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Density of motility-related charge in the outer hair cell of the guinea pig is inversely related to best frequency

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Abstract

Whole cell voltage clamp and freeze fracture were used to study the electrophysiological and ultrastructural correlates of the outer hair cell (OHC) lateral membrane molecular motors. We find that specific voltage-dependent capacitance, which derives from motility-related charge movement, increases as cell length decreases. This increasing non-linear charge density predicts a corresponding increase in sensor-motor density. However, while OHC lateral membrane particle density increases, a quantitative correspondence is absent. Thus, the presumed equivalence of particle and motor is questionable. The data more importantly indicate that whereas the voltage driving OHC motility, i.e. the receptor potential, may decrease with frequency due to the OHC's low-pass membrane filter, the electrical energy ($Q \times V$) supplied to the lateral membrane will tend to remain stable. This conservation of energy delivery is likely crucial for the function of the cochlear amplifier at high frequencies.

Keywords: Outer hair cell; Motility; Gating charge; Capacitance; Cochlea; Membranes

The outer hair cell (OHC) lateral membrane possesses voltage-dependent molecular motors that work at acoustic frequencies to enhance basilar membrane motion [10]. The motors are believed to be proteins represented by ultrastructurally observable intramembranous particles [5,13], which based on electrophysiological determinations of associated voltage-sensor charge movement, possess about 1 equivalent electron charge for each 10 nm particle [1,10,17]. The voltage-dependent nature of OHC motility is predicted to limit the cochlear amplifier's effectiveness at high acoustic frequencies, where the cell's receptor potential is attenuated by the RC properties of the plasma membrane; receptor potentials at threshold levels may provide insufficient drive to evoke enhancement of basilar membrane motion [16,18]. In fact, however, it is the high frequency region that appears to benefit most from the activity of OHCs [14]. In an attempt to reconcile this dilemma, we have analyzed the voltage-dependent capacitance of OHCs isolated from different best-frequency locations along the cochlea spiral. A variety of evidence indicates that the OHC's robust nonlinear charge movement is inextricably related to the cell's mechanical activity [6,11,20].

OHCs were freshly isolated from the organ of Corti of the guinea-pig cochlea, and were whole-cell voltage clamped at room temperature using an Axon 200B (see [9] for details). Recordings were made within 3 min after whole cell establishment to limit any confounding effects of gradual turgor pressure change [12]. Membrane capacitance was evaluated at different potentials by transient analysis of currents induced by a voltage stair step stimulus (–150–100 mV, 10 mV steps), and the capacitance function was fit to the first derivative of a two state Boltzmann function relating non-linear charge to membrane voltage (dQ/dV [9]),

$$c_m = Q_{max}\frac{ze}{kT}\frac{b}{(1+b)^2} + c_{lin} \quad b = \exp\left(\frac{-ze(V - V_{pkCm})}{kT}\right)$$

Q_{max} is maximum nonlinear charge, V_{pkCm} is voltage at peak

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capacitance or equivalently, at half maximal non-linear 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. C_{lin} was estimated from the fit to the above capacitance (C_m) equation, and indicates the membrane's intrinsic linear capacitance. Cell length (L) was measured in 20 cells before whole-cell configuration was obtained. The fitted linear function, $L = 2.9 \times C_{lin} - 0.24$ ($r^2 = 0.89$), was used to obtain length from C_{lin} in all cells ($n = 245$; cochleae > 100). Data collection and analysis was performed with a Windows-based patch clamp program jClamp (http://www.med.yale.edu/surgery/ otolar/santos/jclamp.html).

For ultrastructural studies, guinea pigs ($n = 15$) were deeply anesthetized, and the cochleae fixed by intra-labyrinthine perfusion of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Following dissection, the cochleae were kept in the same fixative overnight at 4°C. Specimens were infiltrated with 30% glycerol for 1–4 h, and frozen in liquid nitrogen slush (–210°C). Fracturing was carried out at 10^{-7} Torr, at –130°C, and rotary shadowing

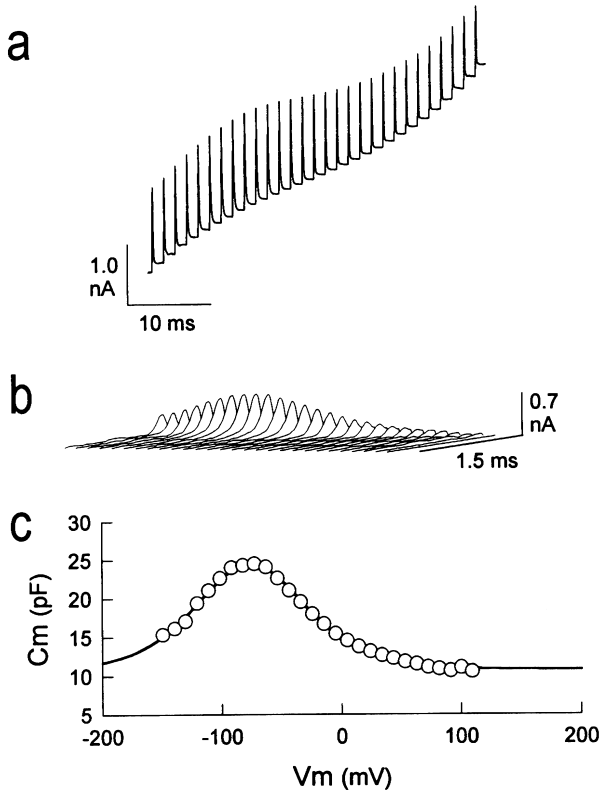

Fig. 1. Non-linear capacitance of an OHC isolated from the basal, high frequency region of the cochlea. (a) Capacitive currents induced by a stair step voltage protocol. (b) Gating currents extracted by subtracting the linear capacitive current obtained at the largest depolarization. (c) Capacitance of the cell (oc), comprised of a bell shaped voltage dependent capacitance riding atop a linear capacitance. The solid line is a fit (see text) indicating V_{pkCm}, –76 mV; Q_{max}, 1.7 pC; C_{lin}, 10.8 pF; z, 0.83. Length is 30.3 μm. Electrode series resistance was 3.7 MΩ.

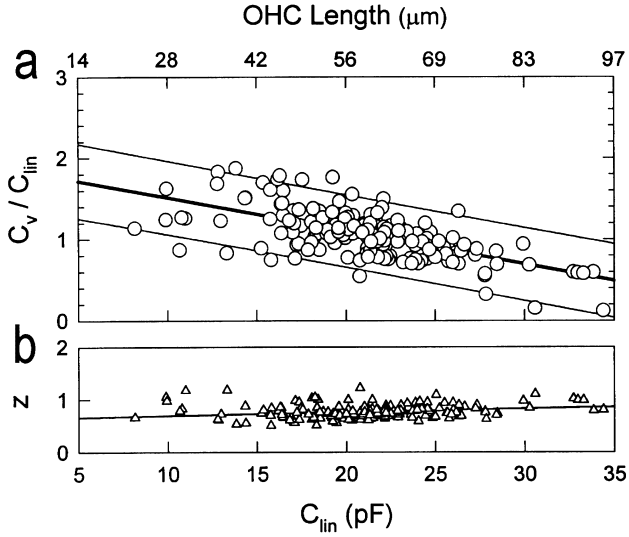

Fig. 2. (a) The ratio of maximum voltage dependent capacitance (C_v) to linear capacitance (C_{lin}) increases as OHC length (or equivalently, C_{lin}) decreases. Total capacitance was determined directly from capacitive currents. C_v is the peak capacitance minus C_{lin}, the latter being determined from Boltzmann fits to the data. (b) The valance z changes little with cell length or C_{lin}. The slight decrease in z may indicate a decreased charge for the voltage sensor and/or a decrease in the electrical distance displaced within the plane of the lateral membrane.

with platinum and carbon backing were made with a vacuum evaporator JEOL JFD-7000. Replicas were examined with a JEOL 1010 or JEOL JEM-100S transmission electron microscope at 100 kV. The protoplasmic fracture face of the OHC lateral membrane was examined at final photographic magnifications of 120 000. Images were divided into 2 cm^2 samples, and the total number of particles was used to obtain densities.

OHC length is inversely related to best frequency; as best frequency decreases from about 40 kHz to 100 Hz in the guinea pig, cell length increases from about 20 to 90 μm. Short, high frequency, OHCs posses a non-linear capacitance whose peak value can be greater than the cell's linear capacitance (Fig. 1). This is markedly different from cells residing in the low frequency region where the magnitude of non-linear capacitance is typically equivalent to or less than the cell's linear capacitance [1,17]. Moreover, as OHC length decreases (indicated by decreasing linear capacitance, C_{lin}), the relative magnitude of maximum voltage dependent capacitance (C_v) increases, as indicated by the ratio of the two (Fig. 2). Boltzmann fits show that the valence, z, changes little with cell length indicating that the parameter responsible for the relative increase in capacitance is the maximum charge, Q_{max}($Q_{max} = 4kT/ze \times C_v$). The specific non-linear charge (Q_{sp}), based on whole cell surface area, changes with a slope of –413 $e^-/\mu m^2$ per pF of linear capacitance (Fig. 3a). This change, however, is an underestimate and does not reflect the highly compartmentalized nature of the OHC plasma membrane. While mechano-electrical transduction channels are restricted to

the OHC apical membrane [15], and voltage-dependent ionic channels are restricted to the basal membrane [19], mechanical responses and associated non-linear capacitance are restricted to the central extent of the cell, the lateral membrane [2,9,13]. Accordingly, a constant 6.2 *pF* (from [9]) of linear capacitance or correspondingly, 620 μm^2, representing the constant apical and basal membrane should be excluded from calculations of Q_{sp}. When only the sensor-motor area is considered, the change in Q_{sp} as a function of cell length is striking, showing an increasing non-linear growth as cell length decreases (Fig. 3b). Q_{sp}'s up to 46 000 $e^-/\mu m^2$ are observed.

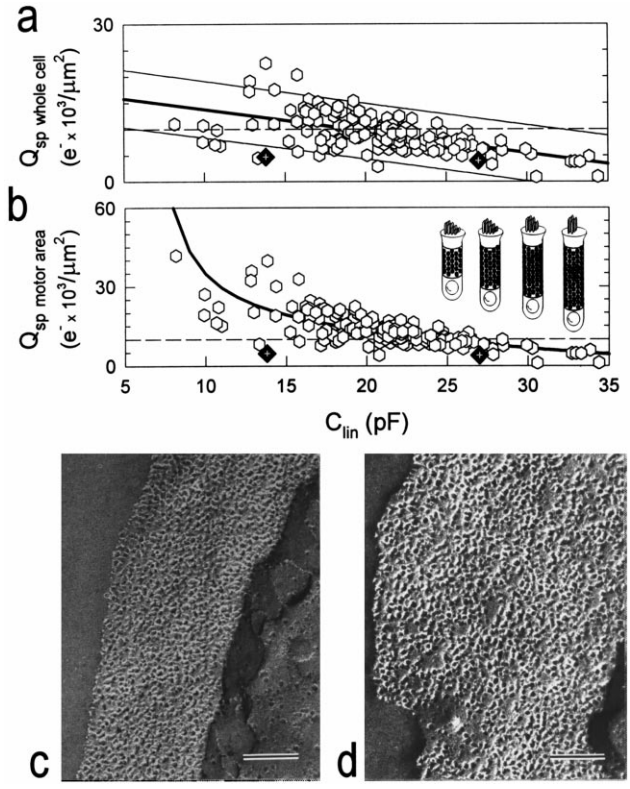

Fig. 3. (a) Non-linear charge density determined from whole cell surface area. Surface area was determined from the equivalence of linear capacitance and membrane surface area ($0.01pF = 1\ \mu m^2$; [9]). Charge density increases as cell length or C_{lin} decreases. The slope, indicated by the solid line fit is –413/*pF*, and the thin parallel lines are 95% prediction limits. The two diamonds indicate the particle densities within the OHC lateral plasma membrane at two disparate frequencies. For values see text. The dashed line is the theoretical maximum density of particles based on 10 nm diameter. (b) Charge density obtained after correction of whole cell surface area for area devoid of sensor-motors. A constant 620 μm^2 was subtracted from whole cell surface area. The solid line is simply the linear fit as above similarly corrected. The OHC schematics illustrate the relative change in sensor-motor containing lateral membrane area as cell length decreases. Other symbols as in (b). (c) Freeze-fracture replica showing the protoplasmic fracture face of the lateral plasma membrane of an OHC from a high frequency region of the cochlea. Densely packed intramembranous particles are characteristically found. Scale bar, 100 nm. (d) Same view obtained from cell in a low frequency region of the cochlea. Density is less than in (c). Scale bar, 100 nm.

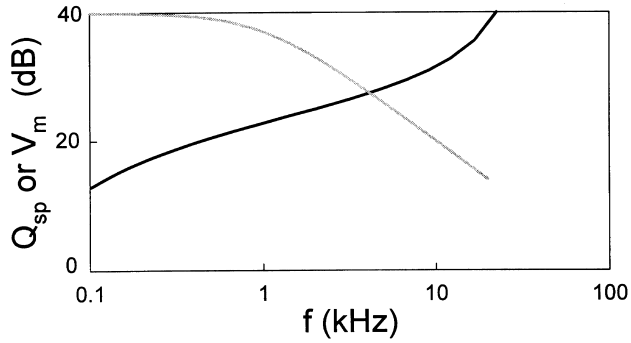

Fig. 4. Relationship between roll-off of receptor potential of a hypothetical OHC and increase of non-linear charge density as a function of frequency. The receptor potential falls at 20 dB per decade above about 1 kHz (gray line), whereas the charge density increases by a similar amount over frequency (black line obtained from b). OHC data were converted to frequency based on the map of Dannhof et al. [4].

A two state Boltzmann model has been successfully employed by several groups to characterize the OHC sensor-motor [1,10,17]. If this model is appropriate for the analysis of OHC charge movement, then the Boltzmann characteristics indicate that the density of independent sensor-motors should increase in correspondence with the specific charge density. A frequency-associated increase in the density of membrane protein is not unusual for the OHC. One K channel current, IK_n, is known to increases as characteristic frequency increases [8], and since this current is limited to the fixed area of the cell's basal pole, channel density must increase [19] (see [7] for an alternative explanation). Using freeze fracture, we measured the density of the putative ultrastructural correlate of the sensor-motor in the lateral membrane from areas of disparate characteristic frequencies (Fig. 3c,d). In a low frequency region (~200 Hz) where cell length averaged 76.3 ± 2.2 μm, particle density was 4033 (±90; 35 samples)/μm^2. In a high frequency region (~4 kHz) where cell length averaged 38.8 ± 0.4 μm, particle density was 4745 (±155; 38 samples)/μm^2. While the densities are significantly different (*t*-test, $P < .001$), they do not correspond to the densities predicted by Q_{sp}. Indeed, even the theoretical maximum density of 10 nm intramembranous particles, namely about 10 000/μm^2, does not approach electrophysiological estimates. Two possibilities may account for the disparity. First, the particles may not represent the sensor-motor molecules. Second, the two-state Boltzmann model inappropriately characterizes the sensor-motor as an independent entity possessing close to one electron charge. However, even if each intramembranous particle possessed more than one charge, the measured growth in particle density still requires that particle (voltage sensor) charge not remain fixed along the cochlea spiral. While a variably charged voltage sensor may not be unrealizable, the simplest explanation is the first.

Finally, the charge density data may help to explain how the OHC is able to overcome the effects of its membrane filter. In vivo measures of OHC receptor potentials show

that above about 1 kHz, AC voltages decrease at a rate 6 dB/octave [3,15]. Thus, the driving force for OHC motility is attenuated at high frequencies. However, our data indicate that, across frequency, motility-related charge increases to nearly the same extent as the decrease in receptor potential magnitude (Fig. 4). Thus, the electrical energy ($Q \times V$) delivered to the OHC lateral plasma membrane remains stable across frequency. It is likely that this frequency-independent energy delivery sustains the wide-band nature of the cochlear amplifier.

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