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Hearing Research 165 (2002) 35–47

HEARING RESEARCH

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The receptor potential in type I and type II vestibular system hair cells: a model analysis

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Received 22 June 2001; accepted 18 October 2001

Abstract

Several studies have shown that type I hair cells present a large outward rectifying potassium current ($g_{K,L}$) that is substantially activated at the resting potential, greatly reducing cell input resistance and voltage gain. In fact, mechanoelectrical transducer currents seem not to be large enough to depolarize type I hair cells to produce neurotransmitter release. Also, the strongly nonlinear transducer currents and the limited voltage oscillations found in some hair cells did not account for the bidirectionality of response in hair cell systems. We developed a model based in the analysis of nonlinear Goldman–Hodgkin–Katz equations to calculate the hair cell receptor potential and ionic movements produced by transducer current activation. Type I hair cells displaying the large $g_{K,L}$ current were found to produce small receptor potentials (3–13.8 mV) in response to mechanoelectrical transducer current input. In contrast, type II cells that lack $g_{K,L}$ produced receptor potentials of about 30 mV. Properties of basolateral ionic conductances in type II hair cells will linearize hair bundle displacement to receptor potential relationship. The voltage to obtain the half maximal activation of $g_{K,L}$ significantly affects the resting membrane potential, the amplitude, and the linearity of the receptor potential. Electrodiffusion equations were also used to analyze ionic changes in the intercellular space between type I hair cell and calyx endings. Significant K^+ accumulation could take place at the intercellular space depending on calyx structure.

Key words: Transduction; Vestibular; Sensory coding; Membrane potential; Afferent synapse

1. Introduction

Hair cells from the inner ear form part of an epithelium separating two fluids with different ionic composition. Their basolateral membrane faces the perilymph (with low potassium and high sodium concentration), and the apical side of the cell is in contact with the endolymph (with high potassium and low sodium composition). This condition implies that in vivo membrane potential generation should be complex. Non-homogeneous currents would arise in different regions of the cell membrane, depending on local permeabilities, and on the ionic concentrations of the surrounding fluid.

Ionic conductance of vestibular hair cells varies de-

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Abbreviations: CR, coefficient of rectification; DV, hair bundle position; e, endolymphatic or apical; E_K and E_{Na}, equilibrium potentials for K^+ and Na^+ respectively; F, Faraday constant; GHK, Goldman–Hodgkin–Katz; g_h, slow inward rectifier; $g_{K,A}$, transient K^+ conductance; $g_{K,DR}$, delayed rectifier K^+ conductance; $g_{K,IR}$, rapid voltage-dependent inward conductance; g_K, lumped K^+ conductance; $g_{K,L}$, low voltage-activated K^+ conductance; g_l, leakage permeability; i, interior of the cell; I_x, specific ionic current; I_{Na} and I_K, lumped Na^+ and K^+ currents; I_{MET}, mechanoreceptor current; $I_{K,MET}$, mechanoreceptor current carried by K^+; $I_{Na,MET}$, mechanoreceptor current carried by Na^+; MET, mechanoelectrical transducer; p, perilymphatic or basolateral; P_e, permeability of the transducer to K^+ ions; $P_{K,h}$ and $P_{Na,h}$, permeabilities to K^+ and Na^+ of g_h; P_K, lumped K^+ permeability; $P_{K,L}$, $P_{K,DR}$, $P_{K,A}$, potassium permeability of $g_{K,L}$, $g_{K,DR}$ and $g_{K,A}$; P_{Na}, Na^+ permeability; P_p, total perilymphatic permeability; P_x, specific channel permeability; P_k^{sc}, P_{Na}^{sc}, P_{Cl}^{sc}, subsynaptic conductor permeability to K^+, Na^+ and Cl^-; R_{MET}, membrane resistance of apical portion of the cell; R, gas constant; T, absolute temperature; V, membrane voltage; V_s, subsynaptic voltage; V_i, intracellular voltage; $V_{1/2}$, half voltage activation

0378-5955/02/$ – see front matter
PII: S0378-5955(01)00418-X

pending on the animal species, the region where the cells originate, and the developmental stage of the animal (Masetto et al., 1994; Eatock and Rüsch, 1997). However, hair cells share some characteristic ionic currents and, in all cells studied to date, potassium currents dominate the basolateral membrane response (Hudspeth, 1986; Hudspeth and Lewis, 1988). Therefore, models considering the cellular location and voltage dependence of ionic currents may be helpful in establishing the range under which different hair cells may operate.

The most striking difference between vestibular hair cell types is the expression of the low voltage-activated, non-inactivating outward K^+ current in type I hair cells (Correia and Lang, 1990; Rennie and Ashmore, 1991; Correia, 1992; Eatock and Hutzler, 1992). This current has been extensively characterized in type I hair cells from the rat utricle, referred to as $g_{K,L}$ (Rüsch and Eatock, 1996a,b), and in pigeon inner ear, where it has been referred to as g_{KI} (Ricci et al., 1996). In many type I hair cells, $g_{K,L}$ is substantially activated at the resting potential, greatly reducing cell input resistance (Rennie et al., 1996; Rüsch and Eatock, 1996b). In fact, mechanoelectrical transducer (MET) currents seem not to be large enough to depolarize type I hair cells to produce neurotransmitter release. Although the real value of the half activation voltage ($V_{1/2}$) of $g_{K,L}$ has been questioned (Lennan et al., 1999), there seem to be substantial data supporting that $V_{1/2}$ value of $g_{K,L}$ can be as negative as -90 mV (range -40 to -90 mV) in physiological conditions (Hurley and Eatock, 1999; Chen and Eatock, 2000).

Even though there is substantial information on voltage-dependent conductances in vestibular hair cells, only a few authors have registered them and maintained the ionic composition similar to the endo- and the perilymphatic fluids (Bracho and Budelli, 1978; Corey and Hudspeth, 1979; Ricci and Fettiplace, 1997, 1998). In most of the works published so far, hair cells have been recorded in vitro while being bathed by a homogeneous low-potassium high-sodium saline solution. Therefore, the receptor potential and ionic currents in vivo cannot be straightforwardly deduced from these data.

Some of the problems to be solved concerning the response of the vestibular system hair cells are related to the fact that $g_{K,L}$ is significantly activated at the zero current potential, thus considerably influencing the gain of the receptor potential. Also, in view of the rectifying nonlinear transducer currents and the limited voltage oscillations found in type I hair cells, it is important to define the degree of rectification of the receptor potential and its participation in determining the bidirectional sensitivity of the system. Finally, the low gain of the receptor potential of type I hair cells poses the question about the mechanism of afferent transmitter release in this cell type. Type I hair cells have a very peculiar afferent synapse which forms a calyceal structure surrounding the basolateral surface of the cell; many authors have wondered what the possible function of this calyx is (Scarfone et al., 1988; Yamashita and Ohmori, 1990; Schessel et al., 1991; Guth et al., 1998). The complex calyx structure suggests that synaptic mechanisms in this cell type are peculiar. The calyx forms a microdomain space between the basolateral region of the hair cell and the afferent neuron. One possibility that has been put forward by Goldberg (1996) is that the basolateral ionic fluxes from type I hair cells may lead to important ionic concentration changes in the synaptic cleft, contributing to determine the hair cell membrane potential and the subsequent neurotransmitter release.

We report a model to determine, from experimental measurements made in a homogeneous environment, the in vivo receptor potential of hair cells. This model made it possible to analyze the influence of basolateral ionic conductances on the hair cell response to the transducer current input. Analysis of basolateral ionic concentration changes in type I hair cells (surrounded by a calyx) that are produced as a consequence of transducer activation was also performed. Some of the results presented here have appeared in abstract form (Soto et al., 1999).

2. Methods

2.1. The model

2.1.1. The apical membrane

For the measurement of membrane conductance changes during mechanical stimulation in isolated hair cells, Rüsch and Eatock (1996a) voltage-clamped the cells with a holding potential of -84 mV. The equilibrium potentials for potassium (E_K) and sodium (E_{Na}) ions according to the intra- and extracellular solutions used by the authors in those experiments were -85 mV for K^+ and 108 mV for Na^+. Consequently, since E_K is almost equal to the holding potential used in voltage clamp experiments, the MET current must be carried mainly by Na^+: hence from the Goldman–Hodgkin–Katz (GHK) equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$I_{MET} \cong I_{Na,MET} = P_{Na} \frac{VF^2}{RT} \frac{[Na]_i - [Na]_o e^{(FV/RT)}}{1 - e^{(FV/RT)}} \qquad (1)$$

where V is the membrane potential; F, R and T have their usual meaning as the Faraday constant, gas constant and absolute temperature respectively; P_{Na} is the permeability to Na^+ and $I_{Na,MET}$ denotes the current through the mechanoreceptor channels.

Although in vivo transducer current (I_{MET}) is mainly carried by K^+ and only a fraction by Ca^{2+}, in isolated cells (surrounded by a uniform extracellular perilymphatic-like medium), transducer current is carried mainly by Na^+.

Substituting for the Rüsch and Eatock (1996a) experimental conditions ($V = -84$ mV, $[Na]_o = 144$, $[Na]_i = 2.5$ mM) and maximal transducer current ($I_{Na,MET} = 250$ pA), we found that the Na^+ permeability of the transducer channels, when they are fully activated, is:

$$P_{Na}\frac{I_{Na,MET}}{VF^2}\frac{1-e^{(FV/RT)}}{[Na]_i-[Na]_o e^{(FV/RT)}} = 50\times10^{-15}\ m^3\ s^{-1} \quad (2)$$

Since (according to Ohmori, 1985) the permeability ratio of the apical membrane for Na^+ and K^+ is $P_K/P_{Na} \cong 1.04$, then, $0 < P_K < 52\times10^{-15}$ m^3 s^{-1}. Other authors have reported similar permeability ratios of the MET channels for Na^+ and K^+ in the hair cells of the turtle basilar papilla ($P_{Na}:P_K:P_{Cs} = 1:0.99:0.79$, Crawford et al., 1991). Although in Eq. 2 the permeability is expressed as a function of the voltage, it has been demonstrated that transducer current exhibits no voltage dependence (Ohmori, 1987). This means that $I_{Na,MET}$ must vary as a linear function of the holding potential, for a given hair bundle position, so the resulting P_{Na} does not depend on the voltage.

Ionic concentrations in vivo are completely different from those used in isolated cell experiments. According to the values measured for the rat in vivo: $[Na]_i \ll [K]_i$, $[Na]_e \ll [K]_e$ (i, intracellular; e, endolymph); $[K]_i \cong [K]_e \cong 140$ mM and $V \cong -80$ mV (Bosher and Warren, 1968; Sterkers et al., 1988; Sauer et al., 1999).

From here, it follows that, in vivo, the MET current will vary between 0 and

$$I_{MET} \cong I_{K,MET} = P\frac{VF^2}{RT}[K] \cong 230\times10^{-12}\ A \quad (3)$$

when the channel open probability is maximal. Membrane resistance of the apical portion of the cell, assuming linearity, can then be calculated from:

$$R_{MET} = \frac{V}{I_{MET}} \cong 350\ M\Omega$$

In the apical membrane, only the MET current was considered and it was used in the model as a source of current. The current versus hair bundle position (DV) relationship was obtained by using a second-order Boltzmann equation (Corey and Hudspeth, 1983), and is used in the model just as a current source.

$$I(DV) = \frac{I_{max}}{1 + e^{k_1(DV_1-DV)}(i + e^{k_2(DV_2-DV)})}$$

where $I(DV)$ is the transducer current, DV is the hair bundle deflection, and k_1, k_2, DV_1 and DV_2 are constants that set the steepness and x-axis position of the function.

2.1.2. The basolateral membrane

At the basolateral surface of the cell the following voltage-dependent permeabilities were considered: (a) the low voltage-activated K^+ conductance ($g_{K,L}$); (b) the slow inward rectifier (g_h); (c) the delayed rectifier ($g_{K,DR}$); (d) the transient potassium permeability ($g_{K,A}$). Both $g_{K,L}$ and $g_{K,DR}$ are K^+-selective outward delayed conductances. They differ in their $V_{1/2}$, $g_{K,L}$ having a very negative value (negative to -60 mV). Since the model is based on thermodynamic equilibrium equations, the relevant parameters of the currents are their voltage dependence and their ionic selectivity. Also a voltage-independent leakage permeability (g_l) was included. We defined lumped conductances as:

$$P_K = P_{K,L} + P_{K,h} + P_{K,DR} + P_{K,A} + P_L$$

$$P_{Na} = P_{Na,h}$$

where P_K and P_{Na} are the permeabilities to K^+ and Na^+ of each of the conductances considered.

2.2. GHK model

In this model, hair cell conductances are nonlinear, and the main simplification resides in accepting the constant field hypothesis (Goldman, 1943). Membrane permeabilities (corresponding to ion channels considered) were calculated as a function of the membrane potential (Rüsch and Eatock, 1996a,b). Reciprocally, using these permeabilities, it was possible to calculate the in vivo membrane potential, taking advantage of the fact that in the vestibular system there is practically no potential difference between the endo- and the perilymphatic compartments (Bracho and Budelli, 1978).

The following assumptions were made: (1) that there is no potential difference between the endolymphatic and perilymphatic regions; (2) transducer permeability follows a second-order Boltzmann dynamics (Corey et al., 1983; Rüsch and Eatock, 1996; Géléoc at al., 1997); (3) the basolateral membrane of the hair cell is predominantly permeable to potassium; (4) the ionic compositions of the cell, the perilymph and the endolymph are constant (for the intracellular space, this is a consequence of the other assumptions. For the endolymph and the perilymph, their volumes far outweigh ionic changes due to hair cell activation.); (5) since no explicit equations about Cl^- currents in hair cells exists, no Cl^- current was taken in consideration; (6) we as-

sume a low Cl^- membrane permeability that contributes to the leakage current.

The transmembrane currents for a given ionic species can be calculated by using the following equation (Hodgkin and Katz, 1949):

$$I_X = P_X\, F\frac{VF}{RT}\frac{[X]_e - [X]_i\, e^{(-FV/RT)}}{1-e^{(-FV/RT)}} \tag{4a}$$

where $X = K^+$ or Na^+, V is the test potential used to record the voltage-dependent currents (the holding potential for the case of MET current recording), P_X is the specific channel permeability and I_X is the ionic current.

Defining:

$$f(V) = \frac{F^2}{RT}\frac{V}{1-e^{(-FV/RT)}},$$

we may express Eq. 4a as:

$$I_X = P_X\left([X]_e - [X]_i e^{(-FV/RT)}\right) f(V) \tag{4b}$$

Consequently, permeabilities are proportional to tail or receptor currents, where the permeability constant can be calculated by the expression multiplying P_X in Eq. 4b. For the $g_{K,L}$ and g_K channels (which are not permeable to Na^+) the permeability constants at the testing voltage (84 mV) are 3.5×10^{-4} and 2.4×10^{-4} $m^3\ s^{-1}$, respectively. In contrast, both MET and g_h channels are permeable to Na^+ and K^+ with permeability constants for K^+ of 2.2×10^{-5} and 1.46×10^{-4} $m^3\ s^{-1}$, respectively. The permeability ratio (P_K/P_{Na}) is about 1.04 for the MET channels (Ohmori, 1985) and 6.95 for the g_h channels. The latter value was estimated from the reversal potential (−45 mV) reported for g_h channels in hair cells (Rüsch and Eatock, 1996a).

The membrane potential of the hair cells can be obtained from the ionic composition of the intracellular, the endo- and the perilymphatic fluids, and from the membrane permeabilities to different ions. The ionic currents due to Na^+ and K^+ from either the endo- or the perilymphatic side of the cell are:

$$I^e_{Na} = P^e_{Na}\left([Na]_e - [Na]_i e^{VF/RT}\right) f(V) \tag{5}$$

$$I^p_{Na} = P^p_{Na}\left([Na]_p - [Na]_i e^{VF/RT}\right) f(V) \tag{6}$$

$$I^e_{K} = P^e_{K}\left([K]_e - [K]_i e^{VF/RT}\right) f(V) \tag{7}$$

$$I^p_{K} = P^p_{K}\left([K]_p - [K]_i e^{VF/RT}\right) f(V) \tag{8}$$

where I_{Na} and I_K are the lumped K^+ and Na^+ currents. P_{Na} and P_K are the permeabilities to Na^+ and to K^+ which are a voltage function $f(V)$ as previously defined. Superscripts in permeabilities and currents indicate the corresponding portion of the membrane (e: endolymphatic or apical; p: perilymphatic or basolateral). Subscripts in permeabilities and currents indicate the corresponding ion. Subscripts in ionic concentrations indicate the compartment (e: endolymph; p: perilymph; i: the interior of the cell). Since $I^e_{Na} + I^p_{Na} + I^e_K + I^p_K = 0$, and considering that the potential difference between the endolymph and the perilymph is negligible, then:

$$e^{VF/RT}\left([Na]_i\,(P^e_{Na} + P^p_{Na}) + [K]_i\,(P^e_K + P^p_K)\right) =$$

$$P^e_{Na}[Na]_e + P^p_{Na}[Na]_p + P^e_K[K]_e + P^p_K[K]_p \tag{9}$$

By assuming that the voltage-dependent permeabilities are all located in the basolateral membrane, and the MET permeability is at the apical side of the cell membrane, we obtain:

$$e^{VF/RT} = \frac{P_e([K]_e + [Na]_e/1.04) + P_p[K]_p + P_{Na,h}[Na]_p}{[Na]_i(P_e/1.04 + P_{Na,h}) + [K]_i(P_e + P_p)} \tag{10}$$

where P_p is the total perilymphatic potassium permeability, and P_e is the permeability of the transducer to potassium ions, obtained from Eq. 2.

Considering that $[Na]_i \ll [K]_i$ and that K^+ permeability (P_p) is much larger than that for Na^+ then Eq. 10 can be simplified to:

$$V = \frac{RT}{F}\ln\frac{P_e([K]_e + [Na]_e/1.04) + P_p[K]_p + P_{Na,h}[Na]_p}{[K]_i\,(P_e + P_p)} =$$

$$\frac{RT}{F}\ln\frac{P_e(140 + 5/1.04) + 5\,P_p + 140\,P_{Na,h}}{140\,(P_e + P_p)} \tag{11}$$

At equilibrium, the voltage calculated by Eq. 11 must be the same membrane potential (V) as that used to calculate the permeability $P_p = P_{K,L} + P_K + P_{K,h}$ and $P_{Na,h}$. This equation has as variable V and the permeabilities, which in turn are functions of V and the position of the hair bundle. Thus, it turns out to be a relation between V and the position of the hair bundle. We developed an iterative Matlab™ program to calculate this function; the program runs iteratively, initially the input current produced by a small hair bundle displacement is calculated; then, the consequent ionic movements and the new membrane potential values were calculated using Eq. 11. With the new voltage, the membrane permeabilities are calculated again and consequently using Eq. 11 a new voltage is obtained. The process continues until iterated voltages become almost constant. In this form, the program allowed for defining the voltage change produced by hair bundle

movements and the consequent changes in permeabilities.

2.3. Subsynaptic compartment ionic concentration analysis

From the model analysis, it became clear that the receptor potential in type I hair cells has a very low gain. This raises the question about the mechanism of the afferent transmitter release in this cell type. To study whether significant ionic concentration changes take place at the synaptic cleft (here designated the subsynaptic space) during transducer current activation, we added a fourth compartment to our GHK model. Thus, the four compartments are: (i) the interior of the hair cell, (ii) the endolymphatic space in contact with the apical surface of the cell, (iii) the perilymphatic space surrounding the external wall of the calyx and (iv) the subsynaptic space (Fig. 1).

The following assumptions were made to model the subsynaptic compartment. (1) The subsynaptic space is connected to the perilymphatic region through the pathway formed between the lateral region of the hair cell and the afferent calyx. Its permeability is variable, reflecting a large or tight calyx, or a small or leaky calyx. We referred to it as subsynaptic conductor; the subsynaptic conductor value is related to the apparent diffusion coefficient (Nicholson and Syková, 1998). (2) Electrodiffusion equations and the constant field hypothesis hold for both the cell membrane and the subsynaptic conductor permeabilities. (3) The only pathway for ions out of the subsynaptic space is by diffusion out of the calyx.

The receptor current through the apical membrane, the current through the basolateral membrane and the current through the subsynaptic ionic conductor must be equal. The current through the apical membrane is the result of the addition of Na^+ and K^+ currents each determined by Eq. 4, and the relevant voltage (V_i) is the potential difference between the interior of the cell and the endolymph.

The current through the basal membrane also results from the addition of Na^+ and K^+ currents determined using the same equation (Eq. 4), but in this case the relevant voltage is the potential difference between the interior of the cell and the subsynaptic (intercellular) region V_s.

The ensuing current through the ionic conductor that connects the subsynaptic space with the perilymphatic space results from the coupled Na^+, K^+ and Cl^- currents also determined by Eq. 4, but considering as the relevant variables the subsynaptic and the perilymphatic ionic concentrations, and the potential difference between the subsynaptic (intercellular) region (V_s) and the perilymph.

The values to be resolved are the intracellular voltage (V_i) and the subsynaptic voltage (V_s). The identification of the three currents provides two equations that determine these voltages. We developed an iterative program to calculate V_i and V_s, at each instant, using Newton's method of root approximation. After obtaining the values for these potentials, other variables were calculated using closed formulae, and changes in the ionic concentrations of the intercellular region at the next instant were calculated.

A third variable (the K^+ concentration in the intercellular region) was thus considered, and new equations and recursive calculations appeared:

$$[K^+]_s = [Cl^-]_s - [Na^+]_s \tag{12}$$

where

$$[Cl^-]_s = [Cl^-]_p \, e^{FV_s/RT} \tag{13}$$

$$[Na^+]_s = [Na^+]_p \, e^{-FV_s/RT} \tag{14}$$

Since these ions are in equilibrium through the subsynaptic ionic conductor (subscripts s and p refer to subsynaptic and perilymphatic spaces).

From these equations we may express $[K^+]_s$ as a function of V_s:

$$[K^+]_s = [Cl^-]_p \, e^{FV_s/RT} - [Na^+]_p \, e^{-FV_s/RT} \tag{15}$$

3. Results

Based on the analysis of the model (Fig. 1) it was found that the variation of the membrane potential due to MET activation is only a few mV (around 7 mV) in type I hair cells and close to 30 mV in type II cells (Fig. 2). Therefore, full activation of the transducer current in type II hair cells may depolarize the cell up to 30–40 mV depending on the magnitude of the transducer current. This voltage displacement would suffice to activate a set of voltage-dependent conductances, including high voltage-activated calcium channels that have been shown to be coupled to neurotransmitter release from hair cells (Issa and Hudspeth, 1994; Perin et al., 2000) (Fig. 2). In comparison, the receptor potential of type I hair cells (about 7 mV) would not be enough to activate voltage-dependent conductances including those that may be coupled to neurotransmitter release (Fuchs et al., 1990; Boyer et al., 1998).

The above mentioned calculations were performed considering that the maximum magnitude of the transducer current is about 250 pA. There are important variations in the magnitude of the transducer current registered in different experimental preparations; however, most of the authors agree on values around 250

pA or less for saturating mechanical stimuli (Géléoc et al., 1997). Recent results obtained using a preparation of the basilar papilla of the turtle indicate that transducer current in vivo could be up to about five times larger (Ricci and Fettiplace, 1998). On this basis, we decided to also study the model response to the extreme value reported in the literature for the transducer current which is 1.2 nA, in order to explore the theoretical limit values attainable by the receptor potential. In this case, it was found that the receptor potential range span for type II hair cells increased 26.3% from 29.5 to 40 mV. For type I hair cells, the receptor potential increased 109% from 6.6 to 13.8 mV, indicating that even in systems in which the transducer current is

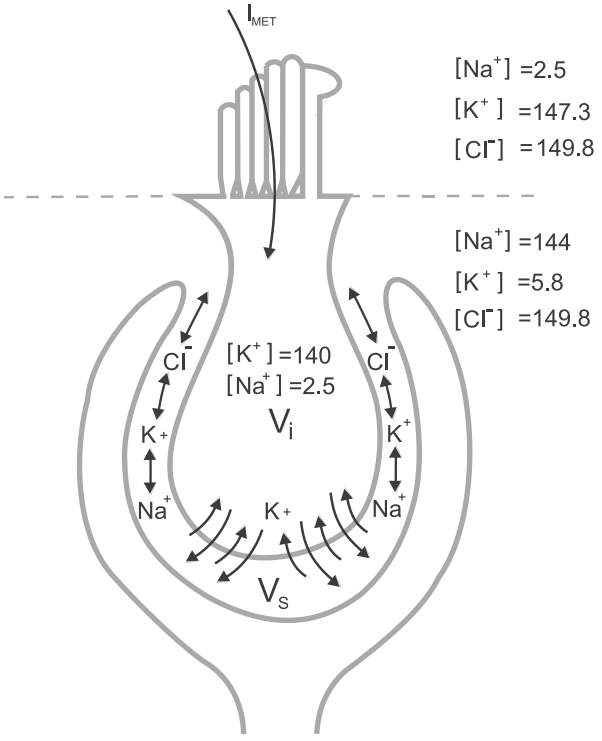

Fig. 1. Schematic drawing of the type I hair cell afferent synapse. The calyx completely surrounds the basolateral portion of the hair cell forming an extracellular subsynaptic microspace. Transducer current activation, mainly carried by K^+ ions in vivo, may lead to a K^+ accumulation in the subsynaptic space. Due to the $g_{K,L}$ conductance which is significantly activated at resting membrane potential, K^+ entering through transducer channels will immediately induce an outward K^+ current through the basolateral membrane of the cell. Thus K^+ can accumulate in the subsynaptic space depolarizing the cell and increasing the gain of the hair bundle position to receptor potential relationship. K^+ accumulation induces coupled ionic fluxes of Cl^- and Na^+ in and out of the subsynaptic space, and water movements. Ionic changes in the subsynaptic space may fade out by diffusion to the perilymphatic space through the apical portion of the calyx ending. Diffusion and active transport to the afferent neuron would also take place. Studies of isolated hair cells disrupt the calyx synapse and the transepithelial ionic gradient. At this moment there seems to be no experimental design that permits the study of the input–output relationship of the hair cell–afferent neuron system while preserving their very peculiar microambient dynamics.

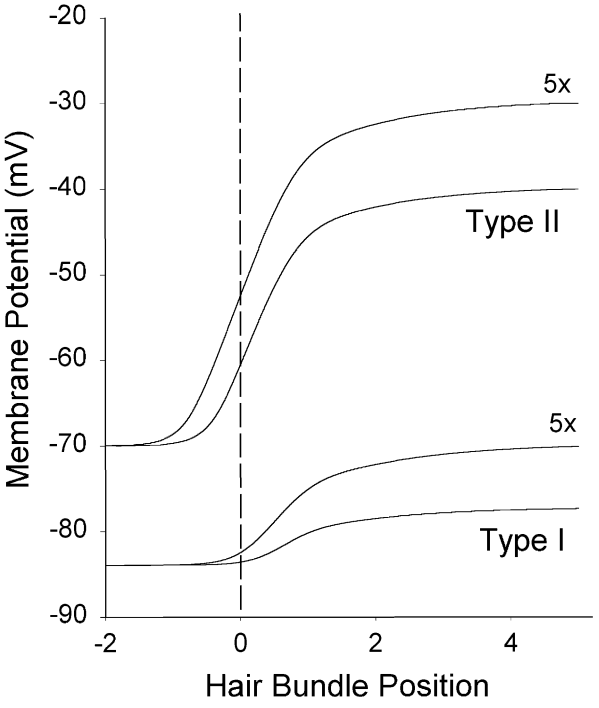

Fig. 2. Relationship between the driving voltage (i.e. position of the hair bundle) and the membrane potential of the hair cell (receptor potential) for type I and type II hair cells. Type I hair cells have a resting potential of −84 mV and type II hair cells have a resting potential of −70 mV. Two curves are shown for each hair cell type, one corresponds to a transducer current input of 250 pA, the other (marked 5×) shows the receptor potential when transducer current input is 1.25 nA. It is worth noting that type II hair cells, apart from producing much larger receptor potentials, exhibit an evident bidirectional response with coefficients of rectification (CR) of 2.27 and 1.27 for the 1× and 5× transducer current input respectively. In contrast, type I hair cells produce a strongly nonlinear response as evidenced by the CR values of 17.3 and 8.28 for the 1× and 5× transducer current input respectively.

much larger, the response of type I hair cells still seems to be of low magnitude, to activate the Ca^{2+}-dependent neurotransmitter release. Except for the data shown in Fig. 2 and marked 5×, the rest of the graphs shown in this work were generated considering 250 pA as the maximum value of the transducer current.

3.1. Rectification

Type I hair cells exhibit a sharp rectification. Voltage changes produced by tilting the hair bundle towards the kinocilium are about 30 times larger than those due to hair bundle displacements in the opposite direction. We defined the ratio between maximal depolarizing and hyperpolarizing membrane potential changes as the coefficient of rectification (CR).

Interestingly, the model predicts that the CR for type II hair cells should be much smaller than that for type I cells (Fig. 2). The sharp rectification present in the

transducer current versus mechanical displacement curve raises an important physiological question: how do the afferent neurons exhibit a bidirectional response with small or no rectification?, given that the transducer current has an almost absolute rectification.

For parameters corresponding to a type II hair cell, an almost completely rectifying transducer permeability (with a CR of 48.7) was transformed into a weakly rectifying receptor potential (CR = 2.22), thus with good bidirectional sensitivity (CR $\approx$ 2 means that the amplitude of the response in the depolarizing direction, when the hair bundle is displaced towards the kinocilium, is two times larger than in the hyperpolarizing direction). In comparison, parameters for type I hair cells produced a receptor potential with much larger rectification (CR = 29.3), thus with poor bidirectional sensitivity.

The difference in the response of types I and II hair cells suggests that the receptor potential CR decreased when the basolateral resting permeability is low (and possibly a nonlinear function of voltage). To examine this possibility, we calculated the receptor potential as a function of the hair bundle position for different values of the basolateral – voltage-independent – permeability (K^+ leakage) (Fig. 3A–D). By changing the basolateral permeability P_p from 1.9×10^{-16} to 1.9×10^{-13} m^3 s^{-1}, the CR changes from 0.37 to 42.4. Using the basal permeability data for a type II hair cell at −60 mV (i.e. 1.9×10^{-15} m^3 s^{-1}), the CR was 2.35 (Fig. 3B), a value which is very close to that calculated when the basal permeability is allowed to vary as a function of the voltage (CR = 2.22). These results indicate that the amplitude of the total K^+ permeability of the basolateral membrane mainly determines the CR. Evidently, a constant K^+ permeability accounts for most of the differences in the CR between type I and II hair cells. However, also, the variations of permeability with the membrane potential contribute to decrease the rectification.

In the resting state, hair cell membrane potential is determined by the balance between the permeability to K^+ of the basolateral membrane, with an equilibrium potential of about −85 mV, and that of the transducer (slightly open), with an equilibrium potential of about

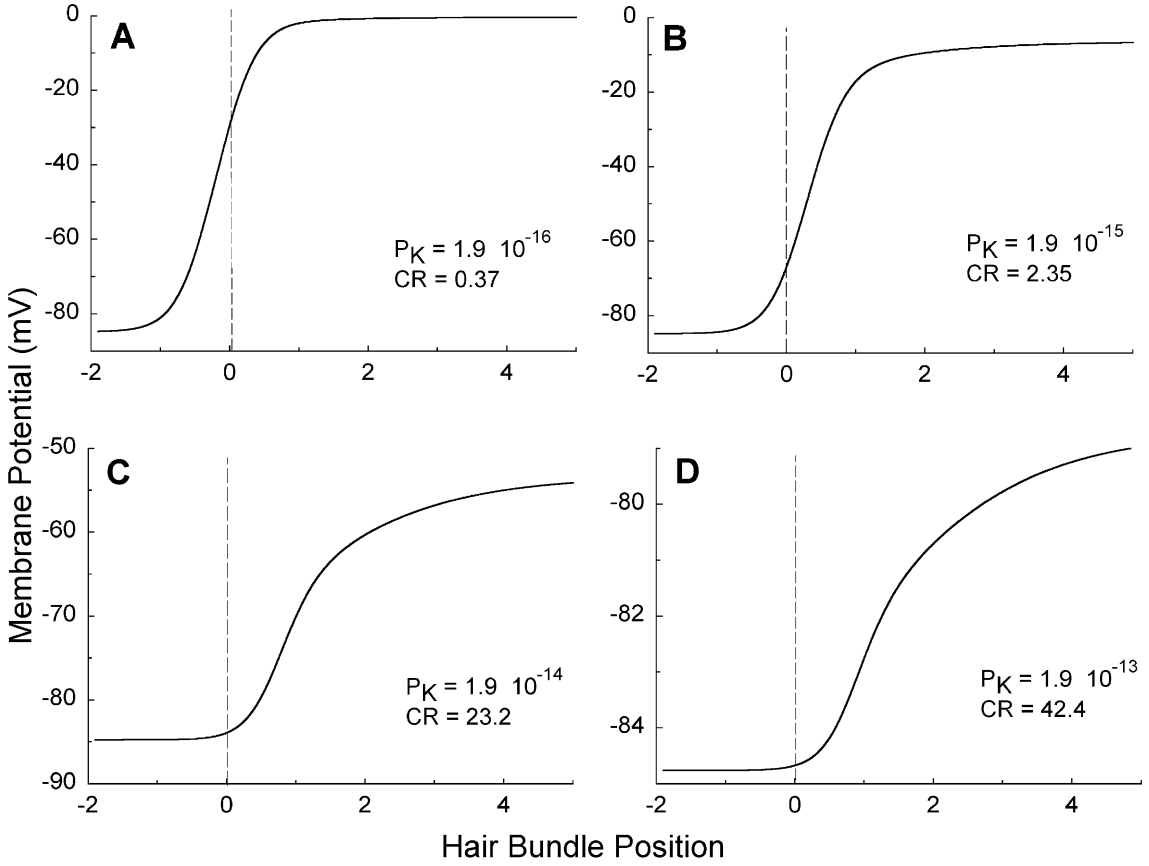

Fig. 3. Relationship between the hair bundle position and the receptor potential for different basolateral K^+ permeabilities and maximum transducer current input is 250 pA. (A) P_K is 1/10 of the normal permeability for a type II hair cell with a membrane potential of −60 mV. (B) P_K is normal for a type II hair cell with a membrane potential of −60 mV. (C) P_K has an intermediate value, between those of type I and type II hair cells. (D) P_K is normal for a type I hair cell with a membrane potential of −75 mV. Permeability values given in m^3 s^{-1}.

0 mV. Consequently, the resting potential is about −62 mV in cells with a basolateral permeability close to the normal permeability of a type II hair cell (Fig. 3B). With hair bundle movements in the hyperpolarizing direction, the transducer channel open probability decreases, and the potential approaches the equilibrium potential of the basolateral membrane (almost −85 mV). When hair bundle displacement is in the opposite direction and the transducer channel open probability increases, the membrane potential becomes ∼−7 mV near the apical equilibrium potential (0 mV). As a consequence, CR is 2.2. However, when the basolateral permeability is 10-fold smaller (Fig. 3A), the resting membrane potential is ∼−25 mV, and rectification is inverted (CR = 0.37). In general, increasing transducer channel open probability drives the membrane potential close to 0 mV, and closing the transducer channels drives the membrane potential close to the membrane potential for the basolateral membrane (which has a limit value determined by the K^+ equilibrium potential, in this case −85 mV).

By using the model with a leakage basolateral permeability close to that of either type I or type II hair cells, the range of membrane potential variation was larger than predicted with the model including all the corresponding voltage-dependent conductances. It should be noted that calculations of the membrane potential with a leakage basolateral permeability did not include a Na^+ current which tends to depolarize the cell. This last effect is larger when the transducer current has its lowest values (hair bundle position −2.0). Simulations including a Na^+ basolateral leakage indicate that this is the case: the membrane potential ranged between −70 and −7 mV for a reasonably high Na^+ permeability (∼3% of the K^+ permeability), compared with a range of −85 and −7 mV when the basolateral membrane is not permeable to Na^+ (Fig. 4). Consequently, Na^+ basolateral permeability reduced the extent of variation of the receptor potentials, thus partially accounting for the results found with the complete model.

To analyze the influence that basolateral currents have on the CR of the receptor potential the cell response was calculated in the presence of only one voltage-dependent basolateral conductance at a time (Fig. 5). Initially, the receptor potential of the cell was studied with a constant permeability g_K as the only basolateral conductance. Activation of the transducer current produced a large receptor potential (∼45 mV) with an inverted CR of 0.85 (Fig. 5A). When the basolateral permeability is produced by both g_K and g_h (type II-like cell), the receptor potential span is about 30 mV and the CR is 2.2. When $g_{K,L}$ was the only conductance in the basolateral membrane, the CR was 13, and the span of receptor potentials ∼7 mV (Fig. 5B).

3.2. Modulation by g_h and $g_{K,L}$

The slow inward rectifier conductance g_h has been reported to be a major target for neuromodulators which, through protein kinase-mediated channel phosphorylation, may modify the $V_{1/2}$ of the conductance activation curve (Fakler et al., 1994; Mo and Davis, 1997). Thus, it seems interesting to analyze the influence of g_h characteristics upon the receptor potential generation in hair cells. For this, the activation curve $V_{1/2}$ of g_h varied between −80 and −120 mV. As a consequence, the span of the receptor potential increased from 22.9 to 38.9 mV, the CR decreased from 5.34 to 1.12, and the resting membrane potential varied from −59.1 to −60.4 mV (Fig. 6A).

In type I hair cells isolated from the rat utricle, $g_{K,L}$ activates with $V_{1/2}$ values ranging from −90 to −50 mV. In ruptured whole cell recording, these values shift positively with time, suggesting that the activation range of $g_{K,L}$ is controlled by intracellular messengers lost during recording (Hurley and Eatock, 1999). Thus, it seems feasible that the $g_{K,L}$ activation range may be modulated with important functional consequences. In the model the $V_{1/2}$ of $g_{K,L}$ was varied from −60 to −110 mV while maintaining the other conductances unchanged. In these conditions, the negative increment of $V_{1/2}$ activation produced a hyperpolarization of membrane resting potential (hair bundle position = 0) from −81.3 to −84.7 mV; a decrease in the receptor potential span from 11.4 to 2.4 mV; and an increase in the asymmetry of the response to positive and negative

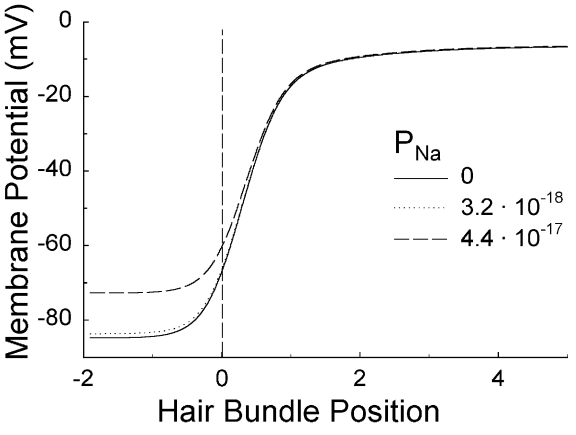

Fig. 4. Influence of basolateral Na^+ permeability upon the receptor potential. Relationship between the hair bundle position and the receptor potential assuming that $P_K = 1.9 \times 10^{-15}$ m^3 s^{-1} and the P_{Na} of the basolateral membrane varies between 0 (when no g_h is present), 3.2×10^{-18} m^3 s^{-1} (corresponding to the g_h permeability at −60 mV) and 4.4×10^{-17} m^3 s^{-1} (corresponding to the g_h permeability at −75 mV). Na^+ permeability of the g_h conductance contributes to determine the resting potential and, also, exerts an important influence in the CR. Maximum transducer current input = 250 pA.

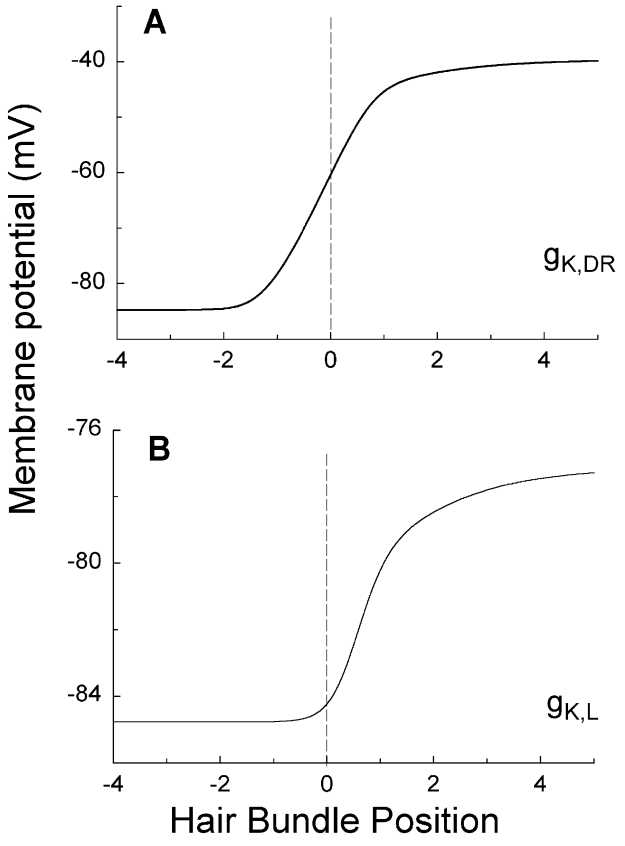

Fig. 5. Relationship between the hair bundle position and receptor potential in the presence of the indicated basolateral conductances. Transducer current (of a maximum of 250 pA) is the only input to the model. (A) $g_{K,DR}$ is the only basolateral conductance. The receptor potential range is 45 mV and CR fell to 0.85. (B) $g_{K,L}$ is the only conductance present on the basolateral membrane. Receptor potential span is ~7 mV and the CR = 13.

stimuli as reflected by a variation of the CR from 8.6 to 45.7 (Fig. 6B).

3.3. Synaptic transmission in type I hair cells

The receptor potentials for different positions of the hair bundle were calculated, assuming different permeabilities through the subsynaptic ionic conductor (Fig. 7). Fig. 7A shows the hair bundle position–membrane potential relationship. When the conductor permeability was relatively high (close to the free K^+ diffusion coefficient), the curve was similar to that obtained without including the intercellular subsynaptic compartment, leading to receptor potentials of less than 8 mV. Since no information is available about diffusional properties of the hair cell calyx, we used values of the subsynaptic conductor permeability corresponding to an apparent diffusion coefficient of 2 (equal to the extracellular space in the cerebellum), 10 and 20 (arbitrary tortuosity values of five and 10 times those of cerebellar extracellular space) (Nicholson and Syková, 1998), receptor potentials of 7.8, 9.6, 11.9 and 16.3 mV were obtained. The voltage of the subsynaptic space (V_s) was negligible in relation to the hair cell potential reaching a maximum value of 0.02 mV. Notice that the

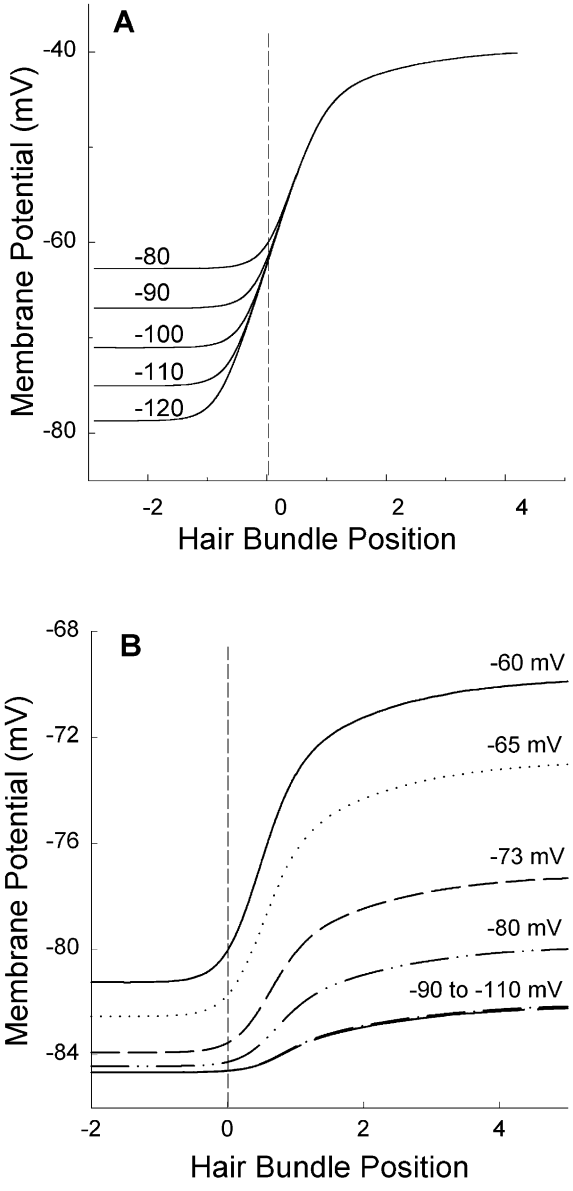

Fig. 6. Effect of the $V_{1/2}$ of g_h and $g_{K,L}$ on receptor potential generation in hair cells with a maximum of 250 pA transducer current input. (A) Changing the $V_{1/2}$ of the g_h while maintaining constant the other voltage-dependent conductances similar to those used to model a type II hair cell. Variations of the $V_{1/2}$ of g_h from −120 to −80 mV depolarized the cell, shifting the membrane potential in the resting position of the hair bundle from −60.4 to 59.1 mV. The CRs varied from 1.1 to 5.3. (1.12, 1.4, 1.9, 3.0 and 5.3 respectively). (B) Simulating a type I hair cell, the $V_{1/2}$ of $g_{K,L}$ was varied from −60 to −110 mV. The membrane potential in the resting position varied from −81.3 to −84.7 mV, the total span of the receptor potential decreased from 11.1 to 2.5 mV, and the CR changed from 8.6 to 45.7

potential through the basal membrane of hair cells ($V_i - V_s$) is the potential sensed by the voltage-gated channels of the hair cell basolateral membrane.

The ionic concentrations in the intercellular region did not change significantly for the highest values of the subsynaptic conductor permeability (Fig. 7B,C). However, for the smallest permeability used in our simulation: $P_k^{sc} = 0.5 \times 10^{-12}$ m^3 s^{-1}, equivalent to a 10-fold decrease of the apparent diffusion coefficient of the cerebellar intercellular space (Nicholson and Syková, 1998), the activation of transducer current produced a K^+ accumulation, its concentration varying from 6.03 to 9.8 mM, and for Na^+ and Cl^- varying up to 2 mM. The potassium accumulation in the subsynaptic space was accompanied by an increase in the receptor potential gain of 108% (from 7.8 to 16.3 mV).

As expected, the total current through the hair cell as a function of the hair bundle was proportional to the permeability of the subsynaptic conductor. The equivalent cord resistance can be calculated for different hair bundle positions from the ratio of the intercellular voltage to the total current of the hair cell. The range of variation of the cord resistance was negligible, except for the smallest permeability used, in which case there was a resistance variation of about 8%.

3.4. Type I hair cell model response to periodic input

The response of the type I hair cell model to sinusoidal transducer currents was also studied. Due to their low membrane resistance, type I hair cell response followed high stimulus frequencies (Rennie et al., 1996). In the model, the evolution of the membrane potential (V) varies in a sinusoidal-like manner for a 1 kHz stimulus, but for larger frequencies a summation potential appears.

We also studied the membrane capacitance influence on the receptor potential generation. In type I hair cells, the small basolateral resistance leads to a time constant between 0.1 and 0.4 ms, several orders of magnitude smaller than other time constants involved in hair cells responses (Goldberg, 1996). Consequently, the influence of the capacitance would be negligible.

4. Discussion

In this work, we present a method to calculate the receptor potential of hair cells in vivo from measurements of both voltage-dependent and mechanosensitive currents made in vitro (homogeneous environment saline surrounding the cells). Receptor potential as a function of the hair bundle position was calculated using the GHK equations and data reported in the literature about outward and inward currents in hair cells. The analysis is valid at the steady state: conductances are assumed to be in equilibrium with the membrane potential.

The calculated variation of membrane potential due to mechanical excitation ranged from 30 to 40 mV for type II, and from 7 to 14 mV for type I hair cells. Our results corroborate previous estimates, emphasizing the

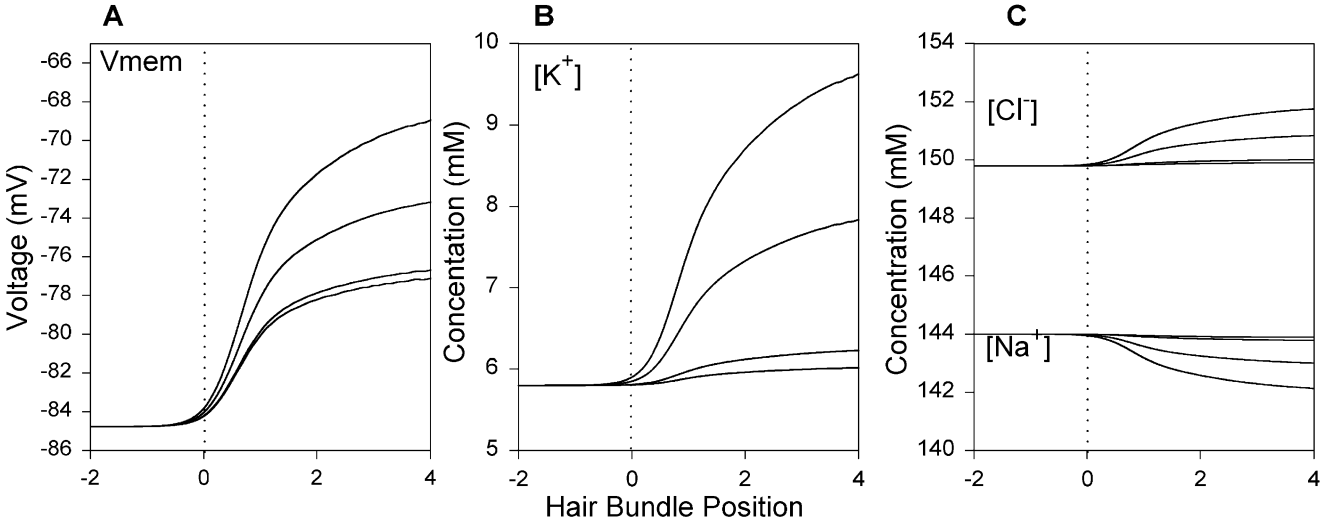

Fig. 7. Concentration changes due to hair bundle displacement and subsequent activation of the MET current. (A) Membrane potential and subsynaptic potential changes in response to hair bundle displacement for four values of the subsynaptic ohmic permeability value to K^+ $P_k^{sc} = x \times 10^{-11}$ m^3 s^{-1} where $x = 1$, 5, 0.1 and 0.5 (permeabilities to Na^+ and to Cl^- were varied by the same amounts, $P_{Na}^{sc} = 0.665 \times 10^{-11}$ and $P_{Cl}^{sc} = 10^{-11}$ m^3 s^{-1} respectively). Depending on the permeability of the subsynaptic ohmic conductor, receptor potential may vary from 7.8 to 16.3 mV. The subsynaptic potential changed less than 0.02 mV for the lower P_k^{sc}. (B) K^+ concentration changes in the subsynaptic space calculated for four values of the ohmic conductor, variations of the K^+ concentration were less than 0.2 to 4 mM. (C) Cl^- concentration increased and Na^+ concentration decreased in a range from 0.1 to 2 mM.

now inevitable question about how type I hair cells transmit information to the afferent neurons (Goldberg, 1996).

Experimentally, it has been found that there are large differences in the membrane potential between type I and type II hair cells. For example, in hair cells isolated from the pigeon semicircular canal, the resting membrane potential of type I cells was -70 ± 3 mV, and that of type II cells was -57 ± 3 mV (Correia and Lang, 1990). Then, MET currents of as much as 1.25 nA could depolarize type I cells 13.8 mV, displacing the membrane potential up to -56 mV. Therefore, for these cells to activate the neurotransmitter release machinery, voltage-dependent Ca^{2+} channels should have a very negative activating voltage, or there should be some other mechanisms contributing to further depolarize the cell, as has been proposed by Goldberg (1996).

An alternative mechanism implies that K^+ entering through the MET channels would leave the hair cell through the basolateral surface. The calyx terminal of the afferent neuron, in close apposition to the hair cell, surrounds its basolateral portion forming a reduced extracellular microspace. Thus, K^+ flowing out from the hair cell by its basolateral surface (following its driving force) would accumulate in the extracellular space, depolarizing the cell and leading to the activation of high voltage-activated calcium channels and subsequent neurotransmitter release (Goldberg, 1996). At the same time, K^+ accumulation in the synaptic cleft may as well depolarize the calyx ending directly influencing the action potential generation in the afferent neuron. In type I hair cells isolated from guinea pig, it has been shown that perfusion with high K^+ solutions led to a significant intracellular Ca^{2+} concentration change, due to the activation of voltage-sensitive calcium channels of the L type (Boyer et al., 1998). Another possibility has been raised recently, it implies the inhibition of $I_{K,L}$ by nitric oxide leading to an increase in the input resistance of the hair cell, and a subsequent increase in the receptor potential gain (Chen and Eatock, 2000).

The model shows that the permeability of the pathway for the ions to diffuse out of the calyx may play a key role in determining the ionic changes that would take place at the subsynaptic space. Thus, the calyx structure seems to be of fundamental importance in the proposed mechanism, since a very tight or large calyx determines a very low permeability for the pathway for ions to diffuse out of the subsynaptic space. In the model we used permeabilities of the subsynaptic ohmic conductor in the range of 10^{-11} m^3 s^{-1} (close to the potassium mobility in a water solution) to 0.5×10^{-12} m^3 s^{-1}, that is more than one order of magnitude decrease in K^+ permeability, equivalent to a tortuosity coefficient of 20 (Nicholson and Syková, 1998) leading to a 62.5% (from 6.03 to 9.79 mM) increase of K^+ concentration in the subsynaptic space.

Also, due to the reduced extracellular volume of the synaptic cleft, it is conceivable that when the Ca^{2+} influx into the pre- or the postsynaptic cell is large, the concentration of Ca^{2+} in the synaptic cleft may drop, leading to a reduction in the amount of Ca^{2+} available for evoking transmitter release. Such a Ca^{2+} concentration depletion due to synaptic activity has been shown in the synapse between the calyces of Held and the cells of the medial nucleus of the trapezoid body in the rat auditory brainstem (Borst and Sakmann, 1999).

Important influences in the hair cell response could be produced by modifications in the characteristics of $g_{K,L}$. Cells which express $g_{K,L}$ showed significantly more negative resting potential. Depending on $g_{K,L}$ magnitude and $V_{1/2}$, the hair cell receptor potential may vary from a few mV, as in type I cells, to tens of mV, as in type II lacking $g_{K,L}$. Also the value of the $V_{1/2}$ of $g_{K,L}$ contributes to determine the resting membrane potential and the total span of the receptor potential, thus controlling the gain of type I hair cells.

Cells expressing $g_{K,L}$ maintain the nonlinearities of the MET. In contrast, the response to mechanical stimuli of those cells without $g_{K,L}$ is partially linearized. We proved that the magnitude and $V_{1/2}$ of a voltage-dependent basolateral conductance might compensate the rectification of the transducer current. The expression of the delayed rectifier $g_{K,DR}$ in the basolateral region decreased the CR, leading to its inversion (Fig. 5A). Adding the slow inward rectifier g_h increased the CR (to values between 1 and 10, depending on the $V_{1/2}$ of g_h activation).

In this work $g_{K,DR}$ and the $g_{K,A}$-type currents were lumped together. Justification for doing so is that since we used GHK equations to develop the model, the relevant parameter is the voltage dependence of the current and not its particular dynamics. In fact, Hodgkin–Huxley-type models of hair cell dynamics using just one lumped basal K^+ conductance reproduce the hair cell voltage response trajectories with a high degree of accuracy (Alexandrov et al., 2001).

Inward rectifying currents have been found in 50% of type I hair cells and 61.5% of type II cells in mammalian vestibule (Eatock and Hutzler, 1992) and in 86% of short oscillatory-type hair cells from the goldfish sacculus (Sugihara and Furukawa, 1995). Both the rapid $g_{K,IR}$ and the slow non-inactivating g_h-type inward currents have been reported in hair cells (Holt and Eatock, 1995). In this work, it has been found that a more negative activation for g_h (permeable to K^+ and to Na^+) increased the receptor potential, and decreased the CR. This indicates that important regulatory influences can take place by modification of the $V_{1/2}$ of this current. Therefore, the expression of g_h may contribute

to differentiate hair cell subsets and constitute an element for plastic and regulatory changes of hair cell response.

Bidirectionality is a fundamental property of hair cell systems that significantly contributes to sensory coding of mechanical stimuli. Hair cell response to hair bundle displacements towards the kinocilium is depolarizing while displacements of the hair bundle in the opposite direction produce a hyperpolarization of the cell. However, the transducer current is highly rectifying, the current produced by hair bundle displacements towards the kinocilium is much larger than the current response to displacements in the opposite direction. It is well known that vestibular afferent neuron response rectifies, but this rectification is not as important as that reported for the mechanoelectrical transduction (Lowenstein and Roberts, 1949; Blanks and Precht, 1976; Fernández and Goldberg, 1976; Hartmann and Klinke, 1980). Then, there is the problem of how to explain the bidirectional sensory coding given the strongly rectifying transducer current. One possibility is that the experimental manipulations of hair cells to record the transducer currents may have some deleterious effect on the response to mechanical stimuli. The resonance of the receptor potential may also contribute to linearize the receptor potential. However, semicircular canal cells have a low resonant frequency and low quality of resonance (Angelaki and Correia, 1991), and type I hair cells show practically no resonance (Rennie et al., 1996). Thus, although resonance could explain the bidirectional coding of some hair cells it is not sufficient to account for bidirectionality as a general property of sensory coding in hair cell systems. Our findings indicate that the type I hair cell receptor potential reflects the shape of the transducer current, thus it is strongly rectifying, and has poor bidirectionality; in contrast, type II hair cells have a high gain bidirectional coding receptor potential.

Sinusoidal transducer current input to the model leads to an interesting observation. When the frequency of stimulation or the cell capacitance increases, the hair cell receptor potential modulation by the sinusoidal stimulus decreases, but an accumulative, slowly rising DC potential develops. This type of DC potential has been described in cochlear hair cells (van Emst et al., 1998).

Finally, it should be taken into account that the transducer current is dynamic, with intracellular Ca^{2+} and cyclic nucleotides regulating its activation and the set point of the response–displacement curve. Our model results indicate that basolateral conductances play a key role in defining the resting membrane potential, the gain and the bidirectional sensitivity of the receptor potential. Also, depending on voltage dependence and the magnitude of the basolateral K^+ currents, and on the diffusional properties of the calyx pathway for ions to diffuse out from the synaptic cleft, significant K^+ accumulation could take place in the synaptic space and contribute to increase the gain of the receptor potential in type I hair cells.

Acknowledgements

This work was partially supported by Consejo Nacional de Ciencia y Tecnología de México (CONACyT), Grant 35525-N to E.S. The authors thank Isabel Pérez Montfort for proofreading the English manuscript.

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