1. Introduction

In the mammalian inner ear, incoming sound is amplified by the mechanics of the cochlear partition. The mechanism depends on the enhanced vibration patterns of the basilar membrane, which, in turn, depends on a specific population of cells, the outer hair cells (OHCs) of the cochlea. These cells lie along the organ of Corti and respond to sound by changing their membrane potential (reviewed in reference [1]). The lateral plasma membrane of each OHC contains a dense array of a protein, ‘prestin’, [2], without which overall cochlear amplification fails [3–5]. In OHCs, preston behaves like a voltage-sensitive mechanoenzyme driven by the induced potential in the cell (reviewed in reference [6]). Although prestin is not unique to mammals, for homologues are found in other hair cell systems as a member of superfamily SLC26 of anion exchangers, mammalian prestin has distinctive properties which ensure that it contributes to the mechanics of the cochlea.

The mechanoenzyme properties of mammalian prestin can be explained by the hypothesis that it is an incomplete transporter [7] where the conformational changes inherent in a transport mechanism occur yet the transport cycle itself occurs at a low rate [8]. As an anion exchange protein, the question arises whether it remains able to transport ions across the OHC basolateral membrane, even though the carrier is operating at low efficiency. However, the high density of prestin expression implies that even a low efficiency transport function may have significant consequences for the OHC biophysics. We have previously shown that prestin is a low turnover electrogenic transporter [9] with a 2 : 1 stoichiometry for bicarbonate : chloride exchange. One consequence of this finding is that the ion fluxes through the cell membrane would change intracellular pH in a voltage-dependent manner.

A phenomenon termed ‘slow motility’ is experimentally observed when OHCs are subjected to ionic perturbations, and, in particular, when OHCs are placed in elevated external K\(^+\) solutions [10]. In addition, there are reported length changes when external buffer changes occur [9,11] or when external chloride changes are made [12]. Under these conditions, the cylindrical cell changes length and diameter consistent with a movement of water into the cell as a result of non-equilibrated osmotic forces. To obtain a quantitative description of such ‘slow’ length changes, we have investigated the movement of protons and other ions across the membrane by modelling the OHC transport processes. We do not include here the effect of depolarization by K\(^+\) which may involve further movement of chloride and which has been considered elsewhere [13]. The model is first developed to include the anticipated in vivo pH regulation system, and then...
the action of prestin as an electrogenic transporter is incorporated. We show below how such a model can predict the intracellular ion concentrations and OHC length changes observed experimentally.

2. Model description

The model consists of two compartments: one extracellular compartment of infinite volume and a finite volume cellular compartment of the OHC. The cell is modelled as simple cylinder allowing a direct calculation of the OHC volume–length relationship (see below). Figure 1 provides an overview of the transport processes incorporated into the model.

2.1. Passive diffusion through the membrane

The flux, $J_X$, of particles of species, $X$, across a surface is defined as the rate of flow of the particles per unit area. In the case of diffusion of charged particles with a valence $z$, the electrical field that exists owing to the potential difference across the cell membrane plays a role. The (inward) flux of these particles may be described by the Goldman–Hodgkin–Katz (GHK) flux equation [14] for a species $X$ with a permeability $P_X$ and is given by (in mol cm$^{-2}$ s$^{-1}$)

$$J_X = P_X z^2 a V \frac{[X]_o - [X]_i}{1 - \exp(zaV)}.$$  

(2.1)

Here, $V$ is the membrane potential, $[X]$, or $[X]_o$, the species concentration inside or outside, respectively, and $a = e/k_BT$, where $e$ is the elementary electron charge, $k_B$ is the Boltzmann constant and $T$ is absolute temperature. In the case that the species is electrically neutral, equation (2.1) reduces in the limit $z = 0$ to Fick’s law

$$J_X = P_X ([X]_o - [X]_i).$$  

(2.2)

2.2. Intracellular pH regulation modulated by bicarbonate permeability

A simple model developed to describe acid transients in the squid giant axon developed by Boron & De Weer [15] was used as starting point onto which other transport processes could be added. The description is as follows, where we use the same notation for component reactants as in the original study.

The bicarbonate anion, $\text{HCO}_3^-$ (represented by $A$ below), is the conjugate base of carbonic acid, $\text{H}_2\text{CO}_3$ (represented by $\text{HA}$), which dissociates with $pK_a = 6.1$ to yield a proton

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow H^+ + \text{HCO}_3^-.$$

The presence of carbonic anhydrase in the cell acts to shift the reaction equilibrium of the hydration of $\text{CO}_2$ to the left and results in the majority of $\text{H}_2\text{CO}_3$ being converted into dissolved $\text{CO}_2$. The total acid concentration, $[\text{TA}]$, is then considered to be the sum of both $\text{H}_2\text{CO}_3$ and $\text{CO}_2$; the apparent dissociation constant of TA is given by $pK_{\text{app}} = 6.3$.

Across cell membranes, the permeation of $\text{CO}_2$ is passive, and the flux of $\text{CO}_2$ (which becomes carbonic acid within cells) can, therefore, be described by Fickian diffusion with a flux $J_{\text{HA}}$. On the other hand, the flux of the bicarbonate ion, $A$ (valence $z = -1$), is subjected to the electrical field of the cell and thus is described as a GHK flux described as in equation (2.1). The sum of the $\text{CO}_2$ and the bicarbonate

Figure 1. A model of the outer hair cell transport mechanisms showing pathways modelled here. (1) Passive diffusion of chloride and bicarbonate across the plasma membrane; (2) $\text{CO}_2$ permeation of the membrane to the cytoplasm leading to acidification of the cell; (3) 2 : 1 bicarbonate : chloride exchange owing to prestin. The inhibitory effect of salicylate, $S$, is shown as an entry of salicylic acid (see text). (4) Water permeation due to osmotic pressure and (5) the resultant cell length change owing to water movement. (Online version in colour.)

fluxes can thus be combined to yield the rate of change of the total acid concentration $[\text{TA}]$, within the cell

$$\frac{d[\text{TA}]}{dt} = \sigma \cdot (J_{\text{HA}} + J_A),$$  

(2.3)

where $\sigma$ is the membrane area of the cell through which the fluxes pass. Using the same arguments as in [15], we find the rate of total acid change and proton change inside the cell as

$$\frac{d[\text{TA}]}{dt} = \sigma \cdot \left[ P_{\text{HA}} [\text{HA}]_o - P_{\text{HA}} \frac{[\text{HA}]}{[\text{HA}]_o + K} [\text{TA}] + P_A aV \cdot (\frac{[\text{TA}] - (K/[\text{HA}] + K) \cdot [\text{TA}] \cdot \exp(zaV)})}{1 - \exp(zaV)} \right]$$  

(2.4)

and

$$\frac{d[\text{H}_2\text{O}]}{dt} = - \frac{2.303 [\text{H}_2\text{O}]}{\beta} \cdot \sigma \cdot \left[ \frac{K}{[\text{HA}]_o + K} \cdot P_{\text{HA}} [\text{HA}]_o - P_{\text{HA}} \right] \cdot \left[ \frac{[\text{HA}]}{[\text{HA}]_o + K} \cdot P_A aV \cdot (\frac{[\text{TA}] - (K/[\text{HA}]_o + K) \cdot [\text{TA}] \cdot \exp(zaV)})}{1 - \exp(zaV)} \right] - J_{\text{HA}},$$  

(2.5)

where $K$ is the acid dissociation constant, (i.e. $10^{-3.6}$ M), $J_{\text{HA}} = P_{\text{HA}} ([\text{H}^+]_o - [\text{H}^+])$ is the passive proton flux entering the cell (described by a proton permeability $P_{\text{HA}}$) and $\beta = d[\text{H}_2\text{O}]/d\text{pH}$ is the buffering power of the cytoplasm defined.
as the amount of acid/base required to perturb the pH by one unit [16,17].

The pair of differential equations (2.4) and (2.5) can be solved numerically yielding the time course for changes in [TA] and pH, once the parameters are fixed. The results are shown in figure 2 where the bicarbonate flux into the cell following acid load was increased by an ad hoc modification of the bicarbonate permeability. Mistrik et al. [9] used a change in HCO₃⁻ permeability in order to mimic the effect of possible HCO₃⁻ secretion by transport. It is clear that an increase in the permeability $P_{HCO_3}$ results in a marked decrease in the recovery time from the CO₂ transients as the additional alkali loading neutralizes the CO₂ acidification.

2.3. Bicarbonate loading by the prestin antiporter

To include a more realistic bicarbonate regulation, a simple model of HCO₃⁻/Cl⁻ exchanger was used in order to produce the effective HCO₃⁻ permeability changes induced by chloride transport. A model of the asymmetrical 2:1 (bicarbonate: chloride) exchanger was adapted from a model of HCO₃⁻ secretion in pancreatic duct cells [18].

The rate of an enzymatically transported substance is determined by its concentration in solution and described by rate equation with a Michaelis constant, $K_m$, a constant indicative of the substrates affinity for the transporter binding site. The value of $K_m$ is assumed to remain constant through both in and out conformations of the transporter. The equation describes the fraction of the maximum transport velocity $V_{max}$ at a specific concentration of substrate, $S$:

$$\frac{d[S]_{out}}{dt} = V_{max} \cdot \frac{[S]_{in}}{K_m + [S]_{in}}. \quad (2.6)$$

To model a 2:1 (HCO₃⁻: Cl⁻) exchanger, we make several assumptions (i) the carrier has a single ion binding site on either side of the membrane; (ii) the ions compete for the single binding site; (iii) the exchanger does not exhibit any constitutive activity in the absence of both ions binding; (iv) the velocity of exchange was equal and opposite for either directions of transport; (v) the ion binding constants ($K_m$) were equal for the site on either side of the membrane; and (vi) the concentration of transporter $[E]$ is conserved. This yields equation (2.7) for the net flux of chloride through the carrier [18,19]

$$J_{carrier} = G \left\{ \left( [Cl^-]_o/[K_{Cl}] \right) \cdot \left( [HCO_3^-]_i/K_{HCO_3} \right)^2 \cdot \exp(aV/2) \right\} - \left\{ \left( [HCO_3^-]_o/K_{HCO_3} \right)^2 \cdot \exp(-aV/2) \right\} \left\{ [Cl^-]_i/K_{Cl}\right\} \cdot [HCO_3^-]_i/K_{HCO_3} \right\} \cdot [Cl^{-}]_i/K_{Cl} \cdot [HCO_3^-]_i/K_{HCO_3} \right\} \right\}

The flux of bicarbonate will then be $-2J_{carrier}$. Equation (2.7) incorporates a constant field correction term $\exp(aV/2)$ which models the effects of the electrical field upon the transporter. Binding constants for prestin were taken as $K_{Cl} = 6.3$ and $K_{HCO_3} = 43.6$ mM [7].

The constant $G$ is effectively the permeability of the total carrier population, $E$, i.e. $G = P \cdot [E]_{tot}$. The permeability coefficient of the antipporter is in units of mol cm⁻² s⁻¹ and was estimated from the density $\rho$ of prestin and the molecular turnover rate of the carrier $K_c$. A protein density of 4000 μm⁻² was estimated from prestin particle density in the basolateral membrane of apical OHCs [20], and a value of 209 s⁻¹ was used for $K_c$ [9]. This yields a total permeability, $G = 1.4 \times 10^6$ mol cm⁻² s⁻¹.

We find the cellular transport rate for each ion can then be calculated by multiplying the flux $J_{bicarb:Cl}$ by the surface area to give a molecular turnover rate in mol s⁻¹, which can then be divided by the compartmental volume to yield a rate of change with respect to ion concentration $C$ in mol dm⁻³ s⁻¹

$$\frac{d[C]}{dt} = \sigma \cdot (J_{carrier} + J_{Cl,leak}) \quad (2.8)$$

and

$$\frac{d[HCO_3^-]}{dt} = \sigma \cdot (J_{bicarb:Cl}) \quad (2.9)$$

Equations (2.8) and (2.9), for generality, also include the effects of leak permeabilities (producing $J_{Cl,leak}$ and $J_{bicarb,leak}$) in the total ion fluxes. For sake of simplicity, we assume these leak permeabilities are zero, although it is likely that some small residual ion movements occur in vivo.

Figure 3 shows the results of simulating the effect of membrane potential on the intracellular levels of chloride and bicarbonate, using equation (2.7), from a holding potential of $-70$ mV. Stepping the potential to $+70$ mV produces a large reduction of intracellular bicarbonate and simultaneous rise in intracellular chloride.

2.4. Water transport and leak channels

In order to model volume changes, the cell is assumed to be an idealized cylinder of constant surface area. The lateral surface area $A_s$ is given by $A_s = \pi \cdot d \cdot L$ and volume $V$ is given by $V = \pi d^2 L/4$ and hence the length change of the cell is related to the
volume change by

$$\frac{\Delta L}{L} = -\frac{\Delta V}{V}.$$  \hspace{1cm} (2.10)

The consequent slow (as distinct from voltage-induced 'fast' 'electomotility') length change of an OHC was referred to as a 'slow motile response' and can easily be measured optically [13]. The volume flow rate into the cell owing to osmotic flux of water is given by (in units of cm$^3$ s$^{-1}$) by the Kedem–Katchalsky equation

$$\frac{dV}{dt} = P_f \cdot V_w \cdot \Delta \varphi,$$  \hspace{1cm} (2.11)

where $P_f$ is the water permeability coefficient in cm s$^{-1}$, $V_w$ is the molar volume of water in cm$^3$ mol$^{-1}$ and $\Delta \varphi = \varphi_{\text{outside}} - \varphi_{\text{inside}}$ is the difference in total osmolarity of the solutes outside and inside across the cell membrane [21,22]. To solve equation (2.11), $\varphi_{\text{inside}}$ includes a correction for the finite volume of the cell and thus the equation is nonlinear $nV$.

2.5. Combining anion exchange and acid transport

The level of bicarbonate in the cell, $[\text{HCO}_3^-]_i$, is sensitive to changes in pH and thus contributions made by the carrier influx and those due to CO$_2$ permeation were integrated together. By mass action

$$[\text{HCO}_3^-]_i = \frac{[\text{H}]_i}{[\text{H}]_i + K_{\text{app}}} \cdot [\text{TIA}].$$  \hspace{1cm} (2.12)

All permeabilities can now be fixed. The total acid concentration in the cell is then described by including the exchanger contributions to the bicarbonate and proton diffusion fluxes described in equations (2.4) and (2.5)

$$\frac{d[\text{TIA}]}{dt} = \frac{d[\text{TIA}]}{dt}_{\text{total}} - \frac{d[\text{TIA}]}{dt}_{\text{diffusion}} + \frac{d[A]}{dt}_{\text{exchanger}}$$  \hspace{1cm} (2.13)

and

$$\frac{d[\text{H}]_i}{dt} = \frac{d[\text{H}]_i}{dt}_{\text{total}} - \frac{d[\text{H}]_i}{dt}_{\text{diffusion}} + \frac{2.303}{\beta} \frac{[\text{H}]_i}{[\text{H}]_i + K} \frac{d[A]}{dt}_{\text{exchanger}},$$  \hspace{1cm} (2.14)

where the subscript 'diffusion' refers to equations (2.4) and (2.5) with a fixed value for $P_{\text{HCO}_3}$ and the exchange fluxes are computed from equations (2.7) and (2.9).

2.6. Simulation methods

Simulations of the model, equations (2.4)–(2.11), were written in Matlab (MathWorks, Natick, MA). Integration of the sets of equations was performed using the inbuilt ordinary differential equation solver ODE23s using default parameters, optimized for stiff equations and using adaptive integration steps for the calculation of the diffusion term. Stepwise integration of the coupled equations was used when the exchange fluxes were included.

2.7. Experimental outer hair cell measurements

Isolated OHCs were obtained by microdissection from the guinea pig cochleas using methods described elsewhere [9,23]. For tissue, animals were killed by rapid cervical
dislocation according to UK Home Office regulations. Cells placed in the experimental chamber were viewed with differential interference contrast microscopy using an inverted microscope through a 40×1.3 NA objective (Zeiss, Germany), and the images captured for subsequent analysis by a substage camera. For the experiments described here, cells attached to the base of the recording chamber were continuously superfused with high Na⁺ extracellular solution buffered with 25 mM bicarbonate and continuously bubbled with CO₂ to pH 7.3. For low chloride solutions, chloride was replaced isotonically by glucuronate. The cell length was computed by tracking the cells’ apex and base using ImageJ software. Experiments were carried out at room temperature (22°C).

3. Results

3.1. Induced pHi changes in outer hair cells

All simulations were carried out at the experimentally observed membrane potential (~70 mV) inferred from intracellular recordings of OHCs in vivo [24]. The free parameters, all traceable to published experimental data, were chosen as described in table 1.

Figure 4 shows that when the extracellular solution around an OHC changes from very low to 25 mM HCO₃⁻, as reported in experimental conditions, there is an initial acidification when the cell is held at negative resting potentials, but the effect of bicarbonate entry is to cancel the acidification produced by the initial passive diffusional entry of CO₂. Intracellular chloride, bicarbonate and total osmotic pressure reach steady-state values within experimental timescales [11] and the model demonstrates that there is reasonable agreement in timescales (minutes) between experimental observations and steady-state values. The similarities between their experimental observations and steady-state values suggest that the model accurately mimics the steady-state dynamics of the OHC at homeostatic equilibrium. As a result of the electrogenic nature of the transporter, the model also predicts that when held even at quite modest potentials (~40 to 0 mV) the effect of bicarbonate exposure should be to produce at steady state a relatively alkaline cytoplasm (pHi ~ 7.5).

3.2. Induced length changes of outer hair cells

Figure 5 shows the effects on the OHC length of a step decrease in the external chloride. The experiment differs from figure 4 in that the external chloride is reduced in the presence of a constant bicarbonate external buffer, but clearly shows how the stoichiometric exchange operation drives bicarbonate into the cell, increasing internal osmolytes, and thereby increasing the water volume to produce a shortening of the cell. The effect of progressively decreasing the exchanger contribution is described below.

3.3. The effects of salicylate on prestin

Salicylate (whose methylated form is aspirin) interacts with prestin by competitively binding to a chloride binding site. We can model its effect if intracellular salicylate binds to prestin to reduce the number of effective molecules. The effective prestin density, \( \rho_{\text{eff}} \), is given by

\[
\rho_{\text{eff}} = \rho_{\text{total}} \left( \frac{K_s}{K_s + [S]} \right)
\]

3.4. Experimental measurement of outer hair cell length changes

To verify that length changes of the magnitude predicted by the model do occur, we performed a limited number of experiments where the effects of lowered Clᵢ could be studied. Figure 6 shows that lowering external chloride in the presence of bicarbonate buffer shortened the cell by approximately 4% with time course of minutes. A similar effect can be seen in reference [9] (figure 1). At each membrane potential, simulations with water permeability coefficients of 0.0001 and 0.001 cm s⁻¹ are shown: better agreement is apparent with higher values of the coefficients, which speed up the osmotic equilibration, and low membrane potentials, compatible with reported data from OHCs.

<table>
<thead>
<tr>
<th>Table 1. Parameter values used in the simulations.</th>
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<tbody>
<tr>
<td>solution concentrations</td>
</tr>
<tr>
<td>chloride, [Cl]ᵢ = 8 mM, [Cl]₀ = 140 mM</td>
</tr>
<tr>
<td>bicarbonate, initial, [HCO₃⁻] = 0.01 mM</td>
</tr>
<tr>
<td>pHᵢ = 7.26, pH₀ = 7.4</td>
</tr>
<tr>
<td>OHC geometry</td>
</tr>
<tr>
<td>( V ) (isotonic volume of OHC) = 3.75 \times 10⁻⁹ cm³</td>
</tr>
<tr>
<td>( L ) (isotonic length) = 50 μm</td>
</tr>
<tr>
<td>( \sigma ) (isotonic surface area) = 15 \times 10⁻⁵ cm²</td>
</tr>
<tr>
<td>( r ) (isotonic radius) = 5 μm</td>
</tr>
<tr>
<td>exchanger model</td>
</tr>
<tr>
<td>( \rho ) (carrier membrane density) = 4000 \mu m⁻²</td>
</tr>
<tr>
<td>( K_{\text{eff}} ) (carrier turnover rates) = 209 s⁻¹</td>
</tr>
<tr>
<td>( K_{b} ) (chloride binding constant) = 6.3 mM</td>
</tr>
<tr>
<td>( K_{B} ) (bicarbonate binding constant) = 43.6 mM</td>
</tr>
<tr>
<td>acid transport</td>
</tr>
<tr>
<td>( P_{CO₂} ) (membrane CO₂ permeability) = 0.001 cm s⁻¹</td>
</tr>
<tr>
<td>( \beta ) (buffering power of cytoplasm) = 1.1 \times 10⁻² mM</td>
</tr>
<tr>
<td>( K_{\text{app}} ) (first apparent dissociation constant of bicarbonate) = 7.94 \times 10⁻⁷ M</td>
</tr>
<tr>
<td>( K_{SA} ) (sulphate binding constant) = 200 μM</td>
</tr>
<tr>
<td>water transport</td>
</tr>
<tr>
<td>( P_{W} ) (water permeability coefficient) = 1 \times 10⁻⁷ m s⁻¹</td>
</tr>
<tr>
<td>( V_w ) (molar volume of water) = 1.8 \times 10⁻² cm³ s⁻¹</td>
</tr>
<tr>
<td>( \phi_w ) (resting osmotic pressure) = 325 mOsm l⁻¹</td>
</tr>
<tr>
<td>leak permeabilities</td>
</tr>
<tr>
<td>( P_{Cl} ) (chloride leak) = 5 \times 10⁻⁸ mol s⁻¹</td>
</tr>
<tr>
<td>( P_{B} ) (bicarbonate leak) = 3 \times 10⁻⁸ mol s⁻¹</td>
</tr>
</tbody>
</table>

An estimate for \( K_{b} \) ~ 200 μM was derived experimentally from measurement of the nonlinear capacitance of inside-out patches from OHCs [7]. Inserting \( K_{\text{eff}} \) into equation (2.7) produces a dose-dependent inhibition of bicarbonate entry. The simulation shows that when the cell is exposed low chloride in the presence of constant extracellular bicarbonate there is dose-dependent reduction of the shortening as increasing levels intracellular salicylate reduce the effect of prestin (figure 5b).
Figure 4. Effect of changing external solution on OHC internal pH. External Cl⁻ reduced from 140 to 1 mM at the same time as HCO₃⁻ is increased to 25 mM, favours extrusion of chloride in exchange for bicarbonate entry. (a) The intracellular pH change is shown at different holding potentials, $V_H$ as $pH_{in}(V_H)$. This experiment can be reported by intracellular pH probes [11]. (b) Time course of the total acid $[TA]_{in}$ inside the cell, with the holding potentials signified as $[TA]_{in}(V_H)$. (Online version in colour.)

Figure 5. Effect of reducing extracellular chloride on the OHC length at constant physiological bicarbonate levels. The chloride gradients are changed at the times shown. The initial conditions (table 1) are such that $[HCO_3^-]_i = 0.01$ mM, and the initial exposure to 25 mM bicarbonate shortens the cells. The cell recovers its pre-exposure length. (a) Exposure to low extracellular chloride at different holding potentials, producing a shortening of approximately 2% after 5 min with negative holding potentials. (b) Exposure to low chloride in the presence of increasing levels of intracellular salicylate, blocking the prestin exchange process. The cell shows a dose-dependent decrease in the rate of shortening. (Online version in colour.)
in vitro [6]. The initial transient shortening may be due to ion equilibration within compartments internal to the OHC, not modelled here: the cell biomechanics is more complex than a simple cylinder, and the initial transient shortening seen here and in other published data may be due to the constrained volume for chloride movement below the prestin-containing lateral membrane. Nevertheless, in the steady state, after 5 min, the length reduction was well fitted by the model if the potential of the experimentally determined cell is close to 0 mV. In vitro, when the cell has been removed from the K⁺ source of the cochlea endolympth, microelectrode studies do suggest that isolated cells do not maintain a negative resting potential [25].

4. Discussion

4.1. An integrated model for outer hair cells

This model describes an integrated model where we have attempted to combine a number of fluxes controlling the contents of the mammalian cochlear OHC. Other fluxes through the cell, in particular, a steady K⁺ flow through the apical transducer to the K⁺ channels at the basal pole and any Ca²⁺ entry from the endolympth via the steroctial transduction channels are not included in this model as there are fewer experimental data about their magnitudes and autoregulation. The present model predicts how intracellular pH and cell length can change under the influence of the transmembrane fluxes postulated to exist in the OHC as result of known biophysical experiments. The simulations show that the presence of a 2 : 1 HCO₃⁻/Cl⁻ antiporter at a high density in the lateral membrane can not only facilitate recovery from a CO₂-induced transient acid load, but also control the osmotic gradients across the cell membrane. The extent to which this occurs is heavily dependent upon the electrochemical gradient of chloride. As a result, the simulations reproduce experimental findings observed when the cell is exposed to low extracellular chloride [11] as well as the cell length changes reported by other authors, e.g. [9,10,12], and shown here for completeness (figure 6). Although prestin and the SLC26 anion exchange family were not discovered until a decade later, the experimental results from Ikeda et al. [11] clearly implicated an anion antiporter in the OHC pH₄ regulation.

The operation of such an exchange system in vivo is likely to be less dramatic than, in this simulation, as it is believed that OHCs are not exposed to the large concentration changes used experimentally. Instead, intracellular pH regulation is likely to occur through extrusion of internally generated HCO₃⁻ as a by-product of metabolism. It should be noted that in very acidic conditions (pH < 6.8) diminished intracellular HCO₃ will create a gradient strong enough to overcome chloride and thus drive HCO₃ into the cell. As an indirect consequence of ion flow, however, OHCs may be able to regulate their length within the cochlear partition and to create the conditions for optimal opening of the apical transducer channel, the positioning of the hair bundle and thereby modify the bias points for cochlear amplification [26].

The reversal potential of the transporter exists around −70 mV at physiological conditions [9]. Thus, depolarization of the cell will drive bicarbonate into the cell and lower of intracellular chloride. Chloride is thought to regulate the voltage sensitivity of prestin when acting prestin acts an area motor so that, in addition, the transport function may act as a homeostatic mechanism to control the OHC electromotility. Depolarization of the cell during sound stimulation might also be able to partially reverse the transporter causing an inward bicarbonate flux. Consequently, chloride will be extruded from the cell and hence reduce the electromotile gain.

The binding constant of chloride for prestin (estimated to be Kₐ = 6 mM) is in an optimal region for the anion to act as a regulator as it is slightly lower than the measured resting [Cl]₀ (less than 10 mM) [27]. The simulations demonstrate that increasing Kₐ by even 4 mM results in severely weakened exchange.

4.2. Modelling the effect of salicylate

There are few known inhibitors of prestin although the salicylate ion, or more clinically relevant aspirin, is one agent which is effective. The mode of action is complex, with indirect effects on the cell biomechanics and cell structure [22]. The main observed effect of salicylate is to block voltage-dependent motility of prestin [23] and to elevate auditory thresholds by removing the OHCs from a cochlear mechanical feedback loop. Although the experimental route of drug application is through the external perilymph, the protonated form, salicylic
acid, is membrane permeable and enters the cell before dissociating into a proton and salicylate, with an effective free concentration $S$. The results shown in figure 5b suggest that in addition to interrupting voltage-dependent motility, salicylate could also act to disrupt the slow time-scale length changes, reducing the shortening (or lengthening) rates. If salicylate is applied externally, the modelling could be further extended by including the trapping of charged salicylate ions (resulting in prolonged osmotic changes when salicylate has been cleared from the extracellular fluid) and the known effects of salicylic acid as weak acid, decreasing the pH$_i$ of the cell [23].

4.3. Homeostasis and metabolism
From the available evidence, in vivo prestin is likely to tie in chloride regulation with the metabolic activity of the cell. Ikeda et al. [11] put forward a model of OHC pH regulation by which CO$_2$ is hydrated to form carbonic acid and then the constituent ions H$^-$ and HCO$_3^-$ are extruded via Na$^+$/H$^+$ or Cl$^-$/HCO$_3^-$ antiporters. This system may act to synchronize the ATP-dependent slow motile response with an increase in the basal chloride level and subsequent increase in electromotile gain. It is possible that metabolic activity occurring in the mitochondria, located along the lateral membrane of the OHC, may be responsible for driving such a process.

Acknowledgements. This work owes much to development of computational models of cochlear hair cells under FP6 'EuroHear'. It also owes a debt to Tom Duke, whose style of extracting simple physical principles to describe complex biological problems remains an ideal, hard to follow.

Reference