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POTASSIUM CURRENTS IN HAIR CELLS ISOLATED FROM THE COCHLEA OF THE CHICK

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SUMMARY

1. Potassium currents were characterized in tall hair cells of the chick's cochlea. Outward potassium currents were found to flow through two distinct classes of channels.

2. Individual hair cells were isolated from 200 μm long segments of the apical half of the chick's cochlea. Whole-cell voltage-clamp and current-clamp recordings were made from these cells.

3. Voltage responses to injected current ranged from high-frequency (100–250 Hz) oscillations in some cells, to slowly repetitive Ca^{2+} action potentials or slow oscillations (5–20 Hz) in others.

4. Ionic currents recorded in voltage clamp also varied in different hair cells. Cells with high-frequency voltage oscillations had rapidly activating Ca^{2+}-dependent outward K^+ current, $I_{K(Ca)}$. Cells that generated action potentials had slow delayed rectifier outward K^+ current, I_K, and inward rectifier current, I_{IR}. All hair cells had inward Ca^{2+} current.

5. $I_{K(Ca)}$ activated positive to -45 mV. Tail currents reversed at the K^+ equilibrium potential. This current was eliminated in Ca^{2+}-free solutions, or when exposed to 10 mM-TEA. This outward current was fully activated within 1–3 ms at 0 mV. The whole-cell current was noisy and ensemble variance analysis suggested a single-channel conductance of 63 pS near 0 mV.

6. I_K activated positive to -50 mV. Tail currents reversed at the K^+ equilibrium potential. This current was not eliminated in Ca^{2+}-free solutions, and was relatively resistant to external TEA. I_K activated slowly, reaching peak values in 10–20 ms at 0 mV. This current showed little variance and the average single-channel conductance based on macroscopic noise near 0 mV was 8 pS.

7. External tetraethylammonium (TEA) or Ca^{2+}-free saline eliminated the high-frequency voltage oscillations seen in many basal cells. In contrast TEA had little effect on the slow action potentials (or low-frequency oscillations) seen in cells with I_K.

8. $I_{K(Ca)}$ was prominent in hair cells originating 1·0–2·0 mm from the cochlear

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apex. I_K and I_{IR} dominated the membrane conductance of tall hair cells originating within 0·5 mm of the cochlear apex.

9. The frequency of voltage oscillation in apical cells was temperature-dependent, nearly doubling for each 10 °C rise in temperature.

10. I_{IR} activated at membrane potentials negative to −75 mV. The average time constant of activation at −100 mV was 2 ms. Tail currents reversed at the K^+ equilibrium potential and did not depend on the external Na^+ concentration. I_{IR} was blocked by 5 mM-Cs^+ or 100 μM-Ba^{2+} in the external saline.

INTRODUCTION

The cochlea of the chicken contains 10000 hair cells distributed along the 4 mm long basilar membrane (von During, Andres & Simon, 1985; Tilney & Tilney, 1986). Hair cells in different regions of the cochlea are stimulated at different frequencies during a complex sound, with cells at the apex subject to the lowest frequencies, and cells at the cochlear base subject to the highest frequencies in the range from approximately 50 to 5000 Hz (von Bekesy, 1960). The morphology of hair bundles (Tilney & Saunders, 1983) and cell bodies varies along the tonotopic axis (length) of the cochlea (von During *et al.* 1985). Across the cochlea there is a progression from tall, cylindrical hair cells on the superior edge of the cochlea to short cells with greatly expanded apical surfaces over the free basilar membrane of the inferior edge (Hirokawa, 1978; Tanaka & Smith, 1978).

We were interested to see if functionally distinct hair cells (from different cochlear regions) had different electrical membrane properties. Any such differences would have important consequences for the form of their receptor potentials *in vivo*, and so contribute to sound processing in the cochlea. Previous work showed that some tall hair cells from approximately the mid-third of the cochlea were electrically resonant, and that electrical resonance arose out of the interaction between voltage-dependent Ca^{2+}, and Ca^{2+}-dependent K^+ currents (Fuchs, Nagai & Evans, 1988), as in electrically tuned hair cells of lower vertebrates (Lewis & Hudspeth, 1983; Art & Fettiplace, 1987; Hudspeth & Lewis, 1988*a*, *b*). In contrast, tall hair cells from the apical tip of the cochlea tended to exhibit slowly repetitive, action potential-like responses to depolarization. Those cells also had inward Ca^{2+} currents; however, their outward currents were dramatically slower, and appeared to be Ca^{2+}-insensitive (Fuchs *et al.* 1988). The experiments presented here were designed to demonstrate the variety of potassium currents found in tall hair cells from the chick's cochlea, and their different roles in shaping the hair cell's membrane potential response to injected current. Some of these results have appeared in abstract (Fuchs & Evans, 1988*a*).

METHODS

Two to eight-week-old post-hatch chicks (*Gallus domesticus*, Leghorn) were used in these studies. Fertilized eggs were obtained from a number of suppliers (predominantly the Colorado State University School of Agriculture) and maintained in a temperature- and humidity-controlled incubator. Hatchlings were left in the incubator until fluffed out, then transferred to warmed brooding cages (12:12 h light:dark cycle) with a continuous supply of feed and water. Animals were killed by cervical dislocation and decapitated. The procedures for cochlear dissection and hair cell isolation have been described previously (Fuchs *et al.* 1988). With these methods hair cells could be isolated from selected regions of the cochlea.

The recording chamber containing isolated cells was placed on the stage of an inverted microscope (Nikon Diaphot-TMD) and single cells were viewed through a 40 × objective modified for Hoffman modulation contrast microscopy. The longest length of the hair bundle, and the length and width of the cell body (and sometimes cuticular plate width), were measured using an ocular micrometer to a resolution of 0·6 μm. Cells were judged to be healthy if their hair bundles were

TABLE 1. Composition of solutions (mM)

	NaCl	KCl	$CaCl_2$	$MgCl_2$
	External saline solution			
Control	154	6	5·6	2·3
Low Ca^{2+}	154	6	0·1	—
Ca^{2+} free	154	6	—	7·9
High K^+	128	32	5·6	2·3
K^+ free	160	—	5·6	2·3
10 mM-TEA	144	6	5·6	2·3
5 mM-Cs^+	149	6	5·6	2·3
154 mM-Tris	—	6	5·6	2·3
5·6 mM-Ba^{2+}	154	6	—	2·3
	Internal (pipette) solution			
	—	140	0·1	2·0

straight and intact, and if the cell body had a 'pearly', three-dimensional appearance under Hoffman optics. Graininess of the cell body, or a clearly resolved nucleus, were taken as signs of trauma and these cells were avoided. The recording chamber was slowly perfused with oxygenated saline using a gravity feed, and the excess was siphoned off under vacuum. Experiments were performed at room temperature, 22–25 °C. In one series of experiments (Fig. 12) the bath temperature was made to range from 10 to 40 °C by altering the temperature of water flowing through a brass chamber surrounding the recording dish.

Recording methods

Whole-cell, tight-seal recordings (Marty & Neher, 1983) were made on single cells. Electrodes of 2–5 MΩ resistance were pulled from borosilicate glass (Boralex, Rochester Scientific, Rochester, NY, USA) on a vertical puller (David Knopf Instruments) and coated with Sylgard (Dow Corning, Midland, MI, USA) or cross-country ski wax (SWIX purple, Astra-Gruppen, A/S, Skarer, Norway) to reduce transmural capacitance. Seal resistances ranged from 2 to 60 GΩ, although the lowest values usually did not provide good whole-cell recordings. In whole-cell recording the input capacitance, clamp time constant and series resistance were calculated as described by Marty & Neher (1983). Series resistance ranged from 5 to 20 MΩ and was compensated 60–80% using the amplifier's analog controls (Axopatch 1A, Axon Instruments, Burlingame, CA, USA; or an 8900, Dagan Corp., Minneapolis, MN, USA). Arithmetic correction for the residual, uncompensated series resistance was made during later analysis of current–voltage relationships. The average input capacitance of the hair cells was 7 pF, resulting in voltage clamp time constants of 35–140 μs.

The external solutions (Table 1) contained 8 mM-glucose, and were buffered to pH 7·4 with 5 mM-HEPES. Barium (100 μM) was added directly, with no substitution. One millimolar EGTA was added to the Ca^{2+}-free saline. The internal solution was buffered to pH 7·2 with HEPES–KOH. Eleven millimolar EGTA and 2 mM-K_2ATP were also added. Experimental solutions were made to flood the immediate vicinity of the cell by opening the stopcock controlling the outflow of one of a set of parallel glass capillaries (80 μm diameter) placed about 200 μm from the cell. Solution exchange was effective and fast (within 0·1 s in the best cases) although positioning and flow rate were critical. One tube in the array contained control saline to provide rapid recovery from the experimental solutions.

Data analysis

Hair cell membrane potential and transmembrane current as well as a trigger pulse and verbal comments were recorded on magnetic tape (Vetter Model D, Rebersburg, PA, USA). The tape-recorder had a low-pass corner (−3 dB) of 9 kHz at 15 in/s. Capacitive transients to small

voltage steps were digitized directly (bypassing the tape-recorder) at 83 kHz for computation of series resistance. All other data were digitized off-line at sampling rates above the Nyquist limit and analysed using DAOS (Laboratory Software Associates, Fitzroy, Victoria 3065, Australia) on a PDP 1123/+ (A–D DT2782A, Data Translations, Marlboro, MA, USA). The usually small linear leak currents and residual capacitive currents were digitally subtracted using currents from opposite polarity voltage steps. All of the membrane potentials reported here have been corrected for the junction potentials between internal and control saline: −4 mV for KCl, −3 mV for CsCl.

Estimates of single-channel size and number were obtained using ensemble variance analysis (Sigworth, 1980). A large number of records of outward current at a given voltage command were collected and averaged together. This averaged current was then subtracted from each individual sweep. The difference current from each sweep was squared and an average variance calculated from the ensemble (see Fig. 8). The background variance in the current at the membrane potential at which no ionic currents were activated was subtracted from the average variance. A plot was then constructed of the instantaneous variance as a function of the amplitude of the mean current and this plot was fitted with a parabola of the form

$$\text{var} = iI - I^2/N, \tag{1}$$

where I = mean current, i = single-channel current and N = channel number. This analysis was carried out on cells that had only one type of outward K^+ current, or in Ca^{2+}-free saline to examine I_K.

When average results are given they are listed ± the standard deviation, unless otherwise stated.

RESULTS

Variance in membrane properties of chick cells

Whole-cell recordings from chick cochlear hair cells revealed striking variability in their electrical properties. Columnar ('tall') cells from well-separated regions of the apical half of the cochlea fell into two general classes with respect to their voltage response to injected current, and in the characteristics of the net ionic current recorded under voltage clamp. Cells from more basal regions (between 1 and 2 mm from the cochlear apex) had resting potentials near −60 mV and exhibited rapid voltage oscillations (ranging from 100 to 250 Hz in different cells) when depolarized by steps of injected current (Fig. 1*A*). In voltage clamp such 'fast' cells displayed pronounced outward rectification at membrane potentials positive to −40 mV (Fig. 1*B*), but were essentially passive at more negative membrane potentials. Cells with high-frequency voltage oscillations had rapidly activating outward currents (Fig. 1*B*). The average length of twenty-three basal cells with rapid voltage oscillations was 14·9 ± 3·3 μm.

Tall cells originating within 0·5 mm of the apical tip of the cochlea had different behaviour. They had more negative resting membrane potentials (near −75 mV) and could generate broad, slow action potentials when depolarized by current steps (Fig. 2*A*). During steady depolarizations to membrane potentials positive to −55 mV these action potentials became smaller, more sinusoidal and repetitive at frequencies between 5 and 20 Hz in different cells (Fig. 11*B*, and see Fuchs *et al.* 1988). Voltage clamp of 'slow' cells revealed a mixture of inward and outward current (Fig. 2*B*). Small inward currents preceded the maintained outward current during depolarization of these cells. In addition to steady-state outward rectification positive to −50 mV, inward rectification was seen at membrane potentials negative to −75 mV. Cells with slowly repetitive action potentials or slow oscillations had slowly activating outward currents (Fig. 2*B*). Twenty-one apical cells that exhibited slowly repetitive action potentials averaged 21·1 ± 3·4 μm in length.

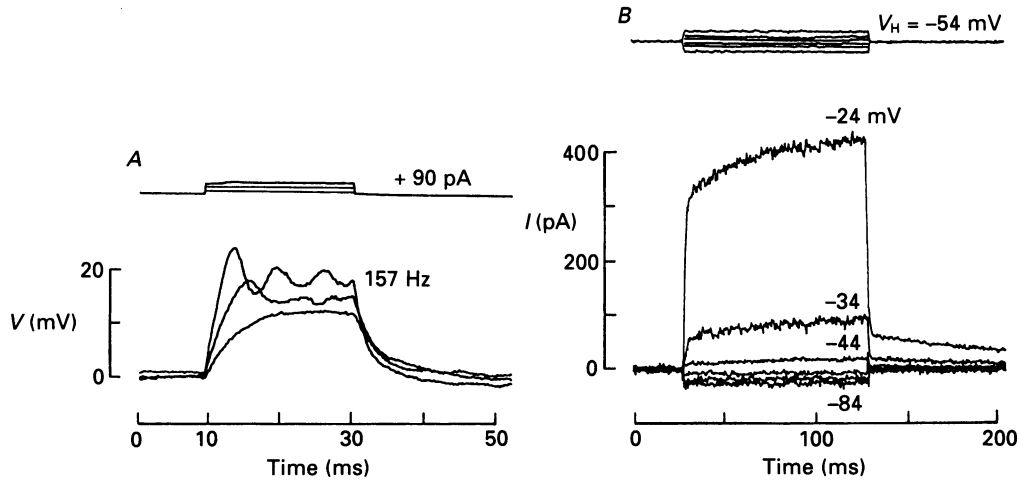

Fig. 1. Basal hair cell. *A*, damped oscillations of the membrane potential at 157 Hz were produced by depolarizing current injection in a hair cell. Successively larger steps of current (30, 60, 90 pA) elicited the three responses shown. The resting potential was −54 mV. *B*, voltage clamp of this same cell from the holding potential of −54 mV. This cell was 14·4 μm long and was isolated from a section 1·3–1·7 mm from the apical tip of the cochlea. Input capacitance was 6 pF; listed voltage uncorrected for uncompensated series resistance of 10 MΩ.

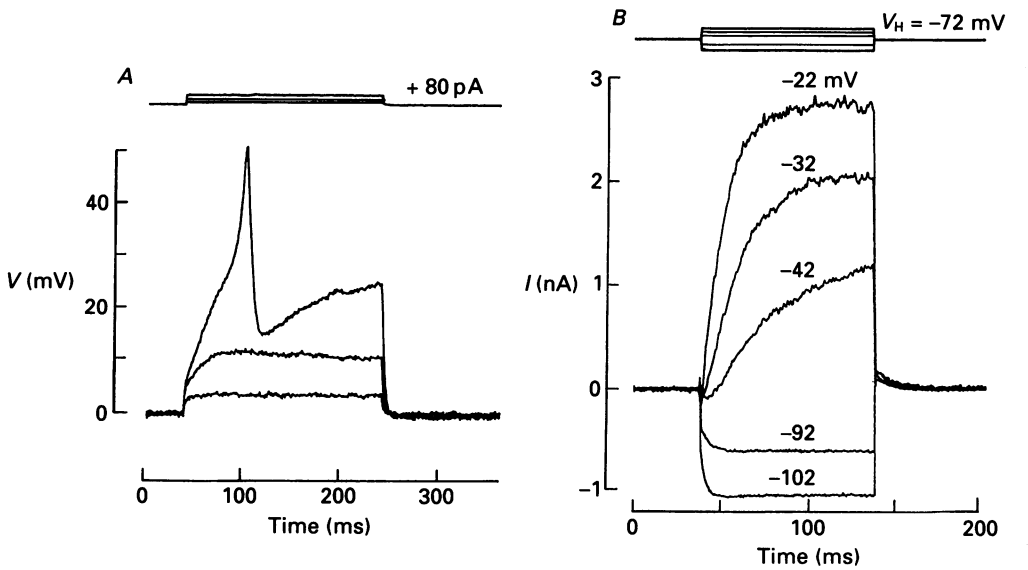

Fig. 2. Apical hair cell. *A*, action potentials were evoked in some hair cells by depolarizing current injection (20, 50 and 80 pA from the resting potential of −77 mV). *B*, voltage clamp of this same cell from the holding potential of −72 mV. This cell was 20 μm long and isolated from the apicalmost 0·3 mm of the cochlea. Input capacitance was 8 pF; listed voltages not corrected for the uncompensated series resistance of 7·1 MΩ.

A comparison of the outward current kinetics recorded from fast and slow cells is shown in Fig. 3. The middle part of the rising phase of each record has been fitted with a single-exponential curve in order to derive a time constant describing the activation rate of the major component of outward current. Such single exponentials

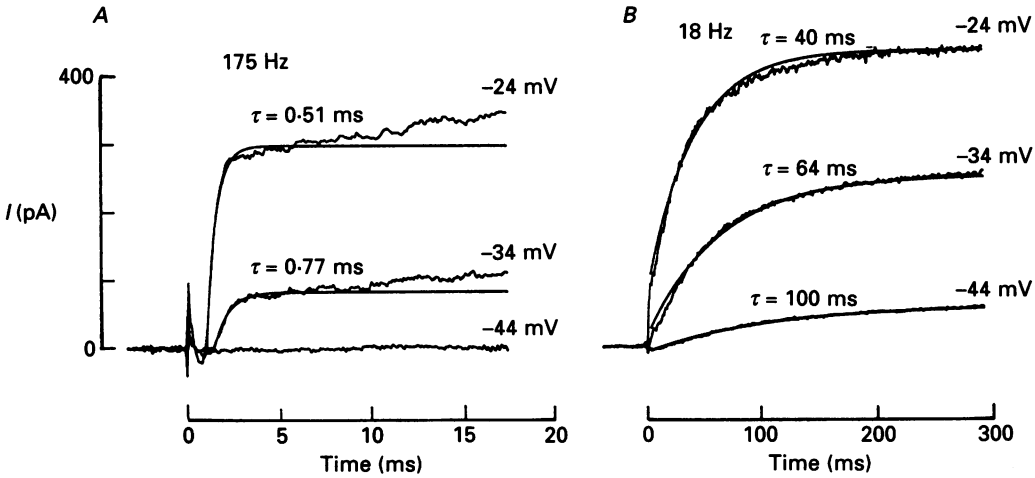

Fig. 3. Comparison of outward current kinetics in fast and slow cells. *A*, in a shorter cell (12 μm long) from a basal region (1·3–1·9 mm from the apex) outward current rose rapidly, reaching 90 % of the steady state within 1 ms. *B*, in a taller cell (17 μm) from an apical region of the cochlea (0·4–0·6 mm from the apex) larger, outward currents that rose slowly were seen. Beside each record is listed the time constant (τ) of a single exponential fitted by eye (the small slow component in *A* was ignored).

do not precisely describe the form of the rising current, but do provide a simple way to compare the kinetics of the predominant outward current in different cells (Fuchs & Evans, 1988*b*). As can be seen in Fig. 3, the cell whose oscillation frequency was 175 Hz had outward currents that activated with time constants of less than 1 ms. There was another, slower component that made a minor contribution to the total outward current and that was ignored in this analysis. The 18 Hz cell had outward current activation time constants nearly two orders of magnitude slower. Also evident in Fig. 3 is the fact that outward current kinetics depended on membrane potential, becoming faster at more depolarized levels.

In Fig. 4 the relationship between the kinetics of the outward current and membrane potential is plotted on semilogarithmic axes for four hair cells with different resonant frequencies. The rate of rise of the outward current varied in an approximately exponential manner with membrane potential in all four cells. Although not shown here, the voltage oscillation frequency in chick hair cells was also voltage dependent, increasing with depolarization (Fuchs *et al.* 1988). An arrowhead indicates the average membrane potential for the best-resolved voltage oscillation for each cell. It is clear that the rate of outward current activation increases with oscillation frequency in a general way amongst these four cells. That is, cells with higher frequency voltage oscillations had more rapidly activating outward currents.

However, we did not observe a continuum in oscillation frequencies or outward current kinetics amongst chick hair cells. Instead, hair cell voltage responses fell into two well-separated classes: broad, often spike-like waveforms that were repetitive at 5–20 Hz, and more rapid, low-amplitude oscillations ranging from 100 to 250 Hz in

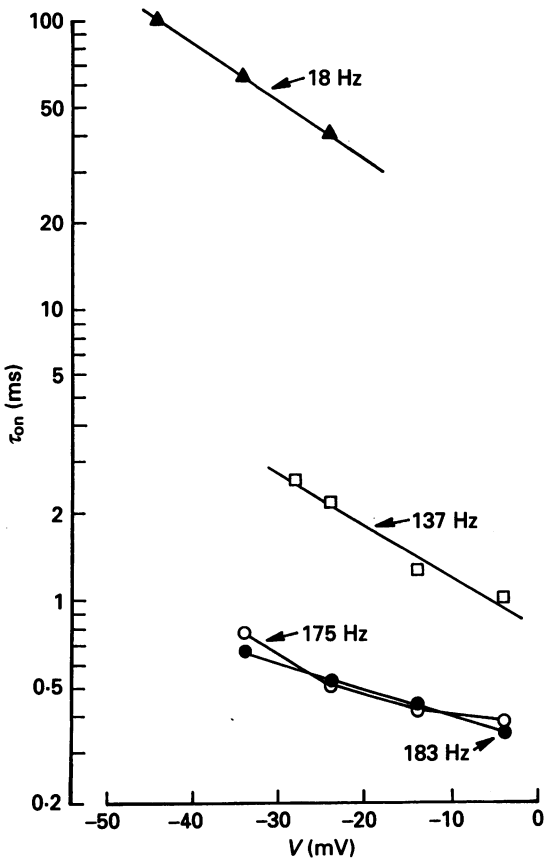

Fig. 4. Activation time constants (τ_{on}) as a function of membrane potential in four hair cells with different oscillation frequencies. The best-resolved oscillation frequency for each cell is listed beside an arrow indicating the average membrane potential at which that oscillation was measured. Time constants were derived from single exponentials as in Fig. 3.

frequency. The gap in frequency of voltage response between 20 and 100 Hz is reflected in the large differences in outward current kinetics between the slowest and next fastest cells seen in Fig. 4. These observations led to the definitions of 'fast' and 'slow' cells, as in Figs 1 and 2.

While we did not observe cells with voltage responses within that intermediate frequency range, it was possible to find cells, particularly in the region 0·5–1·0 mm from the cochlear apex, that exhibited a *combination* of fast and slow voltage responses. These cells had small, irregular, rapid oscillations when depolarized to membrane potentials between −40 and −20 mV, and larger, very slow 'oscillations'

at more negative membrane potentials. Cells with combined voltage responses exhibited both a rapidly activating, and a much more slowly activating outward current in roughly equal proportions under voltage clamp. A small slow component could be seen occasionally in cells that were dominated by fast current, and that generated rapid voltage oscillations in current clamp (Figs 1*B* and 3*A*). Thus, there did not appear to be a continuum in the activation kinetics of outward current in different hair cells. Rather, cells were dominated by either a fast, or a slow outward current, or had mixtures of two kinetically distinct and pharmacologically separable components. It is the intent of the present work to demonstrate that different K^+ channels contribute to the range of electrical behaviours found amongst chick cochlear hair cells.

The ionic dependence of fast and slow outward currents

The identities of the ions carrying the fast and slow outward currents were determined from tail current reversal potential measurements. Outward currents were elicited by a voltage command from the resting potential to 0 mV and their initial value as a function of the membrane potential during a subsequent voltage step was measured. Because hair cells could have a combination of fast and slow outward current, as well as an inward rectifier current (Fig. 2*B*), it proved necessary to measure specific tail currents under conditions where other currents were either absent or blocked. The fast outward currents shown in Fig. 5*A* were the TEA-sensitive component of net membrane current obtained by subtracting the residual current in the presence of 10 mM-TEA from the net current in control conditions. In this example the tail currents decayed rapidly and reversed at an interpolated membrane potential of −81 mV. In another cell the same experiment yielded a reversal potential of −85 mV. The K^+ equilibrium potential, E_K, under these experimental conditions was −80 mV.

Reversal potential experiments in slow apical cells (Fig. 5*B*) were obtained in 5·6 mM-Ba^{2+} saline (Table 1) to block the inward rectifier (see below) and any contribution from the Ca^{2+}-dependent outward current. Inward Ba^{2+} tail currents were much faster than those of the slow outward current (Fuchs, Evans & Murrow, 1990) and so would not interfere with these measurements of slow current tails. In the illustrated cell the tail current reversal potential was estimated to be −79 mV. In a repetition of this experiment on another cell with slow outward current a reversal potential of −80 mV was found. Thus, both the fast and slow tail currents were found to reverse very close to the K^+ equilibrium potential, implicating K^+ as the major charge-carrying species for both currents.

To further test the role of K^+ in carrying the slow outward current, hair cells were bathed in an external solution containing 32 mM-K^+ instead of the usual concentration of 6 mM. Under these conditions the slow outward current tails were found to reverse at an average membrane potential of −32 mV (± 2, $n = 4$). The K^+ equilibrium potential was calculated to be −37 mV. This experiment was not attempted on the fast tail currents due to the difficulty of resolving these in high-K^+ saline.

The *I–V* curve for the fast tail current was non-linear about the reversal potential, as has been noted previously for Ca^{2+}-activated K^+ current (Pallotta, Magleby &

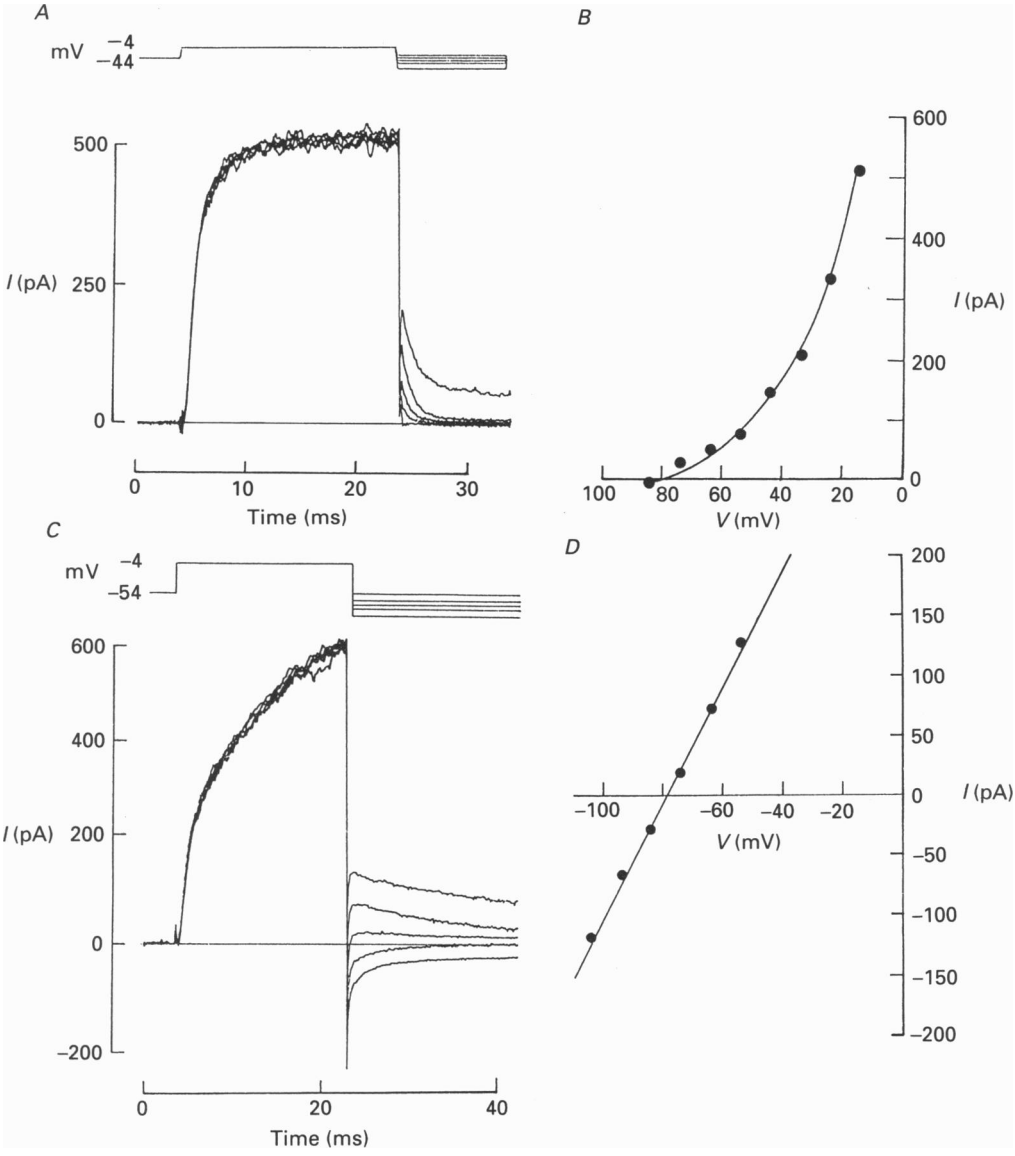

Fig. 5. Reversal potential of outward tail currents. *A*, reversal of rapid, TEA-sensitive tail currents in a cell from a region 1·3–2·0 mm from the apical tip. The activating voltage command was followed by test potentials to various membrane potentials ranging from −14 to −94 mV. *B*, a plot of peak tail current amplitude as a function of membrane potential. Fitted by eye. *C*, reversal potential of slow current tails. Cell from 0 to 288 μm from apical tip. Test potentials ranged from −54 to −104 mV. This experiment was performed in the presence of 100 μM-Ba^{2+} to block the inward rectifier currents. *D*, tail current *I–V* plot fitted by eye.

Barrett, 1981; Art & Fettiplace, 1987). In contrast, the slow current I–V curve was reasonably straight about the reversal potential, similar to the instantaneous I–V curve described for the delayed rectifier in squid axon (Hodgkin & Huxley, 1952). Evidence will be shown later to support the idea that the slow current flows through

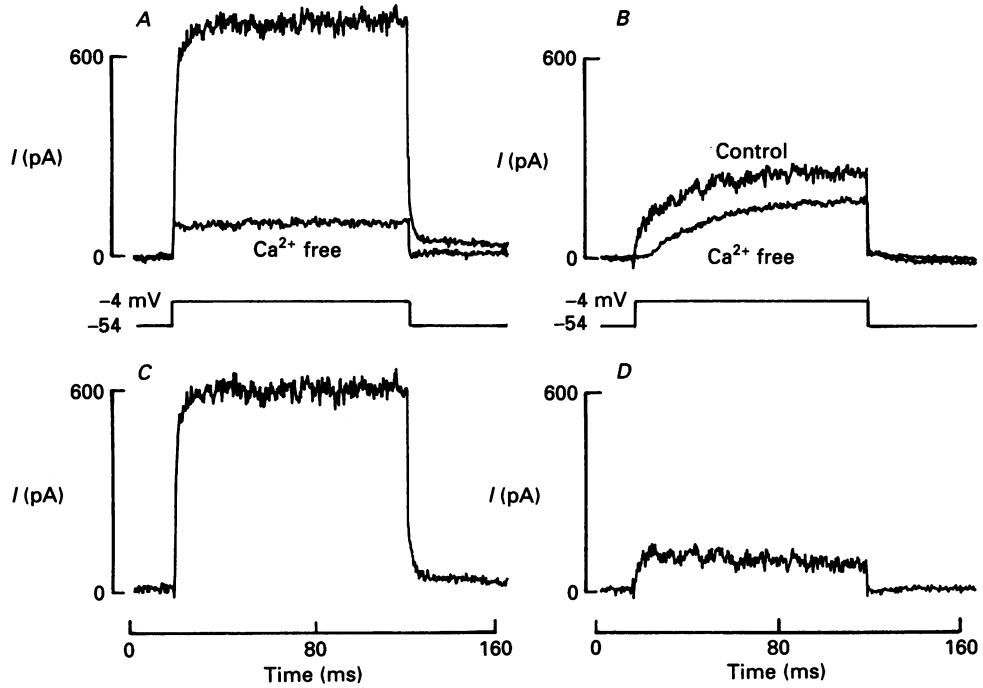

Fig. 6. The effect of Ca^{2+}-free saline on basal and apical cells. During voltage commands to near 0 mV a basal cell and an apical cell from the same cochlea were exposed to saline in which Mg^{2+} substituted for Ca^{2+}. *A*, in the basal cell essentially all the active outward current was eradicated, leaving an instantaneous leak current. *B*, in the apical cell less than half of the outward current was blocked, leaving a slower, less noisy component. *C* and *D*, the Ca^{2+}-sensitive difference currents in the two cells, while of different amplitude, had similar kinetics.

channels that are similar to delayed rectifier channels in other cells. Further support for the distinction between fast and slow current was found in the voltage dependence of tail current decay. The time constant of decay of the fast current increased e-fold with 22 mV hyperpolarization. The slow tail current time constant changed e-fold in 79 mV.

The following experiments were performed on cells containing a combination of fast and slow outward current to illustrate those currents' differential sensitivity to external Ca^{2+} or the K^+ channel blocker TEA.

Differential Ca^{2+} sensitivity of fast and slow outward currents

The Ca^{2+} dependence of outward current in a number of cells was examined using Ca^{2+}-free solutions (Table 1). The rapidly activating outward current of fast basal cells was eliminated by superfusion of the cell with a Ca^{2+}-free saline (Fig. 6*A*). Slow outward currents such as those seen in Figs 2*B* and 3*B* were essentially unchanged

in Ca^{2+}-free saline. This is illustrated in Fig. 6*B* by showing the effect of Ca^{2+}-free saline on a cell that had a combination of fast and slow outward current. Superfusion of the cell with Ca^{2+}-free saline immediately eliminated a portion of the outward current. That Ca^{2+}-sensitive component of the outward current was rapidly

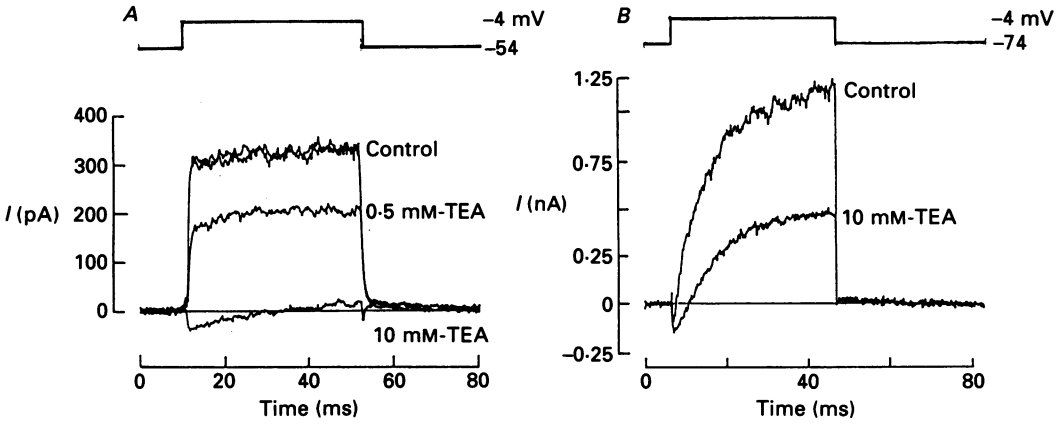

Fig. 7. Effect of TEA on fast and slow outward currents. *A*, in a basal cell approximately 40 % of the outward current was blocked by 0·5 mM-TEA, and 100 % of the net outward current was blocked by 10 mM-TEA. *B*, in an apical cell approximately half of the total outward current was blocked by 10 mM-TEA.

activating and relatively noisy (Fig. 6*D*), similar in these respects to the Ca^{2+}-sensitive current in a fast basal cell (Fig. 6*C*). The outward current that remained in Ca^{2+}-free saline, the Ca^{2+}-insensitive component, was slowly activating, and was considerably less noisy (Fig. 6*B*). This Ca^{2+}-insensitive slow outward current is believed to flow through voltage-activated K^+ channels. The lower noise level of the slow outward current suggests that the single-channel current is smaller than that of the fast component (see below). Another observation suggested that the slow outward current did not require an influx of Ca^{2+} for its activation. While exposure to saline containing Ba^{2+} in place of Ca^{2+} resulted in loss of the outward current in fast cells (Fuchs *et al.* 1990), this was not the case in cells with slowly activating outward current.

TEA sensitivity of the fast and slow outward currents

Fast and slow outward currents in chick hair cells could also be distinguished by their differential sensitivity to the K^+ channel blocker TEA. A characteristic of the rapidly activating $I_{K(Ca)}$ in hair cells of lower vertebrates is that it is sensitive to submillimolar concentrations of TEA. This current was half-blocked by 0·5 mM-TEA in bull-frog hair cells (Lewis & Hudspeth, 1983). The fast outward current in chick hair cells was also sensitive to TEA, showing half-block in a number of cells at an average concentration of 0·7 mM, and complete block in 10 mM-TEA (Fig. 7*A*). (The residual outward relaxation seen in Fig. 7*A* was due to a small amount of slow current in this cell.) In contrast, 10 mM-TEA was relatively ineffective in cells with a large proportion of slow current, blocking approximately half the outward current in the cell shown in Fig. 7*B*. As in the case with Ca^{2+}-free saline, the residual current in TEA rose more slowly, and was less noisy, than the control current. While a

dose–response relation has not been obtained, it was clear from numerous experiments that even 20 mM-TEA was not sufficient to completely block the slow current. Comparison of the effects of Ca^{2+}-free saline and 10 mM-TEA on a number of cells suggested that 10 mM-TEA blocked from 10 to 30% of the Ca^{2+}-insensitive slow outward current.

Thus the rapidly activating outward current of chick hair cells was Ca^{2+}-dependent and relatively sensitive to TEA. It was similar in these respects to Ca^{2+}-activated K^+ current observed in hair cells of lower vertebrates (Lewis & Hudspeth, 1983; Art & Fettiplace, 1987; Hudspeth & Lewis, 1988*a*) and will be referred to as $I_{K(Ca)}$ hereafter. The slow outward current did not require Ca^{2+} influx for its activation, and it was relatively insensitive to TEA. These and other characteristics have prompted its inclusion into the rather broad category of delayed rectifier K^+ currents, and it will be referred to as I_K.

Single-channel conductance of the fast current

The relative 'noisiness' of $I_{K(Ca)}$ in chick hair cells suggested that the single-channel current might be large, perhaps like that of the large Ca^{2+}-activated K^+ channel found in chromaffin cells and muscle (Marty, 1981; Pallotta *et al.* 1981). Estimates of the single-channel conductance underlying the fast outward current of chick hair cells were obtained using the ensemble variance analysis (Sigworth, 1980) of whole-cell, macroscopic currents (see Methods). These measurements were made on cells that had only the fast, Ca^{2+}-activated outward current, as demonstrated by exposure to Ca^{2+}-free solutions. In six cells the single-channel current was approximately 5 pA at 0 mV membrane potential. This corresponds well with that found for the large Ca^{2+}-activated K^+ channel in other cell types (4–6 pA at 0 mV in physiological K^+ concentrations; reviewed in Marty, 1983). The number of channels, N, was quite low, ranging from 30 to 150. Taking the driving force as the difference between the membrane potential and the K^+ equilibrium potential, the average single-channel conductance was 62·8 (±17·5) pS.

The single-channel current, i_{sc}, was also calculated as the ratio of the steady-state variance to the mean current at membrane potentials near −30 mV (eqn (2)). Again, the single-channel conductance was computed from the current amplitude and the driving force on K^+ at that membrane potential.

$$i_{sc} = \text{variance/mean}. \tag{2}$$

From seven cells in which this measurement was made an average single-channel conductance of 38·3 (±11·2) pS was calculated. This smaller conductance at −30 mV membrane potential compared to 0 mV results from the non-linear open-channel current–voltage relation in physiological K^+ concentrations seen in Fig. 5*B*.

Single-channel conductance of the slow current

The slow Ca^{2+}-independent K^+ current, I_K, was much less noisy than $I_{K(Ca)}$ (Figs 6*B* and 7*B*), suggesting that it was carried by a lower conductance channel. Ensemble variance analysis of slow current noise was performed on cells that had only slow current, or were bathed by Ca^{2+}-free saline. This analysis provided estimates of a single-channel current of just under 1 pA at 0 mV (Fig. 9). Taking the

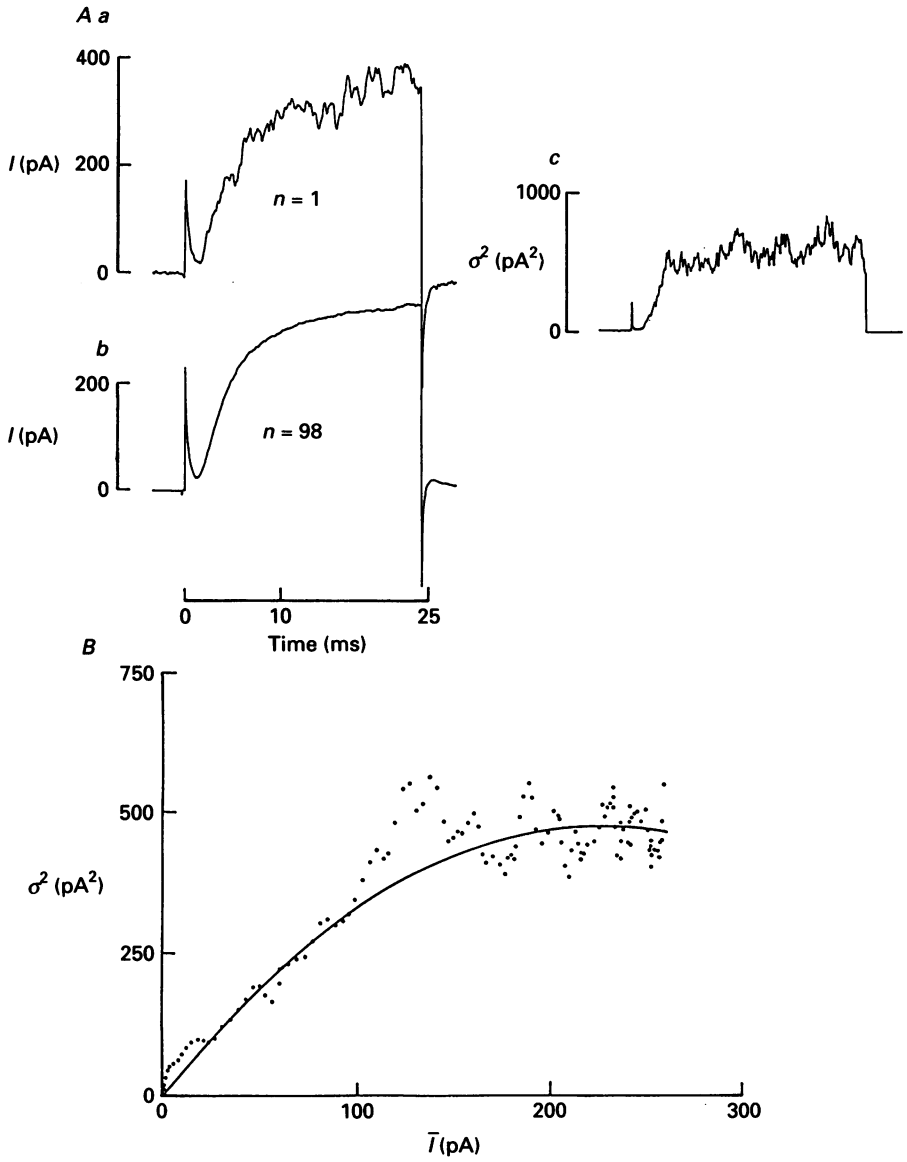

Fig. 8. Ensemble variance analysis of noise on fast outward current in a basal cell. *Aa*, single sweep of outward current observed at −4 mV. *Ab*, average of ninety-eight sweeps as in *Aa*. *Ac*, average instantaneous variance of ninety-eight outward current records obtained by subtracting *Ab* from each record. *B*, plot of instantaneous variance as a function of mean current. Continuous line calculated from eqn (1) (Methods) and fitted using minimum least-squared difference criterion. Single-channel current was 4·2 pA; the number of channels was 109.

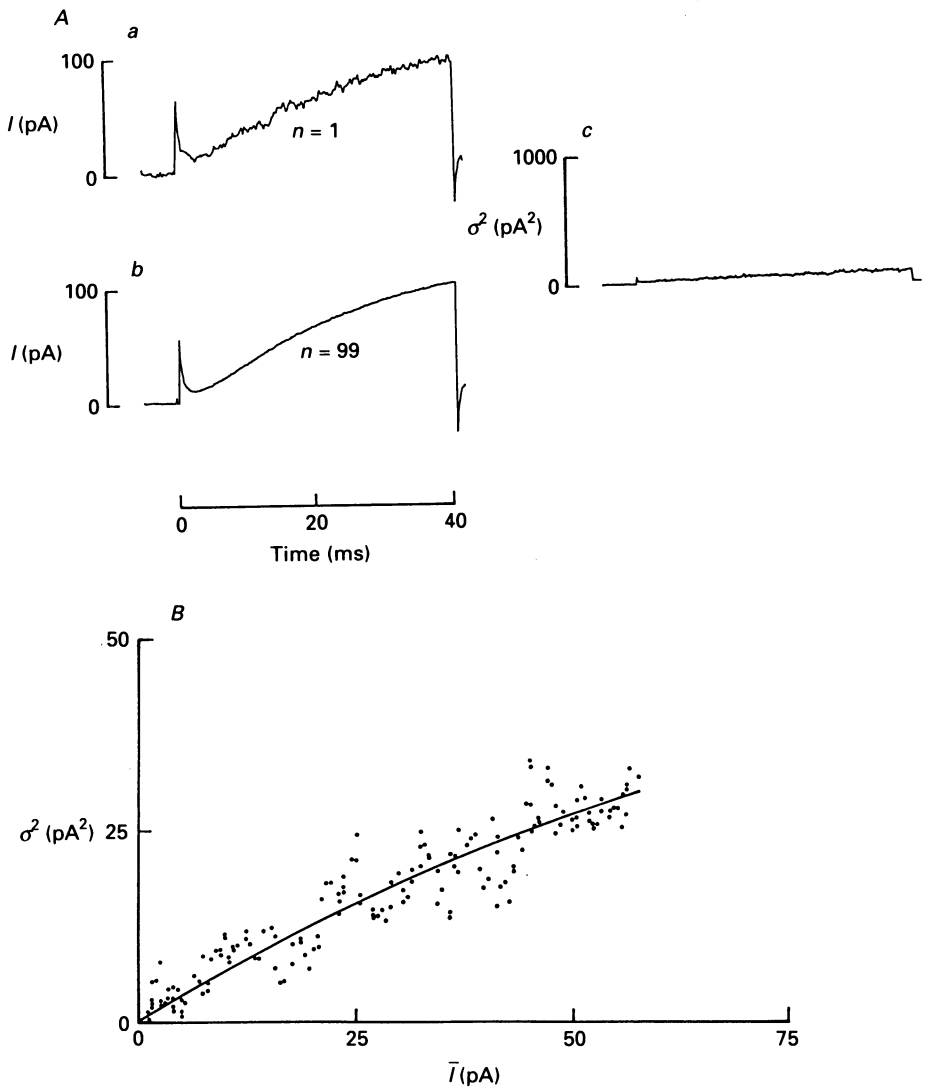

Fig. 9. Ensemble variance analysis of noise on outward current in a slow apical cell, as for Fig. 8. Continuous line in *B* calculated from eqn (1) (Methods). Single-channel current was 0·7 pA; the number of channels was 5000.

driving force on K^+ at this membrane potential, the single-channel current corresponded to an average single-channel conductance of 8·2 (±4·5) pS in six cells. The value of N, the number of channels, ranged from 400 to 8000 in different cells.

Contribution of each current to the hair cell voltage response

Chick hair cells possessed two distinct types of outward K^+ current: a rapid, Ca^{2+}-activated K^+ current, $I_{K(Ca)}$, and a much slower, voltage-activated K^+ current, I_K,

that resembled a delayed rectifier in several respects. The role of these two K^+ channel types in the cell's voltage response to injected current could be distinguished using TEA or Ca^{2+}-free solutions to eliminate $I_{K(Ca)}$. In Fig. 10*A* and *C* is shown the effect of these two experimental solutions on the fast voltage oscillations generated

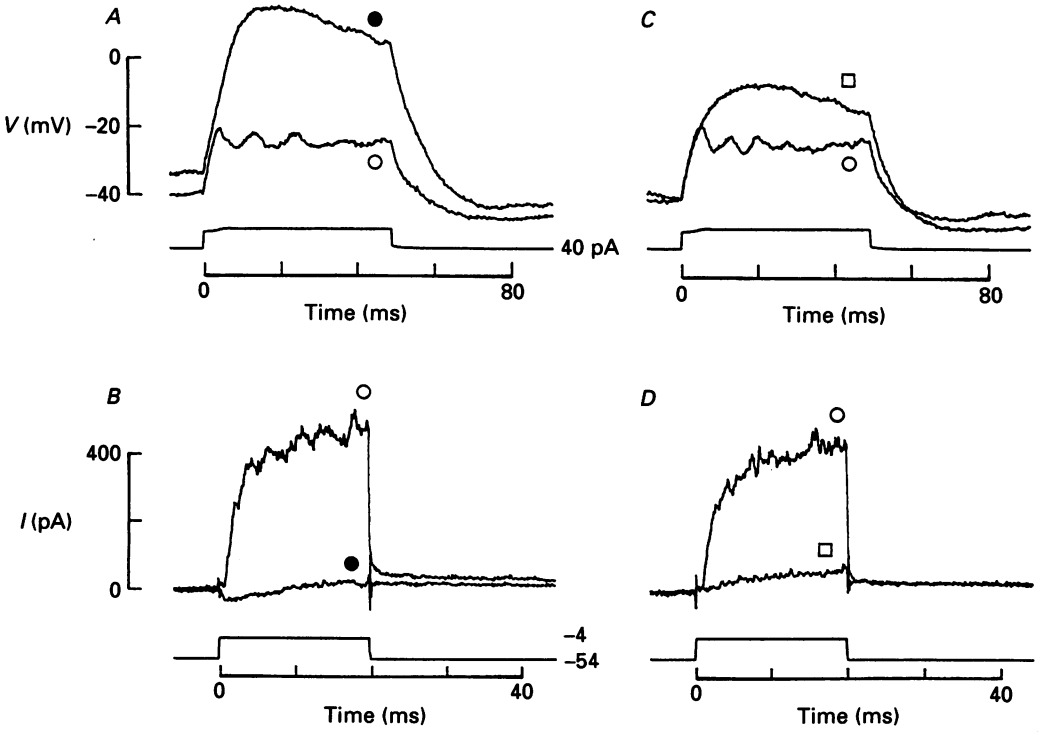

Fig. 10. Effects of zero-Ca^{2+} saline and TEA on fast voltage oscillations. This basal cell was depolarized from an initial resting potential of −60 mV with 40 pA steady current, then a 60 pA step was applied to produce voltage responses seen here. The average ringing frequency was 138 Hz. *A*, 10 mM-TEA caused a 6 mV depolarization of the resting potential and eradicated the oscillatory voltage change. *B*, in voltage clamp 10 mM-TEA blocked all the fast outward current. *C*, replacement of Ca^{2+} with Mg^{2+} also caused the voltage response to become non-oscillatory, and in voltage clamp (*D*) this manipulation blocked most of the cell's outward current. ○, control; ●, 10 mM-TEA; □, zero Ca^{2+} (Mg^{2+}).

in one cell (average frequency 138 Hz). Both experimental conditions prevented the cell from oscillating in response to the same current step, and instead depolarizing current pulses produced large, slow potential changes. In the same cell it was shown that external TEA or the Ca^{2+}-free solution blocked the fast outward current (Fig. 10*B* and *D*). No inward current was seen in the Ca^{2+}-free saline. Thus the small voltage oscillations that occur at frequencies of several hundred hertz depend on $I_{K(Ca)}$.

Since we have not identified a specific blocker of the slow I_K we could not demonstrate the particular effect of its loss on the voltage response of the cell in

current clamp. None the less it was clear that this current was sufficient to generate, in combination with inward Ca^{2+} current, the slow voltage responses found especially in apical cells. This can be appreciated by considering the effect of 10 mM-TEA on a cell exhibiting slow voltage oscillations (Fig. 11). Ten millimolar TEA reversibly

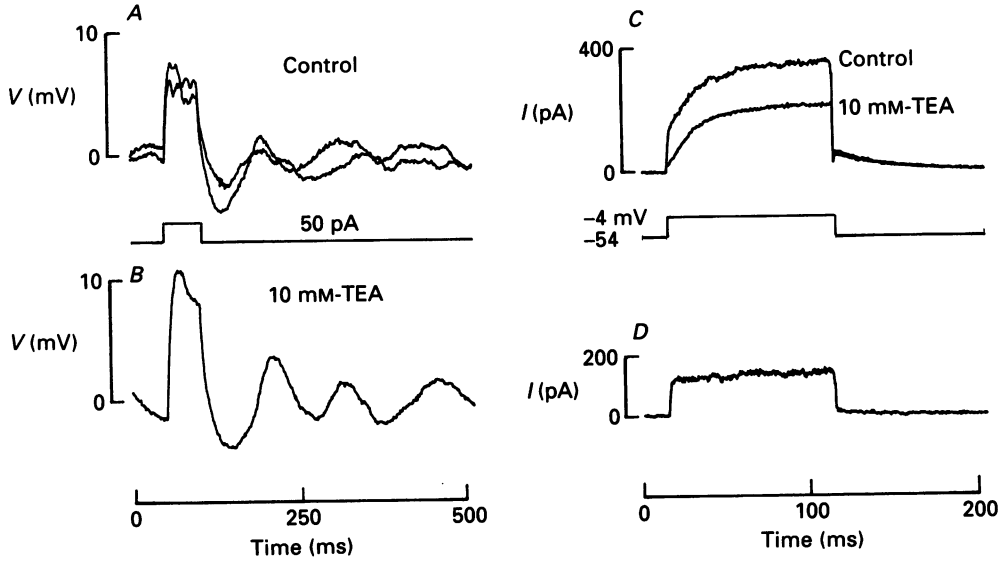

Fig. 11. Slow voltage oscillations persist in 10 mM-TEA. *A*, from a resting potential of −39 mV, a 50 pA step evoked a fast noisy plateau followed by slow oscillatory behaviour. Two responses are superimposed. *B*, when 10 mM-TEA was superfused onto the cell the response to depolarization became a large slow voltage change, and the slow oscillation was enhanced. *C*, in voltage clamp the cell produced an outward current that was partially blocked by 10 mM-TEA (records shown are averages of ten sweeps). *D*, the TEA-sensitive component of the outward current was obtained by subtraction of the residual current in TEA from the unblocked control. This difference current was rapidly activating. A tall cell (30 μm) isolated 0·2–0·4 mm from the cochlear apex.

blocked the $I_{K(Ca)}$ in this cell (Fig. 11*D*), but left a substantial amount of slow I_K (Fig. 11*C*). When the membrane potential of the same cell was recorded in current clamp the slow voltage oscillations persisted in the TEA solution (Fig. 11*B*). Thus, under conditions in which only the slow current remained, the cell could still generate slowly repetitive voltage changes. Calcium-free solutions, while not reducing I_K, nevertheless do prevent slow voltage oscillations (not shown). Presumably these are regenerative responses that require the inward Ca^{2+} current normally present in these cells (Fuchs *et al.* 1990).

Temperature dependence of hair cell voltage oscillations

The hair cell recordings reported in this paper were performed at room temperature (22–25 °C), considerably cooler than bird core temperature (near 40 °C). The frequency of hair cell voltage oscillations arising from gated ionic flux might be

expected to vary with temperature. In order to examine this point, voltage oscillations were recorded from hair cells at different temperatures. Due to the difficulty of recording from one cell during a change of temperature, a number of cells were recorded from at a single temperature and their best oscillation frequencies

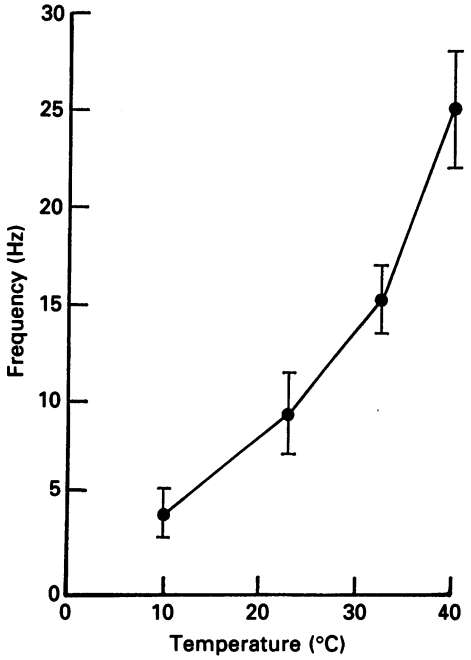

Fig. 12. Temperature dependence of the oscillation frequency in apical cells. Voltage oscillations were measured in apical cells and the resonant frequency determined from the best-resolved oscillation. Mean and S.E.M. shown for three to seven cells at each temperature. Each temperature actually represents a range of ±2 °C. Each mean was significantly different from those adjacent.

averaged. In hair cells isolated from near the cochlear apex the frequency of voltage oscillations rose steeply with increasing temperature, nearly doubling between 10 and 20 °C (Fig. 12). Fewer high-frequency basal cells were studied at warmer temperatures. However, the average oscillation frequency of four cells at 30–33 °C (290 ± 72 Hz) was 1·8 times that of the population of basal cells ($n = 23$) measured near 22 °C (162 ± 40 Hz) suggesting a temperature dependence similar to that shown by slow apical cells. The best frequency of cochlear afferents in pigeons has been shown to vary as a function of temperature, with a Q_{10} of 2 at 30 °C in the range 125 Hz to 2 kHz (Schermuly & Klinke, 1985).

Inward rectifier current (I_{IR}) in hair cells

As previously noted, apically located tall hair cells tended to have marked inward rectification when polarized negative to −75 mV (Fig. 2*B*). This behaviour was similar to that observed in vestibular hair cells of the chick (Ohmori, 1984). For

membrane potentials as large as −120 mV, no inactivation of I_{IR} was observed over 200 ms. With larger hyperpolarizations this current could show an outward relaxation during the step. Inactivation of I_{IR} was seen at all membrane potentials when the cell's internal K^+ was replaced with Cs^+.

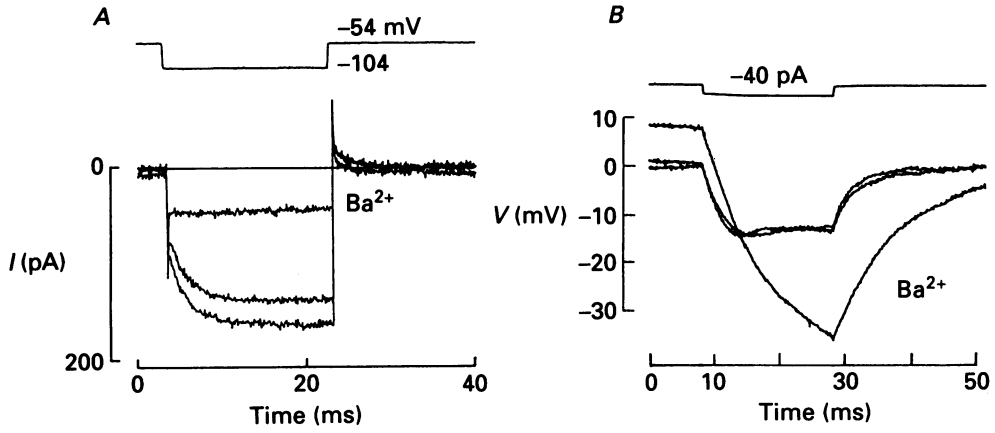

Fig. 13. The inward rectifier was blocked by 100 μM-Ba^{2+}. *A*, in the presence of 100 μM-Ba^{2+} the inward rectifier current was eradicated. Following Ba^{2+} exposure the inward rectifier returned to near control levels (smaller of two inward rectifier currents shown). *B*, in current clamp in the same cell the resting potential was −75 mV, and −40 pA injection caused a hyperpolarization of 15 mV. During superfusion with 100 μM-Ba^{2+} the cell depolarized by 8 mV and the same current step produced a 35 mV hyperpolarization that was not saturated in 20 ms. Returning to control saline produced complete recovery; pre- and post-experimental controls are superimposed.

Tall reversal experiments revealed that I_{IR} reversed very near E_K and shifted to more depolarized levels when the external K^+ concentration was increased. When external K^+ was eliminated, I_{IR} disappeared completely. Substitution of external Na^+ with Tris had no effect on the reversal potential; however, the amplitude of the current was slightly reduced. This may reflect a slight blocking action of Tris on the channel. Substitution of Na^+ by choline resulted in complete abolition of I_{IR}, presumably through a blocking action of choline.

I_{IR} in chick hair cells was blocked by 5 mM-Cs^+ in the external solution. This current was particularly sensitive to external Ba^{2+}, and was blocked at concentrations as low as 100 μM (Fig. 13*A*). The effect of the inward rectifier was to decrease the membrane resistance during hyperpolarizing current steps (Fig. 13*B*). When I_{IR} was blocked by external Ba^{2+} ions, the resistance of the membrane at potentials negative to rest increased dramatically, with a commensurate increase in the membrane time constant (Fig. 13*B*). In addition, external Ba^{2+} caused a 5–10 mV depolarization of the resting membrane potential, suggesting a role for I_{IR} in establishing the resting potential.

The time-dependent inward current could be fitted with a single exponential whose time constant decreased with increasing hyperpolarization. In five cells the time constant of activation ranged from 1·5 to 2·4 ms near −100 mV. No differences in the activation kinetics of this current were noted in cells from different regions of

the cochlea, although fast cells from basal regions of the cochlea had little or no I_{IR} (Fig. 1*B*).

DISCUSSION

Two kinetically and pharmacologically distinct outward currents were found in tall chick hair cells: a rapidly activating, Ca^{2+}-dependent K^+ current, $I_{K(Ca)}$, and a slowly activating delayed rectifier K^+ current, I_K.

Ca^{2+}-activated K^+ current

Basal cells with rapid voltage oscillations appeared to be similar to electrically tuned hair cells in turtles (Crawford & Fettiplace, 1981; Art *et al.* 1986; Art & Fettiplace, 1987) and frogs (Ashmore, 1983; Lewis & Hudspeth, 1983; Roberts, Robles & Hudspeth, 1986; Pitchford & Ashmore, 1987; Hudspeth & Lewis, 1988*a*, *b*). As in hair cells of lower vertebrates, $I_{K(Ca)}$ was the dominant outward current in rapidly oscillating chick hair cells. $I_{K(Ca)}$ in chick cells flowed through channels that appeared to be similar in size to the large conductance Ca^{2+}-activated K^+ channel found in many cell types, including chromaffin cells (Marty, 1981) and muscle (Barrett, Magleby & Pallota, 1982). In a variety of cell types this channel's conductance near 0 mV in physiological K^+ concentrations averages 60 pS (reviewed in Marty, 1983). In chick cells the single-channel conductance estimated from macroscopic noise measurements at −4 mV was 63 pS. The voltage dependence, rapid kinetics and pharmacology of the macroscopic $I_{K(Ca)}$in chick cells were similar to those of the analogous current described in hair cells of the turtle (Art & Fettiplace, 1987) and frog (Lewis & Hudspeth, 1983; Hudspeth & Lewis, 1988*a*). The single-channel conductance at −30 mV was 38 pS in chick hair cells, 37 pS in frog (Hudspeth & Lewis, 1988*a*), and 33 pS at −50 mV in turtle cells (Art & Fettiplace, 1987). A Ca^{2+}-activated K^+ current was also described briefly in vestibular hair cells of the chick (Ohmori, 1984).

Delayed rectifier K^+ current

The slower, voltage-activated K^+ current, I_K, was the dominant current in tall apical hair cells. This current had markedly slower kinetics than did $I_{K(Ca)}$. In cells dominated by I_K the voltage responses were correspondingly slow, consisting of low-frequency Ca^{2+} action potentials or smaller amplitude slow voltage oscillations. I_K activation did not require Ca^{2+} in the external medium, and was not blocked by 20 mM-Ba^{2+}. In addition this current was less sensitive to external TEA than was $I_{K(Ca)}$. The channels underlying this current appeared to be small since there was little noise. Ensemble variance analysis suggested a single-channel conductance of 8 pS.

I_K in chick hair cells appears to fall into the category of delayed rectifier K^+ current described in other cells (Hille, 1984). The voltage activation range of delayed rectifiers is generally similar to that of the chick I_K. Delayed rectifier kinetics vary in different cells, but can be quite slow (Adrian, Chandler & Hodgkin, 1970; Marty & Neher, 1985; Rossman & Trube, 1986). Half the delayed rectifier current of skeletal muscle is blocked by external TEA at 8 mM (Stanfield, 1970). Although we did not

measure a dose–response curve, the slow current in chick hair cells appeared to be only partially blocked by 10 mM-TEA. Delayed rectifier K^+ current can show slow inactivation (Adrian *et al.* 1970; Aldrich, Getting & Thompson, 1979). Inactivation of the slow outward current occurring over the course of seconds was seen in some chick hair cells; however, no attempt was made to characterize this phenomenon completely. In general the channels underlying delayed rectifier current in other cells are small, ranging from 2 to 18 pS (e.g. Standen, Stanfield & Ward, 1985), encompassing the channel size reported here. Thus, by these criteria it seems appropriate to refer to the Ca^{2+}-independent, voltage-activated K^+ current, I_K, of chick hair cells as a delayed rectifier.

Inward rectifier current

The inward rectifier found in hair cells of the chick's cochlea bears close resemblance to the K^+-selective inward rectifier of eggs and some neurones, and as described in chick vestibular hair cells (Ohmori, 1984). Its sensitivity to external Ba^{2+}, in combination with the selectivity for K^+, suggested that the inward rectifier in chick hair cells was most like the inward rectifier of starfish eggs (Hagiwara & Takahashi, 1974; Hagiwara, Miyazaki, Moody & Patlak, 1978) and neurones of the olfactory cortex (Constanti & Galvan, 1983).

This current could contribute to setting the resting membrane potential. In this respect it is significant that tall apical cells tended to have inward rectifier current, and generally had more-negative resting membrane potentials than did shorter basal cells that did not have an inward rectifier. Block of the inward rectifier by external Ba^{2+} resulted in an 8 mV depolarization of the resting potential in one cell. A second role for this current might be to increase membrane conductance during the compressive (hyperpolarizing) phase of sound waves, when other channels in these cells would be closed. As the inward rectifier current turns on it would decrease the membrane time constant and at more negative membrane potentials, tend to clamp the membrane near E_K. In the absence of inward rectification the membrane would become highly resistive, with a correspondingly long charging time, impairing the cell's ability to follow repetitive stimuli.

Distribution of K^+ currents in different cells

Tall hair cells from the chick's cochlea fell into two categories in terms of their membrane properties. 'Slow cells' were dominated by slow delayed rectifier and inward rectifier currents. Tall cells from the cochlear apex commonly exhibited these characteristics. 'Fast cells' had fast Ca^{2+}-activated K^+ current and lacked the inward rectifier. These cells were most common in basal section (more than 1·0 mm from the apical tip of the cochlea) and were on average 6 μm shorter in length than the slow apical cells. In previous work comparing smaller numbers of apical and basal cells the average lengths were 19·8 and 12·7 μm, respectively (Fuchs *et al.* 1988). Both classes of cells had inward Ca^{2+} currents (Fuchs *et al.* 1990). Although many of the hair cells fit into these categories, there were also cells that possessed both fast and slow outward current, implying that there was an overlap in the distributions of these two channel types within the cochlea.

While the differential distribution of I_K and $I_{K(Ca)}$ in apical and basal regions held true generally, it was also the case that cells with slow I_K could be found in basal

segments of the cochlea. Likewise, on rarer occasions cells that had largely $I_{K(Ca)}$ were found within 0·5 mm of the apical tip. Therefore, it may be an oversimplification to describe the distribution of these ionic conductances along a single cochlear axis. In addition to gradients in cell morphology along the cochlear length, hair cell morphology changes dramatically across the cochlear width, from tall cells nearest the neural insertion, to short cells out over the free basilar membrane. The number of tall cells decreases while short cells increase along the cochlea from apex to base (Tanaka & Smith, 1978). Bearing this in mind, it may be significant that apical cells with large $I_{K(Ca)}$ were usually shorter than those with large I_K. Likewise, slow cells from basal regions were among the taller cells from those segments. Thus, there may also exist gradients for channel expression that run *across* the cochlear width, in parallel with changes in hair cell morphology and neural innervation in that direction.

An additional type of K^+ current has been reported in hair cells, the inactivating K^+ current named I_A (Lewis & Hudspeth, 1983; Hudspeth & Lewis, 1988*a*). Rapidly inactivating outward currents were only rarely observed in the present work. Recent experiments have shown that large amounts of I_A are present in short hair cells of the chick's cochlea but not in the tall hair cells that are the subject of the present study (Murrow & Fuchs, 1989).

Functional consequences of different K^+ currents

This paper presents evidence that the ionic currents that underlie electrical tuning found in hair cells of turtles and frogs exist in some cochlear hair cells of the chick. Previous recordings from the cochlear ganglion (Manley, 1979; Manley, Gleich, Leppelsack & Ueckinghaus, 1985) and VIIIth nerve fibres (Gleich, 1987; Temchin, 1988) provided indirect evidence that electrical tuning might occur in the bird cochlea. In addition, the steep temperature sensitivity of hair cell voltage oscillations supports the hypothesis that these processes underlie the temperature sensitivity of auditory tuning in non-mammalian vertebrates (Schermuly & Klinke, 1985).

In addition to the electrical tuning mechanism, chick cells exhibit slowly repetitive depolarization through the interaction of Ca^{2+} current and the slow, delayed rectifier-type K^+ current. In some cells these appear as action potentials. These slow events fall below the range of frequencies over which $I_{K(Ca)}$ was proposed to account for the tuning of turtle hair cells (Art & Fettiplace, 1987). The functional role of these 'slow cells' is presently unresolved. Because I_{Ca} is temporally unopposed in these cells, it provides positive feedback during depolarizing stimuli. This will promote transmitter release, or further enhance the rectifying effect of the transduction current (Crawford & Fettiplace, 1981). Tall hair cells receive the preponderance of afferent contacts in the chick's cochlea (Tanaka & Smith, 1978; Whitehead & Morest, 1981; Manley, Gleich, Kaiser & Brix, 1989).

Alternatively, the slowly repetitive depolarization of these cells may contribute to the 'infrasound' sensitivity known to exist in some birds. A recent study of pigeon auditory afferents revealed low-frequency components in the spontaneous activity autocorrelogram (Temchin, 1988). In another study, single VIIIth nerve afferents that were selective for infrasound were found to innervate the apical portion of the pigeon cochlea (Klinke & Schermuly, 1989). If slow hair cells such as those reported

here do occur in the bird cochlea *in vivo* they could well confer such behaviour onto afferent neurones that contact them.

One interpretation of these results then is that the chick cochlea possesses an electrical tuning mechanism like that found in turtles and frogs. In addition, however, the chick cochlea employs other forms of hair cell excitability, perhaps to aid in the transduction of infrasound, or to discriminate between as yet unknown functions of tall (inner) and short (outer) hair cells.

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