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Temperature dependence of outer hair cell nonlinear capacitance

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Abstract

The temperature dependence of outer hair cell motility-related gating current and capacitance was evaluated under whole-cell voltage clamp. Temperature change caused a shift of these voltage-dependent functions along the voltage axis, with a decrease in temperature causing a negative shift in the voltage at peak capacitance (V_{pkcm}) of 19.2 mV per 10°C. Gating current kinetics showed only mild temperature dependence, the Q_{10} being about 1.5. Temperature is speculated to affect outer hair cell (OHC) mechanical gain and frequency response by alterations in lateral membrane viscoelastic properties. Such temperature-dependent effects on the OHC may mediate known temperature effects on in vivo cochlear physiology. © 1998 Published by Elsevier Science B.V.

Key words: Outer hair cell; Motility; Capacitance; Temperature; Patch clamp

1. Introduction

The discovery that outer hair cells (OHC) can change their length in response to electrical stimuli profoundly influenced the study of cochlear physiology (Brownell et al., 1985). OHC motility is dependent upon transmembrane voltage (Santos-Sacchi and Dilger, 1988; Iwasa and Kachar, 1989); hyperpolarization elongates and depolarization shortens the cylindrically shaped cell. The mechanism underlying this shape change which occurs at acoustic frequencies is unknown. However, it is not based on typical cellular mechanisms of motility (Kachar et al., 1986; Santos-Sacchi and Dilger, 1988; Holley and Ashmore, 1988). In fact, current indications are that the force generating mechanism resides solely within the lateral plasma membrane (Kalinec et al., 1992; Huang and Santos-Sacchi, 1993, 1994), possibly corresponding to the 8–10 nm intramembranous particles observed ultrastructurally (Gulley and Reese, 1977; Forge, 1991).

The mechanical activity of the OHC is mirrored by an electrical signature, a voltage-dependent capacitance or, equivalently, a gating charge movement (Ashmore, 1989; Santos-Sacchi, 1990, 1991; Iwasa, 1993), which indicates that membrane-bound voltage sensor-motor elements control OHC length (Santos-Sacchi, 1990, 1991, 1993; Ashmore, 1989, 1992; Dallos et al., 1991; Iwasa, 1994). In this report we measure the effects of temperature on the OHC motility-related gating currents and nonlinear capacitance. We report that both the kinetics and voltage dependence of nonlinear charge movement are affected. Preliminary accounts of this work have been presented (Santos-Sacchi, 1990; Santos-Sacchi et al., 1995).

2. Materials and methods

Guinea pigs were overdosed with halothane. The temporal bones were removed, the apical two turns of the organ of Corti micro-dissected free, and OHCs isolated enzymatically with collagenase (0.3 mg/ml for 10 min followed by gentle reflux through a tapered polyethylene pipette tip) in medium 199 with Hanks' salts (Gibco). The cell enriched supernatant was then transferred to a 700 μl perfusion chamber, and the cells permitted to settle onto the cover glass bottom. A Ni-

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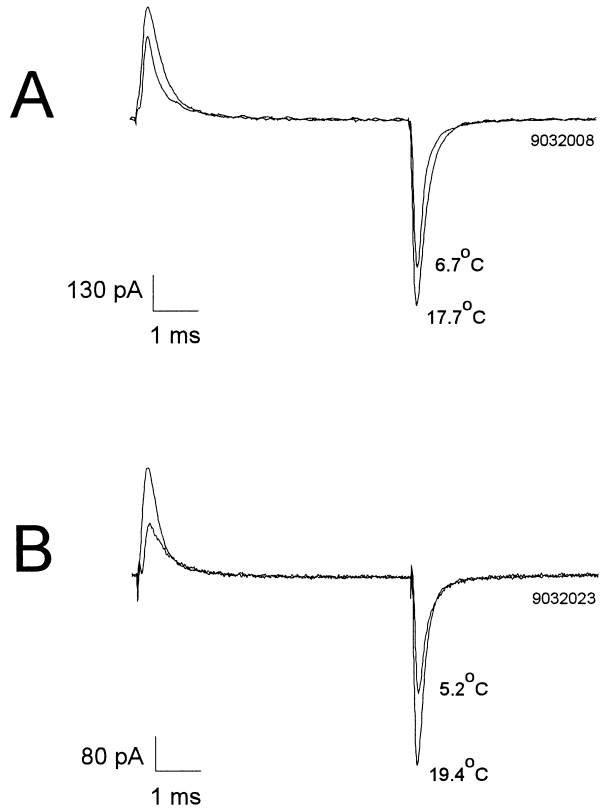

Fig. 1. Two OHCs were clamped to −80 mV and gating currents were extracted with the ±P protocol (40 mV about the holding potential). A drop in temperature decreased both the gating charge moved and the exponential time constant of the current decay. A: Larger amplitude trace: 17.7°C; Q_{off}: 240 fC; τ_{off}: 0.21 ms. Smaller amplitude trace: 6.7°C; Q_{off}: 162 fC; τ_{off}: 0.24 ms. B: Larger amplitude trace: 19.4°C; Q_{off}: 144 fC; τ_{off}: 0.18 ms. Smaller amplitude trace: 5.2°C; Q_{off}: 98 fC; τ_{off}: 0.26 ms. The effects were reversible.

kon Diaphot inverted microscope with Hoffmann optics was used to observe the cells during electrical recording. A modified Leibovitz medium (NaCl 100 mM, KCl 5.37 mM, $CoCl_2$ 2.0 mM, $MgCl_2$ 1.48 mM, 20 mM TEA, 2 μM TTX, 20 mM CsCl, HEPES 5.0 mM, dextrose 5.0 mM, pH 7.2) was used in order to block ionic conductances which might otherwise interfere with capacitive current measures. OHCs maintained normal appearance in this solution for up to 1 h. Osmolarity was adjusted to 300 mOsm with dextrose.

OHCs were whole-cell voltage clamped with a Dagan 8900 patch clamp amplifier at a holding potential of −80 mV, similar to the resting potential recorded in vivo (Dallos et al., 1982). Pipette solutions were composed of 140 mM CsCl, 10 mM EGTA, 5 mM TEA, 2 mM $MgCl_2$, and 5 mM HEPES buffered to pH 7.2. Osmolarity was adjusted to 300 mOsm with dextrose. Gigohm seals were obtained at the nuclear level of the cell membrane; electrode capacitance and series resistance were compensated. A modified version of Clampex (Axon Instruments, CA) utilizing the Labmaster board (Axon) was used to collect data which were saved to disk for off-line analysis. Current was filtered at 5 kHz with an 8-pole Bessel filter. Temperature was controlled with a custom-built Peltier recording chamber, and measured with a micro temperature probe (Sensortek, NJ) placed within 0.5 mm of the recorded cells. In addition, data were confirmed using pre-cooled or pre-warmed solutions in the absence of the Peltier device.

Detailed evaluation of the bell-shaped OHC membrane capacitance function was made at different potentials by transient analysis of currents induced by a voltage stair step stimulus. Step duration was adjusted on-line to 15× the time constant of the capacitive transient to ensure steady-state conditions. The capacitance function was fit to the first derivative of a two-state Boltzmann function relating nonlinear charge to membrane voltage ($\delta Q/\delta V$; Santos-Sacchi, 1991; Huang and Santos-Sacchi, 1993),

$$C_m = Q_{max}\frac{ze}{kT}\frac{b}{(1+b)^2} + C_{lin} \quad (1a)$$

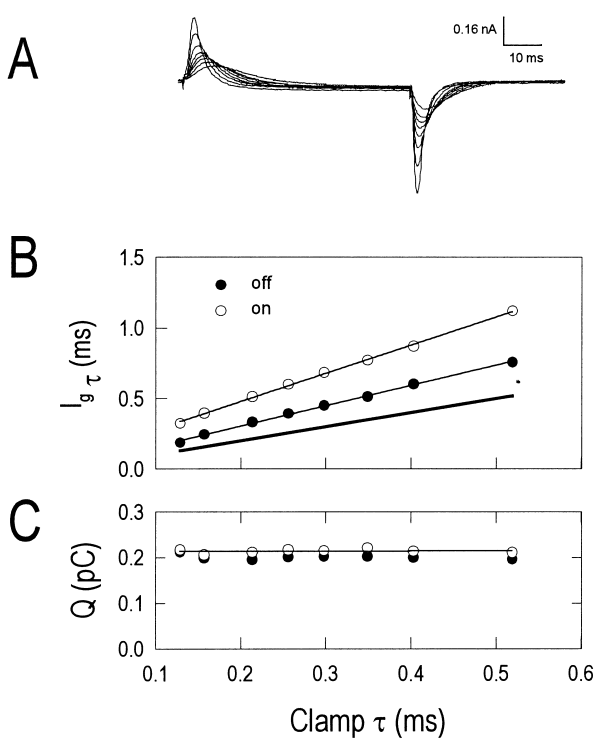

Fig. 2. A: The time constant of OHC gating current decay was modified by altering the speed of the voltage clamp via series resistance compensation. Gating currents were obtained as in Fig. 1. B: Gating charge time constant followed that of the voltage clamp. The thick solid line represents a one-to-one correspondence between clamp τ and gate τ. C: However, changing the gating time constant does not modify total charge moved. The charge is simply redistributed over time. The change in charge moved as a function of temperature (Fig. 1) is therefore not due to a simple effect on the time constant of the clamp amplifier.

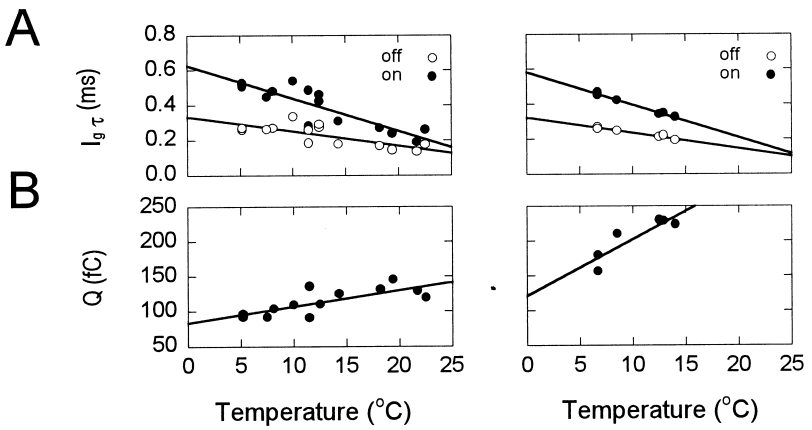

Fig. 3. The effects of temperature on gating currents are presented in detail for two OHCs (left and right panel). A: The time constant of on and off gating current relaxation as a function of temperature. Note the differences between on and off time constants. Left panel: τ^{-1} Q_{10} on: 1.55; off: 1.4. Right panel: τ^{-1} Q_{10} on: 1.62; off: 1.45. B: The average of on and off charge moved as a function of temperature. On and off charge were essentially equivalent.

where

$$b = \exp\left(\frac{-ze(V-V_{\mathrm{pkcm}})}{kT}\right) \qquad (1b)$$

$Q_{\max}$ is the maximum nonlinear charge moved, V_{pkcm} is voltage at peak capacitance or equivalently, at half maximal nonlinear charge transfer, V_{m} is membrane potential, z is valence, C_{lin} is linear membrane capacitance, e is electron charge, k is Boltzmann's constant, and T is absolute temperature. Gating currents were extracted online with the ±P procedure (±40 mV; Armstrong and Bezanilla, 1973) about a holding potential of −80 mV, or with the P/−5 protocol, at a subtraction potential of −160 mV (Armstrong and Bezanilla, 1977). All data analysis was performed with the software package MATLAB (Mathworks, MA). In some experiments, V_{pkcm} was monitored during temperature changes using a previously developed tracking procedure (Kakehata and Santos-Sacchi, 1995). Cell turgor pressure was either maintained constant by pipette pressure, or cells were collapsed so that pressure-induced shift in V_{pkcm} did not interfere with analysis of temperature effects (Kakehata and Santos-Sacchi, 1995). Q_{10} was determined as in Kimura and Meves (1979). The use and treatment of animals was approved by the Yale University animal care committee.

3. Results

Nonlinear gating currents associated with the mechanical responses of OHCs are induced at the onset and offset of voltage steps. Fig. 1 illustrates these currents in two cells obtained at a holding potential of −80 mV, each at two different temperatures. The effect of temperature change is to alter the total amount of charge moved and the time constant (τ_{on} and τ_{off}) of the current decay. Both the rate and quantity of charge moved are decreased as temperature is decreased. Note also that τ_{on} and τ_{off} differ, with τ_{on} being slower. It has been previously shown that the clamp time constant affects the speed of OHC charge movement (Santos-Sacchi, 1990, 1992). However, changing the clamp time constant does not alter the amount of charge moved (Fig. 2). Thus, any affects of temperature upon clamp time constant (Santos-Sacchi, 1990) would not be

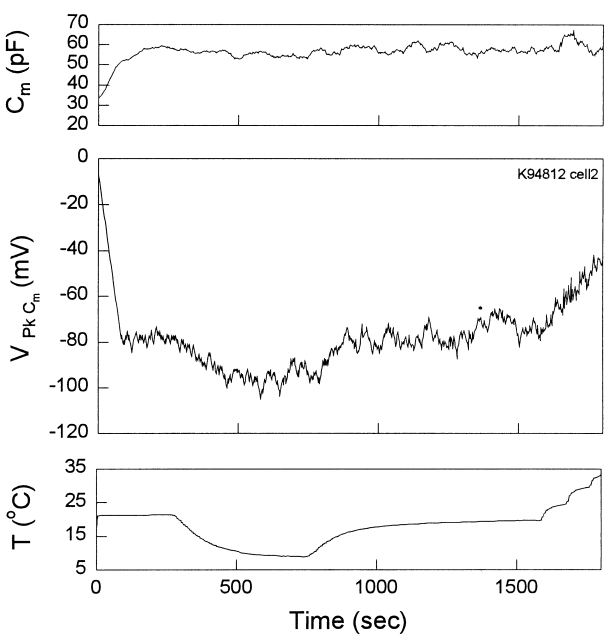

Fig. 4. The voltage at peak capacitance (V_{pkcm}) follows changes in temperature. A tracking procedure developed previously (Kakehata and Santos-Sacchi, 1995) was used to follow changes in C_{mpk} and V_{pkcm} as temperature was reversibly modified over the course of minutes. Tracking initiated at 0 mV. Note close correspondence between changes in temperature and V_{pkcm}.

expected to modify the total charge moved. In Fig. 3, a detailed analysis of the temperature effects is presented for two cells. It can be seen that the gating current time constants (τ_{on} and τ_{off}) and charge movement (Q) are proportional to temperature. The τ_{off} appears less affected by temperature than does τ_{on}. In each cell, the Q_{10} for τ_{on}^{-1} and τ_{off}^{-1} was about 1.6 and 1.4, respectively.

The OHC possesses a bell-shaped voltage-dependent capacitance function as a consequence of its membrane-bound nonlinear charge movement. The voltage where capacitance is maximal (V_{pkcm}) corresponds to the voltage where half maximal charge movement occurs, and where OHC motility is most sensitive to voltage fluctuations. Fig. 4 illustrates with a real time tracking technique that V_{pkcm} closely follows changes in temperature. Decreasing temperature causes a negative shift in V_{pkcm} and visa versa. Detailed capacitance evaluations support these tracking observations. Fig. 5A illustrates a family of capacitance functions obtained from a single OHC during temperature plateaus at 25, 21, 14, 8 and 6°C. V_{pkcm} shifted 26.8 mV between temperature extremes. Peak capacitance also decreased in this case, accompanied by a decrease in valence, z. Average results ($n=6$) are depicted in Fig. 5B. Q_{max} showed little significant systematic change. The valence, z, showed a change of 0.12 per 10°C, and V_{pkcm} changed 19.2 mV per 10°C.

Gating currents were also evaluated using the P/−5 protocol, which more effectively extracts gating currents (Armstrong and Bezanilla, 1977). Fig. 6 provides examples of gating currents from the same OHC evaluated in Fig. 5A. Fig. 6A,B show currents elicited by stepping from −120 mV to −30 and −60 mV, respectively. In both cases, decreasing temperature causes a decrease in the rate and quantity of charge moved. The average ($n=4$) τ_{on}^{-1} Q_{10} was 1.50 ± 0.06 (mean ± S.E.M.). The τ_{off}^{-1} Q_{10} was 1.55 ± 0.08. These results are in accord with the magnitudes obtained with the ±P technique used above; differences between on and off time constant Q_{10} are not significant.

Finally, an attempt was made to obtain gating currents from giant patches of OHC membrane (Hilgemann, 1989). Unfortunately, patch pipettes with tip diameters near 5 μm consistently broke through the membrane to yield whole-cell configurations with poor seals. Nevertheless, recordings under these conditions were sometimes capable of garnering gating current information. Fig. 7 presents such an example, where the cell was held at 0 mV and gating currents extracted with the ±P technique. The very fast time constant of the clamp amplifier under these conditions permitted recording of gating current time constants down to about 50 μs. Deiters cells under the same configuration showed no gating current. As in regular whole-cell configuration, decreases in temperature caused a reduction of OHC nonlinear charge moved, but had little effect on rate of charge movement.

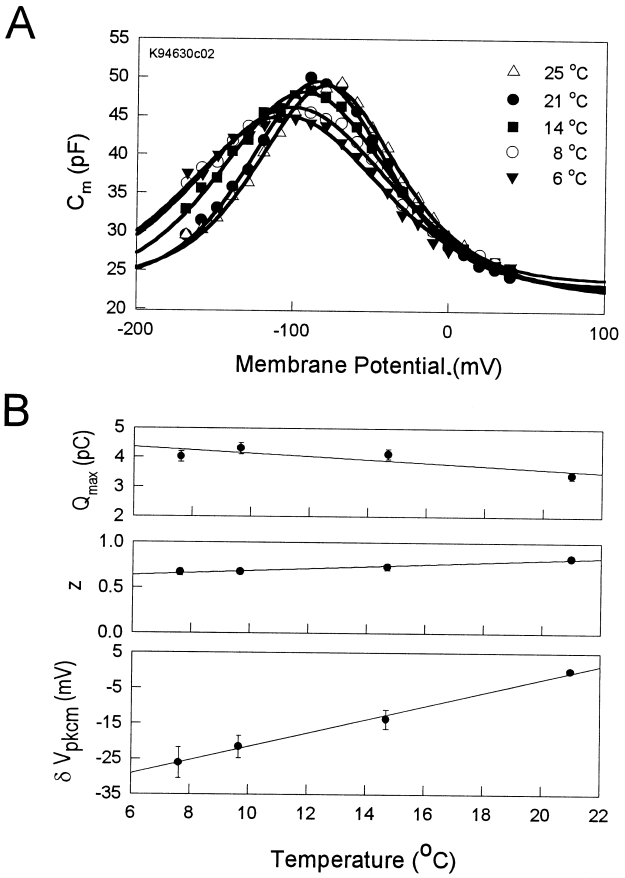

Fig. 5. Effect of temperature on OHC nonlinear capacitance. A: Capacitance was evaluated with the stair step protocol. As temperature is decreased from 25°C to 6°C, the functions shift in the hyperpolarizing direction. Solid lines are fits to Eq. 1. 25°C: V_{pkcm}: −78.8 mV; z: 0.87; Q_{max}: 2.99 pC; C_{lin}: 23.75 pF; 21°C: V_{pkcm}: −84.3 mV; z: 0.87; Q_{max}: 3.1 pC; C_{lin}: 23.12 pF; 14°C: V_{pkcm}: −93.1 mV; z: 0.71; Q_{max}: 3.74 pC; C_{lin}: 22.3 pF; 8°C: V_{pkcm}: −100.5 mV; z: 0.61; Q_{max}: 4.14 pC; C_{lin}: 21.7 pF; 6°C: V_{pkcm}: −105.6 mV; z: 0.62; Q_{max}: 3.77 pC; C_{lin}: 22.4 pF. B: Average results from six OHCs showing changes in parameters Q_{max}, z and V_{pkcm}. Error bars are ±S.E.M. Q_{max} remains fairly stable with temperature, although a slight increase below 21°C may be significant. z changes slightly with temperature. V_{pkcm} is significantly susceptible to temperature, showing a 1.9 mV change per degree centigrade.

4. Discussion

The OHC voltage-dependent charge movement or capacitance is sensitive to alterations in temperature. The most prominent effect appears to be on the position of these functions along the voltage axis, with a decrease in temperature causing a reversible negative shift in V_{pkcm} of 19.2 mV per 10°C. At a fixed holding and step potential, such a shift would be expected to result in a change in magnitude of charge moved. This is illustrated through modeling in Fig. 8. A small com-

ponent of the decrease in charge movement noted in gating current records as temperature is decreased may also be due to a redistribution in time beyond the experimental recording period, since steady-state indications from capacitance measures show little change in total charge moved (Q_{max}). The whole-cell capacitance determinations from stair step stimuli were robust since integrations were made over intervals greater than 15 times the capacitive current time constants (Huang and Santos-Sacchi, 1993). Gating current kinetics are sensitive to temperature, as well, but show only a mild temperature dependence, the Q_{10} being about 1.5. The Q_{10} of ion channel gating current kinetics, on the other hand, is typically between 2 and 3 (Bezanilla and Taylor, 1978; Kimura and Meves, 1979; Adams and Gage, 1979; Schauf and Bullock, 1979; Collins and Rojas, 1982). It should be emphasized, however, that OHC motility-related gating current kinetics are faster than clamp time constants obtainable under whole-cell configuration (Santos-Sacchi, 1990, 1992), and are thus constrained by clamp characteristics. Nevertheless, at least for ion channels, a temperature-dependent shift in the voltage dependence of conformational state often accompanies a temperature dependence of the underlying molecular kinetics. For example, in batrachotoxin treated Na channels, a 7 mV per 10°C shift in the open probability function accompanied a Q_{10} of about 2.3 for mean open times (Correa et al., 1992). For a two-state model, while a shift in steady-state probability reflects a change in the difference between open and closed steady-state energy levels, changes in kinetics are governed by alterations of the activation energy barrier height relative to those steady-state energy levels. Thus, changes in steady-state energy levels which effect shifts in the two-state probability function may also lead to changes in the relative height of the activation energy barrier, thereby affecting gating kinetics. Under the assumption that ionic channel and OHC molecular motor characteristics are similar, it is best to cautiously acknowledge that the large shift in OHC V_{pkcm} may be accompanied by a Q_{10} for the underlying kinetics which is greater than the measured value of 1.5, and that this value is a limitation of clamp speed. Temperature effects on OHC mechanical responses are also probably constrained by clamp speed (Holley and Ashmore, 1988). Recently, Ashmore and Gale (1997) found that gating current kinetics in OHC membrane patches, while remarkably fast (down to 7 μs τ), still evidenced Q_{10}s below 1.5. This is similar to results obtained with very fast clamp characteristics as in Fig. 7. However, they found no change in gating charge magnitude vs. temperature. This may indicate that, unlike our whole-cell results, V_{pkcm} did not shift in their membrane patch recording configuration, or just like our whole-cell results, clamp speed remained limiting.

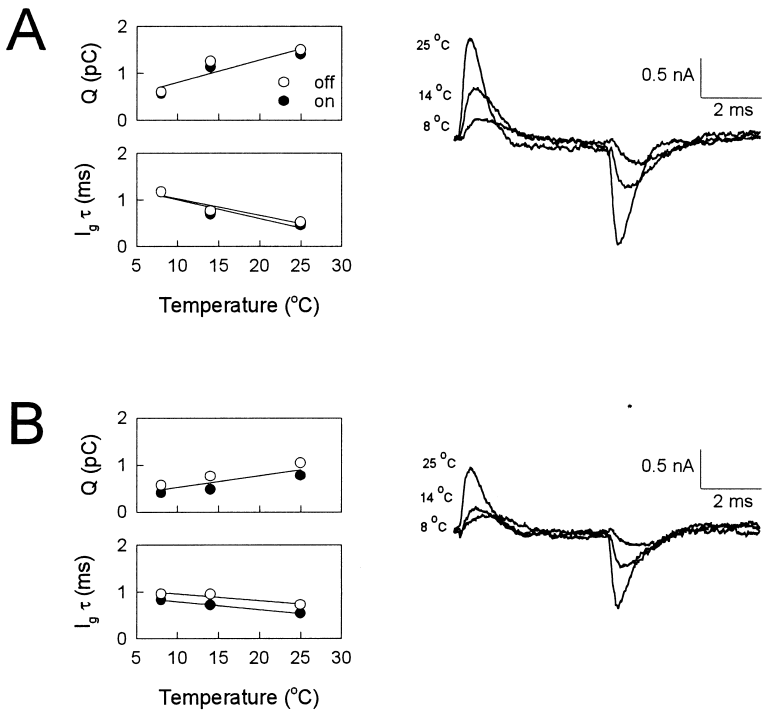

Fig. 6. Effect of temperature on gating currents obtained with the P/−5 protocol. Same cell as in Fig. 5A. A: Step voltage from −120 to −30 mV. A decrease in temperature from 25°C to 8°C decreases both charge moved and rate of movement. Q_{10} determined from the line fits for on and off gate τ was 1.61 and 1.49, respectively. B: Step voltage from −120 to −60 mV. Similar decreases were obtained. Q_{10} for on and off gate τ was 1.26 and 1.18, respectively.

A likely factor which might underlie the effects of temperature upon the kinetics of OHC lateral membrane motors, is a temperature dependence of OHC membrane viscosity. Membrane particle kinetics are governed by frictional forces within the medium, which are proportional to the medium's viscosity and particle size (see Rojas and Keynes, 1975). Interestingly, membrane viscosity may also underlie a component of the double exponential time course in voltage-induced V_{pkcm} shifts that we have recently found (Santos-Sacchi et al., 1996, 1997). It should be the case, then, that factors in addition to temperature, e.g. lipid composition, which influence OHC membrane viscosity or fluidity will have profound effects on OHC function.

OHC voltage-dependent capacitance is known to mirror characteristics of the OHC's mechanical activity (Ashmore, 1989, 1992; Santos-Sacchi, 1990, 1991, 1992; Kakehata and Santos-Sacchi, 1995). Given this relationship, temperature is expected to modify OHC motility and its physiological consequences, namely its contribution to the 'cochlear amplifier'. In this regard it is known that decreases in temperature are able to adversely affect in vivo cochlear frequency selectivity and sensitivity (e.g. Brown et al., 1983). Temperature change will alter the frequency response of OHC motility. In addition, a temperature-dependent shift in the OHC motility function along the voltage axis, which is indicated by the observed shift in V_{pkcm}, will alter the gain of OHC motility. Such effects may be sufficient to explain the in vivo results. However, it has also been shown that temperature modifies the resting length of isolated OHCs, which conceivably could modify basilar

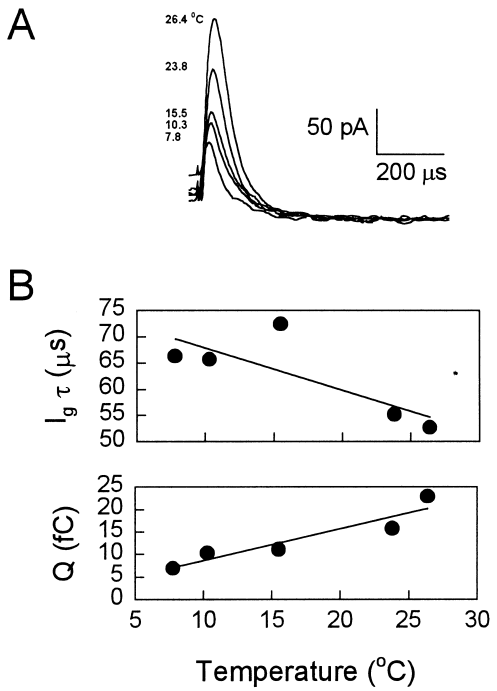

Fig. 7. A: Effect of temperature on gating currents obtained with very fast clamp time constant. During an attempt at giant patch formation with a 5 μm patch electrode tip, whole cell configuration was established. The trace shows off gating currents extracted with the ±P technique at 0 mV holding potential. The actual order of temperature change was 26.4, 10.3, 7.8, 15.5, and 23.8°C. B: Gate time constant and charge movement as a function of temperature. Charge moved decreases with temperature. Gating time constant is little effected by temperature; the fitted line represents a Q_{10} of about 1.2.

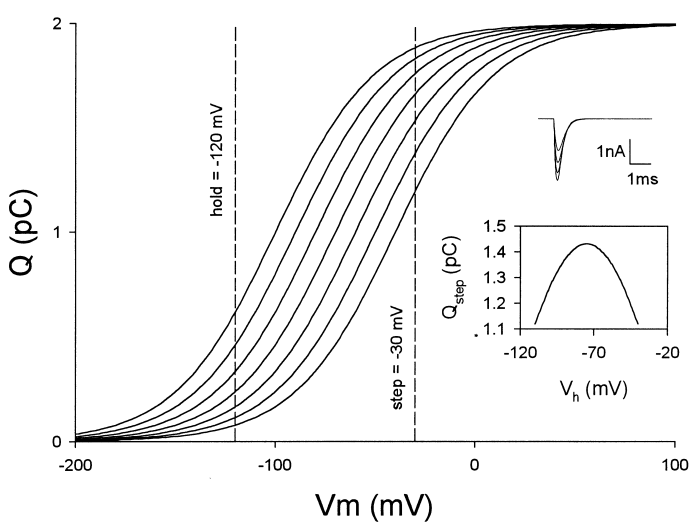

Fig. 8. Model illustrating the expected change in charge moved as a function of V_{pkcm} (V_h) shift. Cell model was as in Santos-Sacchi (1992), with R_s: 5 MΩ, R_m: 100 MΩ, C_m: 29 pF, Q_{max}:2 pC, z: 1, V_{pkcm} (V_h): −40 to −100, in steps of 10 mV. Charge is plotted vs. membrane potential for each value of V_{pkcm}. The dotted lines indicate the holding potential and step potential used to obtain the off gating currents in the inset, which were obtained with the P/−5 technique as for the biophysical data. The traces are plots only from V_{pkcm} of −70 to −100 mV, charge decreasing as V_{pkcm} shifts negatively. Scale: 1 ms, 1 nA. The inserted figure plots charge at the −30 mV step vs. V_{pkcm} (V_h), and illustrates that increases and decreases in charge moved can be obtained depending upon the relationship between holding, step and V_{pkcm} potential.

membrane tension in vivo (Gitter, 1992; LeCates et al., 1995). In addition to affecting the gain of OHC motility, a change in resting length at a fixed resting potential is expected from a shift in V_{pkcm}. This occurs because a displacement of the length vs. voltage function along the voltage axis (i.e. shift in V_{pkcm}) while holding the membrane potential constant will change the degree of contraction of the OHC. Given an OHC mechanical response of 20–30 nm/mV (Santos-Sacchi and Dilger, 1988), the temperature dependence of V_{pkcm} will provide for a resting length change of 0.04–0.06 μm/°C. However, both Gitter (1992) and LeCates et al. (1995) found values nearly an order of magnitude larger, which indicates that something other than a direct effect on the membrane motor was responsible for their observations.

In sum, we provide additional evidence that physical modification of the environment which harbors the molecular motors for OHC motility can profoundly modify the cell's electromechanical behavior. Previously, it had been shown that membrane tension, either externally applied (Iwasa, 1993; Gale and Ashmore, 1994; Kakehata and Santos-Sacchi, 1995) or intrinsically generated by the molecular motors themselves (Santos-Sacchi et al., 1996, 1997), can alter the voltage dependence of OHC capacitance/motility. Taken together, these data attest to the OHC's, and, indeed, the cochlea's, exceptional susceptibility to functional perturbation.

Acknowledgments

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