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THE VOLTAGE RESPONSES OF HAIR CELLS IN THE BASAL TURN OF THE GUINEA-PIG COCHLEA

BY I. J. RUSSELL AND M. KÖSSL

From the School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG

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SUMMARY

1. Intracellularly recorded voltage responses to tones and to current injection were measured from inner (IHC) and outer (OHC) hair cells in the basal turn of the guinea-pig cochlea.

2. The voltage responses of IHCs to tones are increased by negative current and decreased by positive current. For any given current strength, the decrease in the receptor potential amplitude caused by positive current is less than the increase caused by negative current. This was attributed to the voltage-dependent rectification of the membrane conductance.

3. Estimates of the reversal potential of the receptor current were based on extrapolation of the slopes of the current–voltage relations for potentials more negative than −60 mV. The estimated reversal potentials were close to the measured endolymphatic potential.

4. Negative current increased the IHC membrane time constant and increased the transducer driving voltage. When receptor potentials to low-frequency tones were adjusted for constant driving voltage and for the membrane time constant, the positive DC component of the receptor potential was decreased relative to the AC component by current injection but the DC component in response to high-frequency tones near the best frequency of the IHC (16 kHz) was unchanged by negative current.

5. When compensated for constant driving voltage and low-pass filtering due to the basolateral membrane conductance, the proportion of the transducer conductance open in IHCs at rest is increased by negative current.

6. Negative current injection reduced the positive DC components of OHC voltage responses to low-frequency tones and may make them negative. In some cases negative current injection decreased the amplitude of the OHC receptor potential while positive current injection enhanced it. From these observations it is proposed that negative current injection shifts the operating point of the OHC transducer functions towards a positive, saturating region of the relationship and positive current decreases the proportion of the transducer conductance which is open at rest.

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INTRODUCTION

The organ of Corti is maintained in an elaborate electrochemical environment which provides the electromotive force for the flow of receptor current through the mechanically gated, non-selective, cationic transducer channels which are located at the apical pole of the hair cells (Davis, 1958; Corey & Hudspeth, 1979; Russell, 1983; Ohmori, 1985; Russell, Cody & Richardson, 1986; Holton & Hudspeth, 1986). The basolateral membranes of hair cells are the site of voltage- and ion-sensitive conductances whose properties have been predicted to control and alter the voltage responses of the hair cells to the flow of current through the transducer conductance (Ashmore & Meech, 1985; Santos-Sacchi & Dilger, 1988; Kros & Crawford, 1989, 1990). Polarization of the organ of Corti, by extracellular currents passed between electrodes located in the fluid-filled spaces of the cochlea, causes a change in the mechanical properties of the cochlea (Mountain, 1980), the amplitude and distortion of extracellular receptor potentials (Tasaki & Fernandez, 1952; Dallos, Schoeny, Worthington & Cheatham, 1969), and the amplitude of intracellularly recorded receptor potentials recorded from inner hair cells (IHCs) (Nuttall, 1985) and from the auditory nerve (Tasaki & Fernandez, 1952; Teas, Konishi & Wernick, 1970). These electrically induced changes in the electrical and mechanical properties of the cochlea have been attributed to changes in the properties of the OHCs. Support for this supposition has come from the discovery of fast and slow electrically induced changes in the dimensions of OHCs isolated from the cochlea (e.g. Ashmore, 1987). There have been few reports of the responses of cochlear hair cells *in vivo* to intracellular current injection. From studies on apical turn IHCs and OHCs and basal turn IHCs which were stimulated by tones close to their characteristic frequencies (CF), current injection through the microelectrode alters the amplitude of both IHC and OHC voltage responses (Russell, 1983; Nuttall, 1985; Dallos, 1986; Russell *et al.* 1986) but, in the basal turn, intracellular current injection does not alter the frequency selectivity of the IHCs (Nuttall, 1985).

It is now known that OHCs are capable of electrically induced motility and thus it might be expected that hair cell voltage responses to current injection will reflect both the electrical and the electromechanical properties of the hair cells. This paper describes the effect of intracellular current injection on the membrane potential and receptor potentials of IHCs and OHCs in the basal turn of the guinea-pig cochlea and attempts are made to identify those features which might be attributed to the electromechanical properties of the hair cells.

METHODS

Animal preparation and electrical recording

The experiments were conducted on twenty-four pigmented guinea-pigs between 220 and 270 g in weight which were raised in our own colony. The methods used in these experiments have been described in detail elsewhere (Russell & Sellick, 1983; Cody & Russell, 1987). The animals were anaesthetized with sodium pentatobarbitone (30 mg/kg), Operidine (1 mg/kg) and Droleptan (4 mg/kg) (Evans, 1979) and a tracheal cannula was inserted. Operidine was administered every 40 min and the body temperature of the guinea-pig was maintained at 38 °C with a heating blanket. The heart rate was monitored through a pair of skin electrodes placed on either side of

the thorax. The right cochlea was exposed through a lateral opening in the temporal bone and back-illuminated by a fibre optic light guide inserted through a hole made in the basal wall of the bulla.

Micropipettes were pulled from 1 mm o.d. fibre-filled glass tubing and filled with 4 M-potassium acetate and 0·1 M-KCl. Resistances of the micropipettes ranged between 100 and 140 MΩ when measured in the perilymph of the scala tympani. The Ag–AgCl indifferent electrode was inserted in the neck muscles.

The compound action potential (CAP) of the cochlear nerve was recorded with an electrode placed on the round window. The amplified responses of the round window electrode and the micropipette were stored on an FM tape-recorder, with a bandwidth of 4 kHz (Racal Store 4 DS). The recorded data were sampled at 200 μs intervals and analysed using programs written in ASYST (Macmillan) on a microcomputer (Opus).

Acoustic stimulation

Sound was delivered to the tympanic membrane by a calibrated, closed acoustic system. High-frequency tones were delivered through a Bruel and Kjaer 3134½ in condenser microphone and a Beyer DT48 dynamic ear-phone was used to deliver tones below 4 kHz. Continuous tones and tone bursts were presented at a known sound pressure level, expressed in this paper in dB sound pressure level (SPL; dB re 2×10^{-5} Pa).

Experimental procedures

The sensitivity of the cochlea was assessed on the basis of the visual detection threshold of the CAP to pure tones over the frequency range 1–22 kHz. This was done before and after exposure of the basilar membrane and following the penetration of each hair cell. In the experiments described here dissection of the cochlea to expose the basilar membrane did not result in a threshold loss of more than 10 dB. Thresholds occasionally increased following penetration of the basilar membrane, particularly in the region of the OHCs and, if the threshold loss was greater than 10 dB, the experiments were terminated.

The micropipettes were advanced towards the basilar membrane with a custom-built hydraulic microdrive (Evans, 1979). Contact with the basilar membrane was indicated by the increase in the voltage response of the recording system to a 0·15 nA pulse injected by the microelectrode input. The electrodes were withdrawn a few micrometres, the capacitance compensation was adjusted and the frequency response of the electrodes was determined from the time taken by the single-exponential rise of the electrode voltage response to the current pulse to reach 0·63 of its maximum steady-state value. The recording system behaved like a low-pass filter with a corner frequency between 3·5 and 4·5 kHz. The low-pass cut-off frequency of the recording system was confirmed in separate measurements by using the method described by Baden-Kristensen & Weiss (1983). The electrical linearity was assessed by injecting alternate positive and negative current steps in 0·15 nA increments up to $\pm 1{\cdot}8$ nA and by measuring the out-of-balance voltage response of the preamplifier. This procedure was carried out at the beginning of each experimental run in the scala tympani, before penetration of the basilar membrane, and in the extracellular fluid-filled spaces of the organ of Corti before penetrating and after withdrawing the electrode from each hair cell. If, at the commencement of a run, the electrodes were out of balance or excessively noisy they were rejected. If the voltage response of an electrode was found to be out of balance after it had been withdrawn from a cell then the membrane voltage responses of that cell to current injection were rejected.

Estimates of the IHC membrane time constant were based on the time taken by the membrane voltage to reach a new steady-state value in response to a current step. This measurement is complicated by contributions from the time constant of the recording system (which was taken to be 45 μs, see above) and the activation or inactivation times of the voltage-dependent conductances. The membrane response to the current step approximates a single-exponential function and for the purpose of this analysis is treated as such. The IHC membrane time constant was determined by measuring the time taken by the membrane potential to reach 0·63 of its final value in response to negative current steps minus the time constant of the recording system. This estimation of the membrane time constant was compared with that derived from the product of the membrane slope resistance and the total capacitance of the IHC (taken as 10 pF, Russell &

Sellick, 1978; Kros & Crawford, 1990) and from the time to decay of the DC receptor potential at the offset of 16 kHz tone bursts (fall time of tone burst < 60 μs). Estimates of the time constant based on the three methods were found to be in close agreement.

RESULTS

Voltage dependence of inner hair cell slope conductance

The voltage responses of an inner hair cell to intracellular current steps and 300 Hz tones at 90 dB SPL are shown in Fig. 1*A*. Successive current steps of alternating polarity were delivered through the recording electrode in 0·15 nA increments and, over the range +1·2 nA to −1·5 nA, the electrode impedance appeared to be balanced completely by the amplifier bridge circuit when the recording pipette was in the tunnel of Corti before the IHC was penetrated, when the pipette was intracellular and when the electrode was withdrawn from the IHC. The resting membrane potential was −45 mV and the potential remained within 1 mV of this value from the time of penetration for about 25 min. The mean resting potential of IHCs in this study is -39 ± 6 mV (S.D., $n = 12$).

The membrane voltage plotted as a function of current in Fig. 1*B* shows that the IHC membrane potential was strongly rectifying. The slopes of the relationship (slope resistances) were calculated for a minimum of four points for regions where the relationship between the injected current and the voltage response of the IHC remained relatively constant. For potentials more negative than −80 mV the slope resistance of the IHC is $59{\cdot}1 \pm 1{\cdot}9$ MΩ (S.D., $n = 5$). For potentials more positive than this but more negative than −60 mV, the resistance decreases to $44{\cdot}7 \pm 0{\cdot}5$ MΩ (S.D., $n = 4$). Around the resting membrane potential (−45 mV), the slope resistance is $23{\cdot}9 \pm 1{\cdot}9$ MΩ (S.D., $n = 5$), and the slope resistance decreased to $17{\cdot}4 \pm 0{\cdot}2$ MΩ (S.D., $n = 4$) for potentials more positive than −35 mV. Current–voltage relationships are shown in Fig. 1*C* and *D* for another IHC during tone burst stimulation at the best frequency (16 kHz at 30 dB SPL). For membrane potentials more negative than −80 mV the slope resistance was $48{\cdot}0 \pm 0{\cdot}6$ MΩ (S.D., $n = 4$). This decreased to $44{\cdot}7 \pm 0{\cdot}4$ MΩ (S.D., $n = 4$) for potentials more positive than −80 mV but more negative than −60 mV. For potentials around the resting membrane potential (−40 mV) the resistance fell to $19{\cdot}8 \pm 3{\cdot}9$ MΩ (S.D., $n = 5$) and to $15{\cdot}5 \pm 1{\cdot}1$ MΩ (S.D., $n = 7$) for potentials more positive than −35 mV.

Reversal potentials

Attempts were made in these two IHCs (Fig. 1*B* and *D*) to determine the reversal potential of the receptor current. Direct measurements of the reversal potential were precluded because of the strongly rectifying electrical properties of the IHC membrane and the limited current injection range of the recording pipettes. To obtain an estimate of the reversal potential, regression lines were drawn through the current–voltage relationships of the peak positive and negative voltage responses to stimulation by tones and the membrane potential of the cell at rest. This analysis was confined to potentials where the negative phase of the receptor potential was more negative than −80 mV because, at these potentials, the basolateral conductance is largely inactivated (Kros & Crawford, 1989) and should not contribute towards the IHC voltage responses to tones. Furthermore, the electrical time constant of the

membrane for potentials more negative than −80 mV is fixed at a maximum and should not introduce a proportional error in the slope of the regression lines measured through the peaks of the voltage responses to low-frequency tones. In fact, measurements of the DC receptor potential to the 16 kHz tone (Fig. 1*C*) will not be

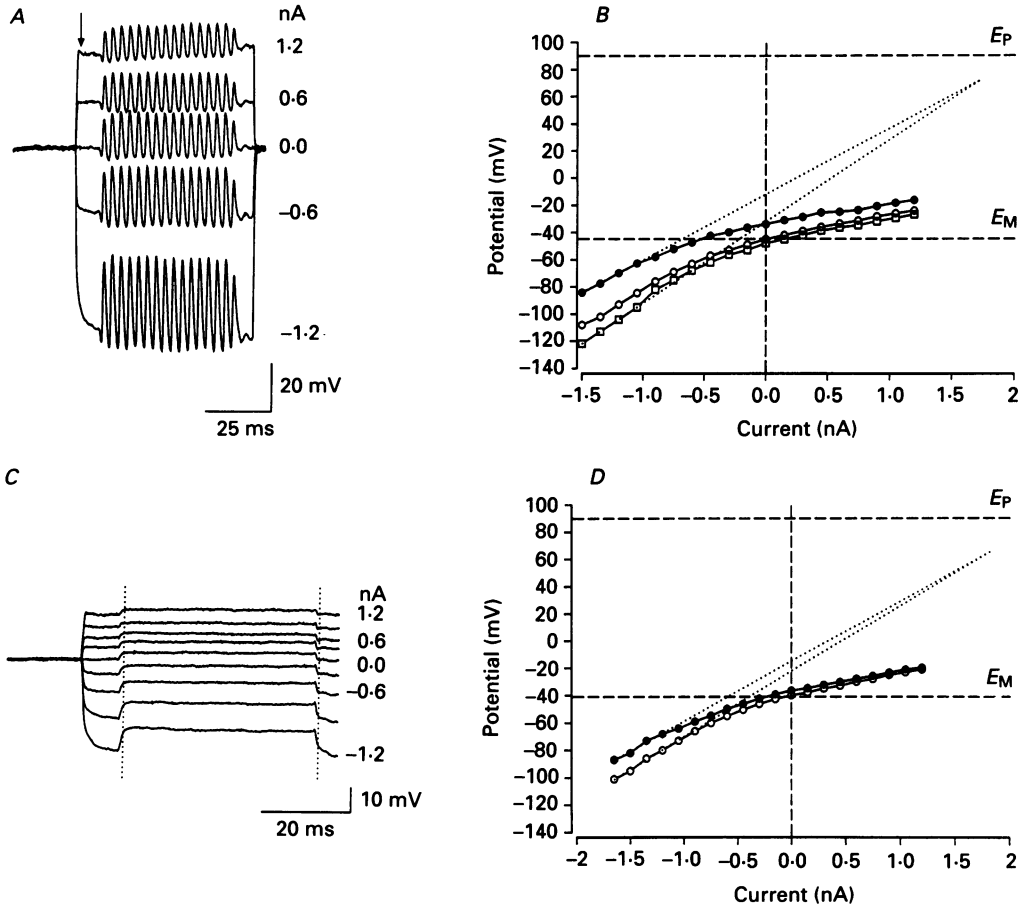

Fig. 1. Responses of an inner hair cell to positive and negative current injection. *A*, voltage responses to 300 Hz tones at 90 dB SPL; *C*, responses to 16 kHz tones at 30 dB SPL. *B* and *D*, voltage plotted as a function of current for the responses measured at 300 Hz and 16 kHz respectively. The arrow in *A* indicates the small time-dependent voltage response and the vertical dotted lines in *C* indicate the termination of the onset and offset responses to tones when the IHC is at the resting membrane potential (−45 mV). The dotted lines in *B* and *D* are regression lines drawn through the curves for points more negative than −60 mV. E_P indicates the level of the recorded endocochlear potential and E_M indicates the resting membrane potential. In *B* and *D* the ●, ○ and □ represent the peak positive phase of the receptor potential, the membrane potential in the absence of sound stimulation and the peak negative phase of the receptor potential, respectively.

influenced by the time constant. The regression lines in Fig. 1*B* and *D* intersect at values of +71 and +65·5 mV, respectively, which is less than the measured endocochlear potential (+90 mV).

Inner hair cell AC receptor potentials and current injection

The records in Fig. 1*A* show that the peak-to-peak amplitude of the receptor potential increases with negative current injection and decreases when the current is positive. Current injection produced an asymmetrical effect with negative current producing by far the greatest changes in the amplitude of the receptor potential. The rate of growth of IHC receptor potentials with increasing negative current injection was frequency dependent. This is illustrated in Fig. 2*A*, *B* and *C* by records of receptor potentials of an IHC to tones at 300 Hz and 80 dB, 600 Hz and 80 dB and 16 kHz (best frequency) and 50 dB SPL, and by the curves in Fig. 2*D* which shows the receptor potentials as a function of injected current. The receptor potentials in Fig. 2*D* have been normalized so that their peak-to-peak response amplitudes in the absence of current injection are unity. Stimulus levels were chosen such that the amplitudes of the receptor potentials elicited by the different frequencies were similar and, for positive current injection, the normalized responses of the receptor potentials were very similar and declined slowly in amplitude with increasing increments (Fig. 2*D*). However, the amplitudes of the receptor potentials change more rapidly with increasing increments of negative current and the growth rate of the DC voltage responses of 16 kHz tones is much steeper than that of the AC voltage response to either the 300 or 600 Hz tones. In contrast, the effects of current amplitude and polarity on the peak-to-peak amplitude of the receptor potential were independent of the level of the acoustic stimulus (see Fig. 2*E*).

A likely explanation for the steeper growth of the DC response to 16 kHz tones (Fig. 2*D*) is that the AC voltage responses to the low-frequency tones are attenuated by the membrane time constant which increases with increasing hyperpolarization. For the IHC illustrated in Fig. 2, the membrane time constant increased from 0·3 ms at the resting membrane potential to 0·6 ms when injected with −1·2 nA current. The predominantly DC voltage responses to the 16 kHz tone will not be attenuated by the membrane time constant. This hypothesis was tested by compensating the amplitude of the AC voltage responses for the voltage-dependent membrane time constant of the IHC by calculating the attenuation due to a single-pole low-pass filter:

$$V_0 = V_i/(1 + w^2t^2)^{\frac{1}{2}}, \quad (1)$$

where V_0 and V_i are the attenuated and unattenuated voltage responses respectively, t is the time constant and $w = 2\pi f$ where f is the stimulus frequency. The curves in Fig. 2*F* illustrate that the normalized AC voltage responses and the high-frequency DC voltage response behave similarly during current injection when the AC voltage responses are compensated for the membrane time constant.

Inner hair cell DC receptor potentials and current injection

A DC component could be measured from the low-frequency receptor potentials by calculating the mean voltage response to the tone (sampled at 100 μs intervals) relative to the membrane potential. When plotted as a function of the injection

current for receptor potentials to 600 and 300 Hz tones the growth of the DC component with increasing negative current injection tends to be less than, or similar to, that of the AC component of the receptor potential and far less than that of the DC receptor potentials to 16 kHz tones. As a consequence of similar growth rates

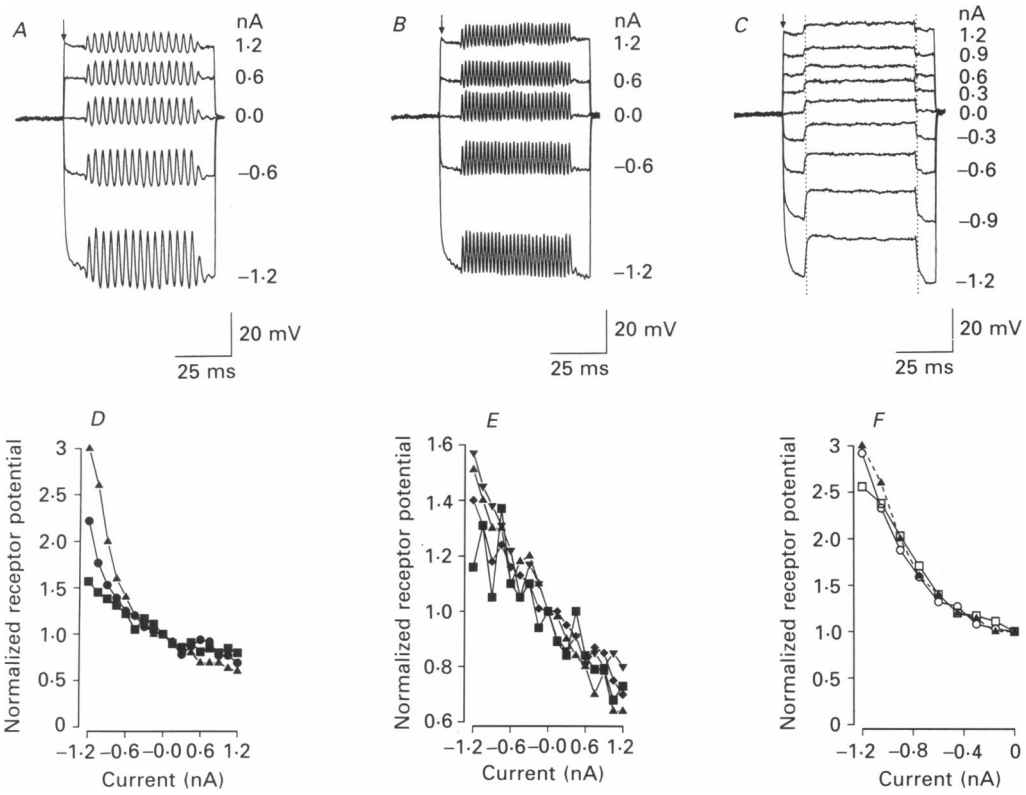

Fig. 2. Receptor potentials recorded from an inner hair cell stimulated with 300 Hz (*A*) and 600 Hz (*B*) tones at 80 dB SPL and to 16 kHz tones at 50 dB SPL (*C*) with positive and negative current injection steps. The arrows in *A*, *B* and *C* indicate the small time-dependent voltage response and the vertical dotted lines in *C* indicate the termination of the onset and offset responses to tones when the IHC is at the resting membrane potential. *D*, peak-to-peak amplitude of receptor potentials at 300 Hz, 80 dB SPL (●), 600 Hz, 80 dB SPL (■) and DC receptor potentials at 16 kHz, 50 dB SPL (▲), normalized to their responses without current injection, as a function of the injected current. *E*, peak-to-peak amplitude of the receptor potential generated by an inner hair cell to a 600 Hz tone at 60 dB SPL (■), 70 dB SPL (◆), 80 dB SPL (▼) and 90 dB SPL (▲) normalized to the amplitude at resting membrane potential (with zero current injection) as a function of injected current. *F*, normalized receptor potentials from an inner hair cell stimulated with 300 (○) and 600 Hz (□) tones at 80 dB SPL and a 16 kHz tone at 50 dB SPL (▲) as a function of injected current. The responses to 300 and 600 Hz tones are compensated for the low-pass filtering due to the membrane time constant.

with current injection, the ratio of the uncompensated AC and DC components remains remarkably independent of the current injected, although the DC/AC ratio is dependent on stimulus level. This level dependence arises because the waveform of

the receptor current grows from an almost symmetrical response to tones just above the noise level of the recording to responses which are very asymmetrical for stimulus levels above this (Russell & Sellick, 1983). When expressed in double logarithmic co-ordinates (Fig. 3*B*), and for non-saturating stimulus levels, the slope of the DC

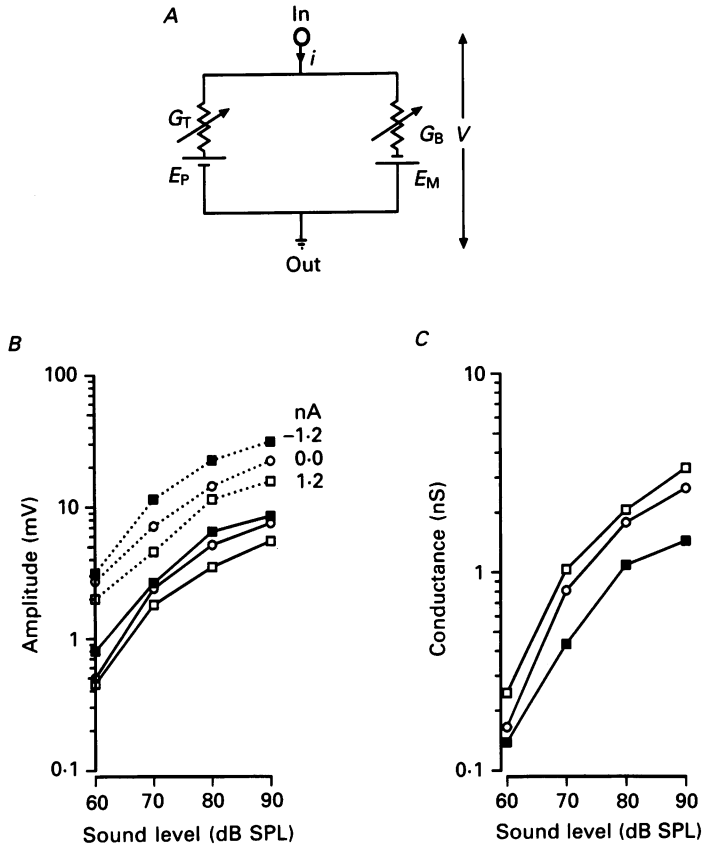

Fig. 3. *A*, a simple electrical model of a hair cell. *B*, amplitude–level functions of AC (dotted lines) and DC (continuous lines) components of the receptor potential of an inner hair cell stimulated with 300 Hz tones at 80 dB SPL and when injected with zero (○), +1·2 nA (□) and −1·2 nA (■) of current. *C*, transducer conductance associated with the DC receptor potentials shown in *A* as functions of sound level.

amplitude–intensity function is twice that of the AC amplitude–intensity function and approximates a square-law relationship (Goodman, Smith & Chamberlain, 1982; Russell & Sellick, 1983; Dalos, 1985). Not unexpectedly, current injection shifts the curves along the vertical axis with positive current injection causing a downward shift and negative current causing an upward shift of the curves. These difference are seen most clearly in the ways positive and negative current influence the transducer conductance associated with the DC receptor potential. An estimation of the transducer conductance was performed on data from five IHCs where the series resistance of the micropipette was apparently fully compensated in the voltage

responses to currents over the range $\pm 1{\cdot}2$ nA. The estimate was based on the simple electrical model of a hair cell (Weiss, Mulroy & Altman, 1974) shown in Fig. 3*A*. The membrane potential V is given by:

$$V = \frac{G_T}{G_B+G_T}E_P + \frac{G_B}{G_B+G_T}E_H + \frac{i}{G_B+G_T}, \quad (2)$$

where G_T and G_B are the transducer and basolateral conductances respectively, E_P is the endocochlear potential, E_H is the IHC resting potential and i is the injected current. For the data illustrated in Figs 3, 4 and 5, E_P was found to be +80 mV and E_H was −45 mV. G_B was estimated from the slope resistances measured when the IHC was injected with $-1{\cdot}2$, 0 and $+1{\cdot}2$ nA of current. The slope resistance was that measured during the negative phase of the receptor potential to a 90 dB, 300 Hz tone when it is assumed that the transduction channels are closed and that these channels do not contribute to the total conductance of the IHC. The values of G_B thus obtained were $1{\cdot}5\times10^{-8}$ S ($-1{\cdot}2$ nA), $4{\cdot}11\times10^{-8}$ S (0 nA) and $5{\cdot}75\times10^{-8}$ S ($+1{\cdot}2$ nA). The transducer conductance G_T, derived from eqn (2), is shown at different sound levels in Fig. 3*C*. It can be seen that negative current caused a 50% reduction in the amplitude of the DC transducer conductance and reduced the initial slope of the function from 1·8 without current injection to 1. Positive current injection caused a 20% increase in the amplitude of the DC transducer conductance without an appreciable change in the initial slope of the amplitude–level function.

Inner hair cell transducer functions

The DC component of the receptor potential is derived from the shape of the IHC transducer function, i.e. the relationship between the IHC transducer conductance and the sound pressure measured at the tympanic membrane. Strong hyperpolarization of the IHC might alter the transducer conductance to yield a smaller DC component of the receptor potential in relation to the AC component. This could be achieved, for example, if the IHC transducer function became more symmetrical about the origin. To test this hypothesis, first the relationship between the peak sound pressure and the peak voltage response was plotted for a single hair cell for tones at 300 Hz and with injection currents of $+1{\cdot}2$, 0 and $-1{\cdot}2$ nA (Fig. 4*A*). The transducer conductances associated with the peak negative and peak positive receptor potentials were estimated using eqn (2). However, the receptor potentials measured when the IHC is injected with $-1{\cdot}2$ nA current will be attenuated by the low-pass filter properties of the IHC membrane because the frequency of the tones used in these experiments (300 Hz) was found to be close to or above the corner frequency of the filter when the membrane potential was more negative than −80 mV. The membrane filter corner frequencies were 796 Hz at the resting membrane potential and 265 Hz when injected with $-1{\cdot}2$ nA. The peak voltage responses obtained during negative current injection were adjusted for the membrane time constant according to eqn (1) and then the transducer conductance was estimated according to eqn (2). In Fig. 4*B* the transducer conductance is expressed relative to the hair cell membrane conductance where the lines through the relationships are pairs of hyperbolic tangent functions. The method for deriving these functions is given elsewhere (Russell & Sellick, 1983) and the constants are given in the legend

to Fig. 4. For positive sound pressure the transducer conductance increased and it decreased for negative sound pressure. The decrease in conductance is greater and the increase is smaller during negative current injection than for either zero or positive current injection. From studies of hair cells *in vitro*, the transducer channels

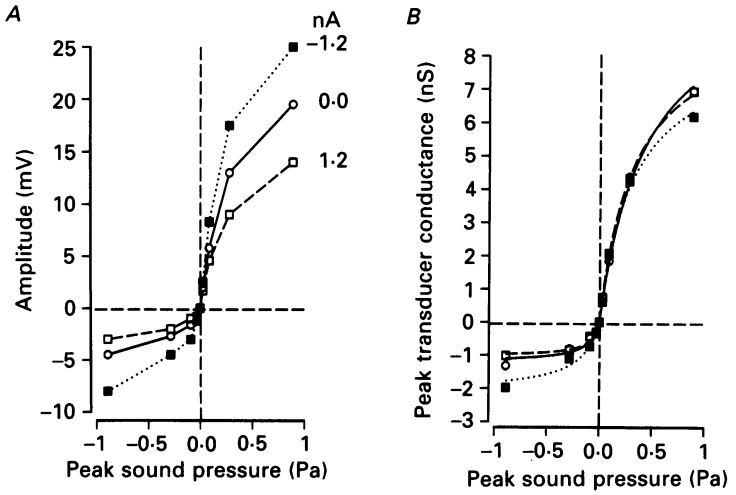

Fig. 4. *A*, relationship between peak sound pressure and the peak negative and peak positive receptor potentials of an inner hair cell, the amplitude–level functions of which are shown in Fig. 3. ○, zero current injection (continuous line); □, with +1·2 nA (dashed line) current injection; and ■, with −1·2 nA (dotted line) current injection. Stimulating frequency 300 Hz. *B*, peak transducer conductance estimates for the receptor potentials shown in *A* as functions of peak sound pressure and compensated for the IHC membrane time constant. The electrical time constant of the IHC membrane was measured from the rise time of the voltage response to the step change at the onset of the current step. The lines and symbols have the same meaning as in *A* and the curves are composed of two hyperbolic tangent functions whose equations are:

$$V_D = V_{max,D}\frac{p}{\bar{p}+p} \text{ for } p \geqslant 0,$$

and

$$V_H = V_{max,H}\frac{p}{\bar{p}_H - p} \text{ for } p \leqslant 0,$$

where V is the peak receptor potential, p is the peak sound pressure and the subscripts D and H refer to the depolarization and hyperpolarization of the membrane potential. The constants are computed from linear functions of the data (see Russell & Sellick, 1983). $V_{max,D}$ and $V_{max,H}$ (the peak positive and negative values of the receptor potential) are 9·31, −1·04; 9·46, −1·23 and 7·89, −2·13 for +1·2, 0 and −1·2 nA currents, respectively. The sound pressures, in Pascals, at which the voltage response reaches half-saturation for rarefaction and compression phases of the sound pressure (P_D; P_H), are 0·32, −0·07; 0·36, −0·08 and 0·27, 0·17 for +1·2, 0 and −1·2 currents, respectively.

are opened by displacement of the hair bundle towards the kinocilium and closed by displacements in the opposite direction (Hudspeth & Jacobs, 1979; Russell *et al.* 1986). Thus, a parsimonious explanation of the apparent downward vertical shift of the IHC transducer function with negative current injection is that negative current injection causes an increase in the proportion of the transducer conductance open at

rest. Conversely, positive current injection causes a smaller upwards vertical shift of the transducer function in the opposite direction corresponding to a decrease in the resting transducer conductance. The three curves shown in Fig. 4*B* can be aligned by moving them along the vertical axis so that the minimum conductances coincide (see Fig. 5*A*). To achieve this, the curve obtained with negative current injection was moved up 0·67 nS and the curve obtained with positive current injection was moved down 0·14 nS. If it is assumed that the transducer conductance is completely turned off during very negative sound pressure, then without current injection the resting transducer conductance represents 11·4% of the total transducer conductance. This increased to 21·2% with negative current injection and decreased to 10·1% with positive current injection. The transducer functions can be aligned in the horizontal plane so that the zero crossing points coincide (see Fig. 5*B*). This was achieved by moving the negative current curve by +0·08 Pa (72 dB SPL) and the positive current curve by −0·005 Pa (48 dB SPL). Thus an injection of −1·2 nA of current, and a corresponding hyperpolarization of the IHC membrane potential to −95 mV, is equivalent to an excitatory bias of the stereocilia bundle which would be produced by a static pressure change of +0·08 Pa. Current injection with +1·2 nA, and a corresponding depolarization to −24 mV, produced the equivalent of a negative bias of the hair bundle which would be produced by a static pressure change of −0·005 Pa. For a total of five IHCs from which measurements were made in these experiments, the resting conductance expressed as a percentage of the total estimated conductance (7·8±2·5 nS; mean±S.D.) without current injection, with −1·2 nA current injection and with +1·2 nA current injection, were 10·8±1·1, 19·6±3·2 and 9·0±0·8% (mean±S.D.), respectively.

Anomalous responses to current injection

In the vast majority of cells from which voltage responses to tones were recorded (> 150), current injection did not alter the amplitude of the voltage response. From the location of these cells adjacent to the basilar membrane, the size of their resting potentials (approximately −85 mV) and the similarity of the tone-evoked voltage responses in amplitude and form to the Cochlear microphonic potential recorded extracellularly in the organ of Corti, the cells were assumed to be supporting cells.

In addition to the IHCs, a further fifteen cells were encountered in these experiments where current injection altered the amplitude and form of the voltage responses to low-frequency tones. The resting potentials of these cells had a mean value of 73±5 mV and they were encountered after the micropipette had first passed through at least one supporting cell and the pipette often penetrated the scala media if it was advanced beyond these cells. Tone-evoked voltage responses from these cells varied in amplitude between 2 and 3 times that of the organ of Corti microphonic. The peak-to-peak amplitudes of the voltage responses varied between 6 and 12 mV for 300 Hz tones at sound levels between 80 and 90 dB SPL. In contrast to the IHC receptor potentials where, for all frequencies and sound levels, the amplitude of the depolarizing voltage response dominates that of the hyperpolarizing response, the depolarizing and hyperpolarizing voltage responses of these cells were approximately equal for 300 Hz tones at sound levels between 80 and 90 dB SPL. In relation to previous measurements where cells with these electrophysiological characteristics

have been identified morphologically, the cells were assumed to be outer hair cells (OHCs) (Russell & Sellick, 1983; Cody & Russell, 1987).

It was not possible to remain in the OHCs long enough under stable recording conditions to balance accurately the electrode impedance in these cells and so no

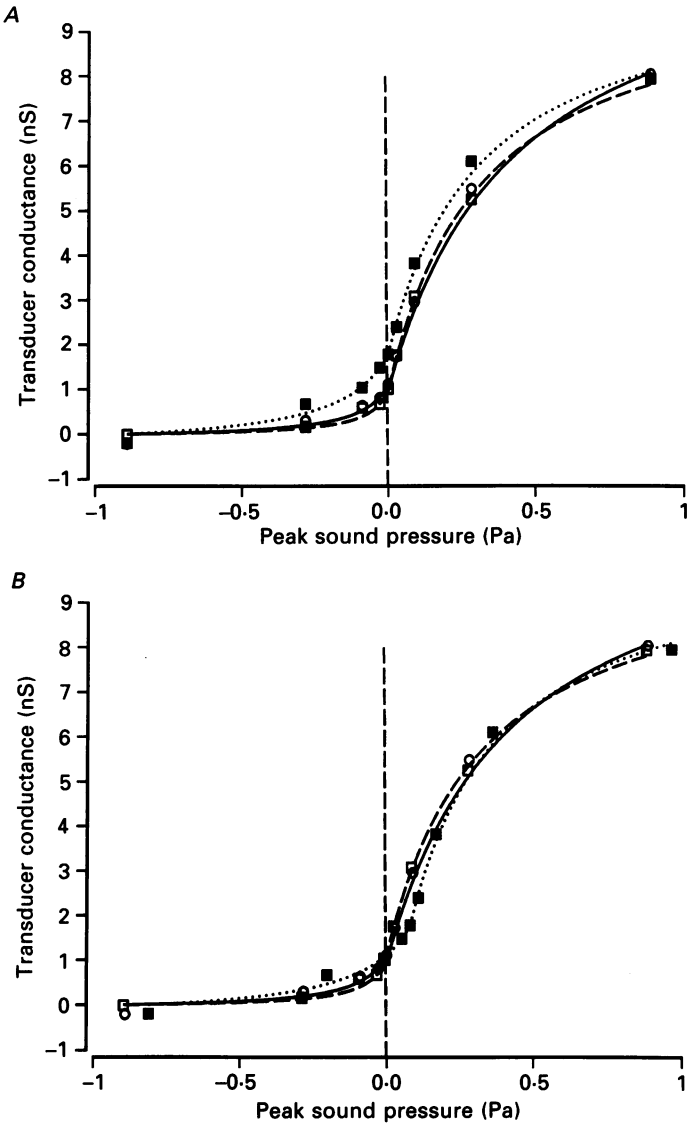

Fig. 5. The relationships shown in Fig. 4*A* but aligned along the vertical axis (*A*) so that the transducer conductances asymptote to zero for negative sound pressure and realigned along the horizontal axis (*B*) so that the resting transducer conductances of all three curves are the same at zero sound pressure. The smooth curves are hyperbolic tangent functions which are based on constants given in the legend to Fig. 4.

current–voltage curves were derived. An example of the voltage responses of a presumed OHC to 300 Hz tones at 80 dB SPL and current injection is shown in Fig. 6*A* and *B*. With increasing levels of negative current injection the voltage responses became smaller in amplitude and the waveform of the responses either remained

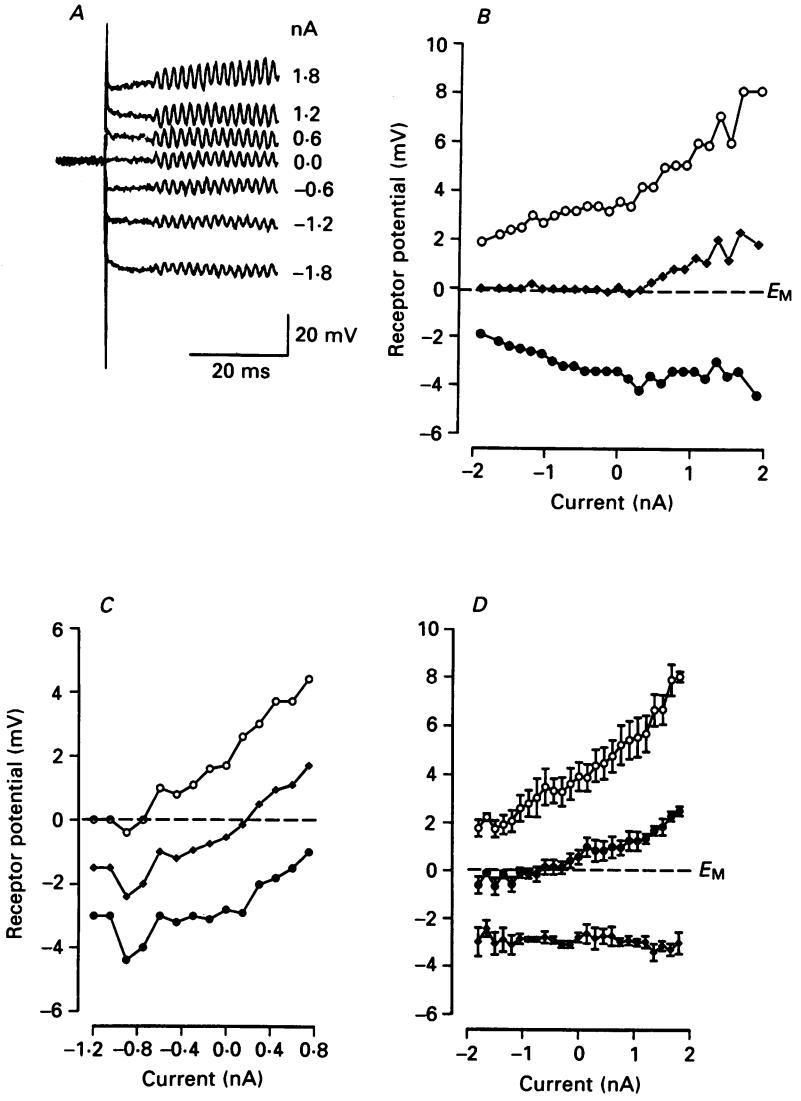

Fig. 6. *A*, receptor potentials recorded from an outer hair cell when stimulated with 600 Hz tones at 80 dB SPL and during current injection. *B* and *C*, peak positive (○), peak negative (●) and DC (◆) receptor potentials of the outer hair cell and those of a further OHC in another preparation as a function of injected current. E_M indicates the membrane potential. *D*, similar data pooled from five OHCs in two preparations. Each point represents the mean and standard deviation of the pooled data.

symmetrical (Fig. 6*B*) or the negative phase of the responses dominated, thus producing a negative DC potential (see Fig. 6*C* and *D*). With increasing levels of positive current injection, the voltage responses became larger and more asymmetrical so that the depolarizing phase dominated (Fig. 6). These responses to current injection were regarded as anomalous. This is because positive current injection would tend to reduce the driving voltage for the flow of current through the transducer channel.

DISCUSSION

The principal finding reported in this paper is that the proportion of the hair cell transducer conductance open at rest, when cochlear hair cells are not receiving mechanical stimulation, is increased when the hair cells are injected with negative current and decreased when they are injected with positive current injection. The experiments also confirm earlier findings, that the mean resting membrane potential of IHCs recorded in this study (−39 mV) is similar to that recorded previously from IHCs in the guinea-pig cochlea (Russell & Sellick, 1978; Dallos, 1985) and that the current–voltage relationships are outwardly rectified when the membrane potential is more positive than about −60 mV (Russell *et al.* 1986). The rectification is similar to that reported by Kros & Crawford (1989, 1990) who voltage clamped isolated IHCs using whole-cell recording techniques and found that the rectification is due to voltage-dependent potassium conductances in the basolateral membranes which activate when the membrane potential is made more positive than −55 mV. These conductances, which are responsible for the strongly damped oscillatory behaviour of the membrane potential to current steps (Kros & Crawford, 1989, 1990), may be responsible for the small positive peaks which appear at the onset of the voltage response to current injection when the membrane potentials of IHCs *in vivo* are stepped to potentials more positive than −30 mV (see Figs 1 and 3).

The strong outward rectification of IHCs and the current-passing limitations of the micropipettes prevented a direct measurement of the reversal potential of the transducer current in the experiments reported here. However, the indirect technique used in these experiments indicates that the reversal potential approaches the measured E_P which is in agreement with earlier measurements (Russell, 1983).

The effects of current injection on receptor potentials

Inner hair cells

The IHC membrane resistance and the membrane time constant are both increased by negative current injection. Thus AC receptor potentials to tones above the cut-off frequency determined by the time constant (*ca* 0·6 ms for membrane potentials more negative than −60 mV) might be expected to be attenuated with respect to the DC component of the receptor potential with hyperpolarizing current injection. In fact the AC/DC ratio of the receptor potential remains independent of the injected current, at least over ranges of 1·2 nA, and this ratio increases with hyperpolarizing current if the magnitude of the AC component is compensated for the low-pass filtering due to the membrane time constant. The apparent decrease in the DC component is ascribed to an increased symmetry in the IHC transducer function such that a greater proportion of the transducer conductance is open at rest when the IHC

is hyperpolarized (20%) than at the normal resting potential (11%) and when depolarized (9%) (Figs 4 and 5). In fact the increase in symmetry of the IHC transducer function with negative current injection accounts for the 50% decrease in the transducer conductance associated with the DC receptor potential (see Fig. 3*C*). The reduction in slope of the DC conductance–level function with negative current injection seen in Fig. 3*C* is probably a consequence of the change in shape of the transducer function with negative current injection (Figs 4 and 5). In particular, the negative-going saturation of the function is less steep than for transducer functions obtained either without current injection or with positive current injection. Furthermore, the transducer function obtained with negative current injection is less sensitive to small positive sound pressure changes (see Fig. 5*B*). Both of these characteristics of the transducer function would account for the lower slope of the DC conductance–level function with negative current injection.

Negative current injection has the equivalent effect of displacing the stereocilia bundle in the excitatory direction, i.e. towards the tallest row of stereocilia, while positive current injection has the opposite effect. Effects equivalent to those produced by injecting the IHC with −1·2 and +1·2 nA of current could be achieved with static pressure changes delivered to the external auditory meatus of +72 dB SPL and −48 dB SPL respectively (assuming that static pressure changes can be transmitted to the IHCs). These static pressure changes are equivalent to displacing the basilar membrane in the basal turn by 3·5 nm towards scala vestibuli and 0·22 nm towards scala tympani respectively (Sellick, Patuzzi & Johnstone, 1982). When related to the changes in membrane potential caused by the injected currents, the change in the resting transducer conductance caused by positive current injection is about 1% of the total transducer conductance per 10 mV change in membrane potential and about 2% per 10 mV change for negative current injection.

For frequencies approaching the characteristic frequency of the cell, the DC component of the IHC seems to depend only on the driving potential and the basolateral conductances (see Fig. 1*C*) and to have no other voltage dependence. Thus, it is possible that the mechanism of the voltage-dependent shift of the operating point takes into account the instantaneous state of the transducer conductance but is not fast enough to cope with transduction at very high frequencies.

Outer hair cells

The effect of current injection on the receptor potentials of OHCs is regarded as anomalous because negative current injection causes a decrease in the amplitude and a shift in the symmetry of the receptor potential (and, hence, the polarity of the DC component) in the hyperpolarizing direction. Positive current injection causes an increase in the amplitude of the receptor potential and displaces the symmetry and the DC component of the receptor potential in the positive direction (see Fig. 6). These observations cannot be explained in terms of the known properties of the basolateral conductances which have been measured in isolated OHCs (Ashmore & Meech, 1986; Santos-Sacchi & Dilger, 1988). Here, negative current injection decreases the basolateral conductance, increases the driving voltage for the receptor current across the apical membrane and should, therefore, increase the amplitude of

the intracellularly recorded receptor potential. These predictions are supported by measurements made on OHCs in the apical turns of the guinea-pig cochlea (Dallos, 1986) where there appears to be little influence of current injection on the transducer conductance. However, electrical polarization of the cochlear partition changes the distortion levels recorded in the cochlear microphonic (Dallos *et al.* 1969), in the acoustic signal measured at the tympanic membrane (Mountain, 1980) and in the mechanical input to the inner hair cells (Nuttall, 1985). All of these effects have been attributed to voltage-dependent mechanical changes in the OHCs.

The observations reported in this paper are in accord with the explanation that hyperpolarization causes an increase in the resting transducer conductance equivalent to a displacement of the OHC stereocilia bundle towards the tallest row so that the OHC now operates in a saturating and less-sensitive region of the transducer function. Depolarization causes a decrease in the transducer conductance equivalent to displacing the stereocilia bundle in the inhibitory direction which results in a shift in the operating point of the transducer function to a more sensitive region of operation. Thus the proposed effects of current injection on OHCs are similar to, and a stronger manifestation of, the effects of current injection which have been observed on the receptor potentials of IHCs reported in this paper and in OHCs in organ cultures of the neonatal mouse cochlea (Russell, Richardson & Kössl, 1989).

Mechanism of the shift

The voltage-dependent shift in the transducer functions need not involve displacement of the stereocilia bundle (see Crawford, Evans & Fettiplace, 1989) but the bundles may move directly as a consequence of current injection (Crawford & Fettiplace, 1985; Hacohen, Assad, Smith & Corey, 1989; Rüsch & Thurm, 1990). Voltage-dependent movements of sterocilia bundles have been observed in hair cells of the vestibular systems of lower vertebrates where positive current injection (Hacohen *et al.* 1989) or depolarizing transepithelial currents (Rüsch & Thurm, 1990) cause the stereocilia bundle to be displaced away from the kinocilium. Here, the voltage-dependent shift in the transducer function has been associated with sensory adaptation and the influx of Ca^{2+} through the transducer channels (Crawford *et al.* 1989; Hacohen *et al.* 1989). Adaptation and the shift in the transducer function are strongly Ca^{2+} sensitive in that both are strongly reduced and may disappear when the hair cells are made very positive, when the intracellular Ca^{2+} levels are increased to 10 mM and when the extracellular Ca^{2+} is diminished to 0·5 nM (Crawford *et al.* 1989; Hacohen *et al.* 1989; Evans, Fettiplace & Crawford, 1990). The Ca^{2+} concentration of cochlear endolymph is 20 μM (Boscher & Warren, 1978) and thus it is unlikely that the current-induced shifts in the IHC transducer function reported here involve the same calcium-dependent mechanism which has been postulated for adaptation and hair bundle displacement in lower vertebrate hair cells.

In OHCs at least, the stereocilia bundle may be moved as a consequence of voltage-dependent changes in the length of the cell body (e.g. Ashmore, 1987; Santos-Sacchi & Dilger, 1989). This suggestion has already been made to account for the shift in the operating point of the transducer function with current injection which has been observed in OHCs in cultures of the neonatal mouse cochlea (Russell *et al.* 1989). In the cochlear cultures the shift in the transducer function is in the same direction as that observed for IHCs (a positive shift with hair cell hyperpolarization). Changes in

the symmetry of sinusoidal, voltage-induced movements by positive (shortening) and negative (lengthening) transcellular currents have been observed in OHCs isolated from the guinea-pig cochlea (Santos-Sacchi, 1989; Evans, Hallworth & Dallos, 1990). These changes are analagous to sound level-induced changes in the symmetry of OHC receptor potentials recorded *in situ* (Dallos, Santos-Sacchi & Flock, 1982; Cody & Russell, 1987) and the current-induced changes in IHC and OHC receptor potentials reported here.

Functional significance of the shift

OHCs have been attributed with an interactive role in cochlear transduction where, through electromechanical feedback of energy to the cochlear partition, they have been proposed to control mechanical input to the IHCs (Davis, 1983). In the basal turn of the guinea-pig cochlea, the sensitivity of the IHC responses to tones near the CF is optimal when the DC receptor potential responses of OHCs to tones of the same frequency are minimal (Cody & Russell, 1987; Kössl & Russell, 1990). This is taken to indicate that the operating point of the OHC transducer, and hence the mechanical input to the IHCs, is somehow regulated to achieve maximum efficiency of the electromechanical feedback. Sensitivity is greatly reduced at high sound levels (> 90 dB SPL) when the OHCs generate slow depolarizing DC receptor potentials and the symmetry of the low-frequency receptor potentials is depolarizing (Cody & Russell, 1987). On the basis of the experiments reported here and from observations on OHCs in neonatal mouse cochlea cultures (Russell *et al.* 1989), it is proposed that the voltage control of the OHC transducer operating point forms part of a negative feedback system concerned with minimizing the DC component of the OHC receptor potential. Accordingly, any tonic displacement of the OHC bundle which tends to increase the probability of channel opening, thereby causing the OHCs to be depolarized, leads to OHC shortening and to displacement of the stereocilia bundle in the negative direction. This decreases the probability of channel opening and counters the depolarization. Tonic displacements which tend to hyperpolarize the OHCs are opposed by increases in length of the OHCs which lead to displacement of the stereocilia bundles in the positive direction, thereby increasing the probability of transducer channel opening, and repolarization of the OHC membrane potential.

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