + p_{C_1} + p_{C_2} = 1.0$, and rate constants (in ms$^{-1}$) that depend on the displacement, $X$, in $\mu m$ are

$$\begin{align*}
    k_i &= k_1 \cdot e^{[A_{0}(X_r-X_s)/2]} \\
    k_o &= k_2 \cdot e^{[A_{0}(X_r-X_s)/2]} \\
    \beta_n &= \beta_1 \cdot e^{[-B_{0}(X_r-X_s)/2]} \\
    \alpha_n &= \alpha_1 \cdot e^{[-B_{0}(X_r-X_s)/2]}
\end{align*}$$ (A4)

where $k_0 = 10.0 \text{ ms}^{-1}$, $A_0 = 18.072 \text{ mm}^{-1}$, $B_0 = 1.9 \text{ ms}^{-1}$, $B_O = 6.024 \text{ mm}^{-1}$, and $X_r$ in $\mu m$ is the position of the set point (the adaptation displacement) regulated by other processes as described later (Eq. A10). These values were derived from fits to experimental records giving a half-activation for the conductance at 0.18 $\mu m$ with a slope 2.42 $\mu m^{-1}$.

**Ca$^{2+}$ currents and fluxes**

Ca$^{2+}$ influx was derived from measured transducer currents and channel permeabilities for hair cells tuned to ~300 Hz in various extracellular Ca$^{2+}$ concentrations (Ricci and Fettiplace 1998). The average Ca$^{2+}$ current per stereocilium, $i_{Ca}$, is given by

$$i_{Ca} = \frac{i_{MT} \cdot p_{Ca}}{n_s} = i_{MT} \cdot p_{Ca}$$ (A5)

where $i_{MT}$ is the maximum transducer current, $i_{MT}$ is the maximal transducer current per stereocilium, $p_{Ca}$ is the Ca$^{2+}$ permeability and $n_s$, the total number of stereocilia, is 90 (Hackney et al. 1993). Calculations were performed for four external Ca$^{2+}$ concentrations in which the values of $i_{MT}$ and $p_{Ca}$ were: 2.8 mM Ca$^{2+}$, 7.8 pA, 0.58; 1 mM Ca$^{2+}$, 9.3 pA, 0.42; 0.35 mM Ca$^{2+}$, 10.6 pA, 0.28; and 0.07 mM Ca$^{2+}$, 11.9 pA, 0.13.

The rate of change of free Ca$^{2+}$ concentration due to the opening or closing of transducer channels is

$$\frac{\partial [Ca^{2+}]}{\partial t} = \frac{-i_{Ca}}{2 \cdot F \cdot V_c}$$ (A6)

in which $i_{Ca}$ is the maximal Ca$^{2+}$ current per stereocilium defined in Eq. A5, $F$ is Faraday’s constant, $p_{Ca}$, $i_{MT}$ (computed from Eq. A3) is the open probability of the transducer at time $t$, and $V_c$ is the volume of the compartment into which Ca$^{2+}$ enters.

**Ca$^{2+}$-dependent modulator**

The Ca$^{2+}$-dependent modulators are assumed to be uniformly distributed over $n$ compartments along $z$ direction from $z_1$ to $z_n$, and
of Ca\(^{2+}\) active form specified region was used cylindrical region, was 1.5 nm for both sites. Thus the overall dis-
site 2

er tors are given in parentheses.

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The proportion of the site, \(f_{SCa}\), that is Ca\(^{2+}\) bound in each compartment subsequently regulates the conformational change of a modulator from inactive \(M\) form to active \(M^*\) form described as follows:

\[
M_i(r, z) = f_{SCa} M_i(r, z) + \frac{k_{Mj}^i}{k_{Mj}^i} M_j^*(r, z) \quad (A8)
\]

\(M_j^*(r, z)\) is the proportion of active modulator at position \((r, z)\) and must be converted into a displacement of the transducer channel’s set point \(X_i\). As a first approximation, a linear transfer function over the specified region was used

\[
x_i = k_i \int_0^{r_2} \int_0^{z_2} M_j^*(r, z) \cdot r \cdot dr \cdot dz + \lambda_i \quad (A9)
\]

where \(k_i\) and \(\lambda_i\) are constants in \(\mu\)m, and \(r_2\) the radius of the cylindrical region, was 1.5 nm for both sites. Thus the overall displacement of the set point based on the assumption of uniform distribution over \(n\) regions can be generalized as follows:

\[
X_i = \sum_{i=1}^n x_i \quad (A10)
\]

from which the total displacement of the set point, \(X_n\), can be directly substituted into Eq. A4. Model parameters are listed in Table A1.

**Calcium extrusion and buffering**

Ca\(^{2+}\) is extruded by CaATPase pumps (Crouch and Schulte 1995; Tucker and Fettiplace 1995; Yamaoh et al. 1998) that are assumed to be uniformly distributed in the hair bundle membrane and bind Ca\(^{2+}\) with a dissociation constant \(K\) = 0.5 \(\mu\)M. An inward Ca\(^{2+}\) leak maintains the steady state at the stereociliary base (Sala and Hernández-Cruz 1990). The combination of Ca\(^{2+}\) extrusion and leakage is defined as

\[
\begin{align*}
\frac{d[Ca^{2+}]}{dt} &= \frac{v_{max} \cdot A(r, z)}{V_C} \left( \frac{[Ca^{2+}]_o - [Ca^{2+}]}{[Ca^{2+}] + K_m} \right) \\
\end{align*}
\]

where \([Ca^{2+}]_o = 0.1 \text{ } \mu\text{M}\) is the initial steady-state concentration, \(v_{max} = 3.32 \times 10^{-6} \text{ moles } \text{s}^{-1}\) (based on 100 ions \(\text{s}^{-1}\) pump and 2,000 pumps \(\text{mm}^{-2}\)) is the maximal velocity of transport, and \(A(r, z)\) is the effective pumping area of a compartment \((r, z)\) and \(V_C\) is the volume of that compartment. A 10-fold increase or decrease in the pump density from its control value of 2,000/\(\mu\text{m}^2\) had little effect on the time course of transducer adaptation for an isolated stimulus. Ca\(^{2+}\) binding to fixed buffers is described by

\[
[Ca^{2+}] + B_i \approx \frac{k_{0i}^F}{k_{i}^F} CaB_i \quad (A12)
\]

where \(B_i\) and \(CaB_i\) represent the Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound fixed buffers. The dissociation constant \(k_{0i}^F\) is equal to \(k_i^F/k_{i}^F\). The rate of change of free [Ca\(^{2+}\)] by the fixed buffer is

\[
\frac{d[Ca^{2+}]}{dt} = \frac{k_{0i}^F}{k_{i}^F} \cdot ([Ca_{i}^F] - [B_i]) - k_i^F \cdot [Ca^{2+}] \cdot [B_i] \quad (A13)
\]

where \([Ca_{i}^F]\) is the total concentration of \(B_i\). The rates of change of free and Ca\(^{2+}\)-bound buffers also can be related to Eq. A13

\[
\frac{d[B_i]}{dt} = \frac{d[Ca^{2+}]}{dt} = \frac{d[Ca^{2+}]}{dt} \quad (A14)
\]

Because the fixed buffer is uniformly distributed, \([Ca_{i}^F]\) is a constant for all compartments. The kinetic scheme for the diffusible buffer is similar to the fixed buffer

\[
Ca^{2+} + B_i \approx \frac{k_{0i}^D}{k_{i}^D} CaB_i \quad (A15)
\]

where \(B_i\) and \(CaB_i\) are the Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound diffusible buffers, \(k_{0i}^D\) and \(k_{i}^D\) the binding and unbinding rate constants and \(k_{0i}^D\) (\(= k_i^D/k_{0i}^D\)) the dissociation constant. If the \(B_i\) and \(CaB_i\) are treated as a single species, the net exchange of \([B_i]\) and \([CaB_i]\) between compartments is 0; i.e., the spatial distribution of total buffer remains fixed (Neher 1986; Roberts 1994). Then the rate of change of free [Ca\(^{2+}\)] produced by the diffusible buffer can be defined as

\[
\frac{d[Ca^{2+}]}{dt} = \frac{k_{0i}^D}{k_{i}^D} \cdot ([Ca_{i}^D] - [B_i]) - k_i^D \cdot [Ca^{2+}] \cdot [B_i] \quad (A16)
\]

where \([Ca_{i}^D]\) is the total concentration of \(B_i\). The rates of change of Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound buffers also can be related to Eq. A16

\[
\frac{d[B_i]}{dt} = \frac{d[Ca^{2+}]}{dt} = \frac{d[Ca^{2+}]}{dt} \quad (A17)
\]

where \(\nabla^2[B_i]\) is the differential operator defined in Eq. A1. Parameters for Ca\(^{2+}\) buffering are listed in Table A2.

**Integration**

A set of ordinary and partial differential equations (ODEs and PDEs) was integrated to calculate the spread of free Ca\(^{2+}\). For each compartment, ODEs computed the open probability of transducer channels (Eq. A3), Ca\(^{2+}\)-dependent modulation processes (Eqs. A7 and A8) and the reaction of fixed buffers (Eq. A13). All PDEs were related to the diffusion processes. One PDE (Eq. A17) determined the concentration of Ca\(^{2+}\)-free diffusible buffer and one PDE described the total rate of change of [Ca\(^{2+}\)] and is a summation of Eqs. A1, A6, A11, A13, and A17:
TABLE A2.  

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_F^1$</td>
<td>Forward rate constant for the fixed buffer</td>
<td>100 µM$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$k_F^2$</td>
<td>Dissociation constant for fixed buffer</td>
<td>100 µM</td>
</tr>
<tr>
<td>$[B_0]$</td>
<td>Total concentration of fixed buffer</td>
<td>4 nM</td>
</tr>
<tr>
<td>$k_0$</td>
<td>Rate constant of Ca$^{2+}$ binding to BAPTA</td>
<td>500 µM$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$k_{ATP}$</td>
<td>Rate constant for Ca$^{2+}$ binding to ATP</td>
<td>500 µM$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>$[B_1]$</td>
<td>Total concentration of BAPTA</td>
<td>0.1, 1, 10 mM</td>
</tr>
<tr>
<td>$[B_2]$</td>
<td>Total concentration of ATP</td>
<td>1 mM</td>
</tr>
<tr>
<td>$k_0$</td>
<td>Dissociation constant for BAPTA</td>
<td>0.2 µM</td>
</tr>
<tr>
<td>$k_{ATP}$</td>
<td>Dissociation constant for ATP</td>
<td>2300 µM</td>
</tr>
<tr>
<td>$D_m$</td>
<td>Diffusion coefficient of free Ca$^{2+}$ ions</td>
<td>400 µm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_B$</td>
<td>Diffusion coefficient for BAPTA</td>
<td>200 µm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_A$</td>
<td>Diffusion coefficient for ATP</td>
<td>220 µm$^2$ s$^{-1}$</td>
</tr>
</tbody>
</table>

Finite difference equations and boundary conditions are analogous to those described earlier (Wu et al. 1996). Compartment sizes increased with distance from the source, from 1 nm in the r and z directions near the channel to 10 nm at 200 nm from the channel. Computations were performed at a variable integration interval (0.5–50 µs).

The sources of the values for most of the parameters are given in Wu et al. (1996). Values for ATP are taken from Naraghi and Neher (1997). BAPTA, bis(2-aminophenoxy)N,N,N',N'-tetraacetic acid.

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