

## ARTICLE

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**A theory for the membrane potential of living cells**

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**Abstract** We give an explicit formula for the membrane potential of cells in terms of the intracellular and extracellular ionic concentrations, and derive equations for the ionic currents that flow through channels, exchangers and electrogenic pumps. We demonstrate that the work done by the pumps equals the change in potential energy of the cell, plus the energy lost in downhill ionic fluxes through the channels and exchangers. The theory is illustrated in a simple model of spontaneously active cells in the cardiac pacemaker. The model predicts the experimentally observed intracellular ionic concentration of potassium, calcium and sodium. Likewise, the shapes of the simulated action potential and five membrane currents are in good agreement with experiment. We do not see any drift in the values of the concentrations in a long time simulation, and we obtain the same asymptotic values when starting from the full equilibrium situation with equal intracellular and extracellular ionic concentrations.

**Key words** Membrane potential · Cells · Cardiac pacemaker · Osmotic pressure

**Introduction**

The purpose of the work we present here is to obtain a model for the membrane potential of a single cell which is reasonably realistic, and yet so simple that it can be used in practice to simulate numerically single cells or several coupled cells. For simplicity, experimentally

observed currents (Boyett et al. 1996) are omitted if they seem too small to have a significant influence on the intracellular ion concentration, or at least too small to change the dynamics of the cell. On the other hand, we try to make the theory realistic by using equations that are compatible with, or can be derived from, basic physical principles.

It is a basic assumption of most models (Wilders 1993) for the electrical activity of cells that only the motion of positive ions, and specifically those of potassium, calcium and sodium, influence the membrane potential. This assumption is usually expressed as a differential equation for the time dependence of the potential. We observe that this differential equation can be integrated exactly, and argue that the integration constant is given by the requirement that the potential is zero when the ion concentrations on both sides of the membrane are equal, as the density of negative charge happens to be the same on both sides. Then it follows that the potential is directly proportional to the excess number of positive ions inside the cell, a formula which is nothing but the one for an electric capacitance that follows from Gauss's law in electrostatics.

We derive equations for ionic currents flowing through channels, exchangers and electrogenic pumps. These are based on the Boltzmann distribution law (Boltzmann 1868), which states that a particle in thermal equilibrium spends less time in states of higher energy than in states of lower energy, the Markov assumption (Markov 1906) which says that the transition probabilities of a stochastic system (of Markov type) is only dependent on its present state, and the principle of detailed balance (Onsager 1931) which says that the microscopic laws of physics are invariant with respect to the reversal of time. Our equations were inspired by Ehrenstein and Lecar's model of channel gating (1977), Nonner and Eisenberg's model for channel current (1998), Mullins' model of the  $\text{Na}^+, \text{Ca}^{2+}$  exchanger (1977), and Chapman's model of the  $\text{Na}^+, \text{K}^+$  pump (1978). In particular the book by Mullins (1981) "Ion transport in heart" has been a major source of inspiration for us.

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The theory is illustrated with a simple model of spontaneously active cells in the rabbit sinoatrial node. The observable parameters in the model are based on the experiments of Shibasaki (1987), Hagiwara et al. (1988), Muramatsu et al. (1996) and Sakai et al. (1996). The non-observable parameters in the model are determined numerically, in the same way as in an earlier study (Endresen 1997a), by comparing the action potentials generated by the model with the shape of the action potentials recorded by Baruscotti et al. (1996).

By using an algebraic equation for the potential in place of the standard differential equation, as mentioned above, we obtain a model which is stable against a slow drift of the intracellular ion concentrations, sometimes seen in other models. Furthermore, by fixing the integration constant for the voltage we obtain from the model a prediction of the steady state ion concentrations in the cell. It is even possible to predict these steady state concentrations by starting with an initial state having equal concentrations inside and outside the cell, and integrating the equations of motion over a long time interval.

From the equations of motion we obtain an equation that explicitly demonstrates the energy balance in the process of moving ions in and out of the cell. The energy to make the cell function comes from breakdown of ATP that runs the  $\text{Na}^+, \text{K}^+$  pump. Part of this free (or useful) energy is dissipated while the rest enters the cell. In the cell some of this energy is used to create a potential energy that depends upon the ionic concentrations in the cell, while the rest is dissipated by the currents in the ionic channels and the  $\text{Na}^+, \text{Ca}^{2+}$  exchanger. This potential energy function is thus such that the work associated with ionic currents balances exactly the change in potential energy. In a numerical integration of the differential equations one may compute separately the work and potential energy, comparing the two in order to check (and maybe control) the accuracy of the numerical integration. In our long time integration we observe indeed a balance between work and change in cell membrane potential energy.

## Derivation of the equations

### The Nernst equilibrium potential

Simple diffusion is described by the empirical law of Fick (1855):

$$\vec{\phi} = -ukT\nabla[S] \quad (1)$$

where  $\vec{\phi}$  is the ionic flux,  $[S]$  the concentration of ions and  $u$  is the mobility,  $T$  is the absolute temperature and  $k$  is Boltzmann's constant. The empirical law of Ohm (1827) describes the net motion of charged particles in an electric field:

$$\vec{\phi} = -zeu[S]\nabla U \quad (2)$$

where  $z$  is the valence,  $e$  the elementary charge and  $U$  the electrical potential. The fact that the mobility in Fick's law must be identical to the mobility in Ohm's law was first noticed by Einstein (1905). If we combine Eqs. (1) and (2), we obtain the Nernst–Planck equation for the total flux of ions due to diffusion and electric forces.

$$\vec{\phi} = -ukT \exp\left(-\frac{zeU}{kT}\right) \nabla \left[ [S] \exp\left(\frac{zeU}{kT}\right) \right] \quad (3)$$

The Nernst (1888) equilibrium potential for which the flux is zero is

$$v_S = U_i - U_e = \frac{kT}{ze} \ln \frac{[S]_e}{[S]_i} \quad (4)$$

It can be found by setting  $\vec{\phi} = 0$  in Eq. (3) and integrating from the extracellular (e) to the intracellular (i) side of the membrane. Here  $U_i, U_e, [S]_i$  and  $[S]_e$  are the intracellular and extracellular potentials and concentrations. The same formula can be derived in a more general way using the Boltzmann factor (Boltzmann 1868). The relative probability at equilibrium that an ion is at the intracellular or extracellular side of a cell membrane is

$$\frac{p_i}{p_e} = \frac{[S]_i}{[S]_e} = \exp\left(-\frac{ze(U_i - U_e)}{kT}\right) \quad (5)$$

where  $ze(U_i - U_e)$  is the energy difference between the two positions of the ion. Solving Eq. (5) for  $U_i - U_e$  gives Eq. (4). The equilibrium potentials for the predominant cellular cations are then:

$$v_K = \frac{kT}{e} \ln \frac{[K]_e}{[K]_i} \quad (6)$$

$$v_{Ca} = \frac{kT}{2e} \ln \frac{[Ca]_e}{[Ca]_i} \quad (7)$$

$$v_{Na} = \frac{kT}{e} \ln \frac{[Na]_e}{[Na]_i} \quad (8)$$

### Ionic channels

#### Ionic channels gating

Imagine that ionic channels are either completely open or completely closed and randomly fluctuate between these states in a simple Markov process (Markov 1906), described by the first-order kinetics (Ehrenstein and Lecar 1977):

$$C \xrightleftharpoons[\beta]{\alpha} O \quad (9)$$

where the rate constants  $\alpha$  and  $\beta$  are functions of transmembrane voltage and control the transitions between the closed (C) and the open (O) states of the gate. The rate for a closed channel to open is  $\alpha$ , and  $\beta$  is the

rate for an open channel to close. Let  $x$  denote the average fraction of channels that are open, or, equivalently, the probability that a given channel will be open. We may say that the ionic flux through an ensemble of channels is regulated by a sliding door whose position is  $x$ . This yields:

$$\frac{dx}{dt} = \alpha(1-x) - \beta x \equiv \frac{x_\infty - x}{\tau} \quad (10)$$

where

$$x_\infty = \frac{\alpha}{\alpha + \beta} \quad (11)$$

$$\tau = \frac{1}{\alpha + \beta} \quad (12)$$

Here  $x_\infty$  denotes the steady state fraction of open channels and  $\tau$  the relaxation time. Let us assume that the energy difference between the open and closed positions is given by

$$\Delta G = G_{\text{open}} - G_{\text{closed}} \equiv q(v_x - v) \quad (13)$$

where  $q$  is a gating charge, usually  $q \approx \pm 4e$ , such that  $qv$  represents the change in electrical potential energy due to the redistribution of charge during the transition, and where the term  $qv_x$  represents the difference in mechanical conformational energy between the two states. At equilibrium,  $dx/dt = 0$ , and the ratio of the probabilities for a single channel to be in the open state or the closed state is

$$\frac{x_\infty}{1 - x_\infty} = \frac{\alpha}{\beta} \quad (14)$$

This relation is known as the principle of detailed balance (Onsager 1931). The same ratio is given by the Boltzmann distribution (Boltzmann 1868):

$$\frac{x_\infty}{1 - x_\infty} = \exp\left(-\frac{\Delta G}{kT}\right) \quad (15)$$

Thus, from Eqs. (13), (14) and (15), with  $q = +4e$ :

$$x_\infty = \left[1 + \exp\left(\frac{4e(v_x - v)}{kT}\right)\right]^{-1} \quad (16)$$

The simplest possible choice for  $\alpha$  and  $\beta$  is

$$\alpha = \lambda \exp\left(-\frac{2e(v_x - v)}{kT}\right) \quad (17)$$

$$\beta = \lambda \exp\left(+\frac{2e(v_x - v)}{kT}\right) \quad (18)$$

where  $\lambda$  is a constant. Assuming  $\lambda$  to be constant gives the maximum relaxation time at the voltage where  $x_\infty = 1/2$ . The relaxation time as a function of  $v$  is then

$$\tau = \frac{1}{\alpha + \beta} = \left[2\lambda \cosh\left(\frac{2e(v_x - v)}{kT}\right)\right]^{-1} \quad (19)$$

### Ion channel current

Here we want to obtain the current  $i$  through a one-dimensional ionic channel of length  $d$ . We will allow the cross sectional area  $A$  to vary with position, i.e., we take  $A = A(x)$ . By definition,  $x = -d/2$  is the inside and  $x = d/2$  the outside of the membrane. Let  $\phi = \phi(x)$  be the  $x$ -component of the flux  $\vec{\phi}$ , the other components are negligible as long as the variation of  $A$  with  $x$  is smooth and slow. This is the analogue of water flow in a pipe of varying cross section. By stationary flow, the current  $i$  must be the same through all cross sections, i.e. independent of  $x$ . Thus the flux  $\phi$  is inversely proportional to the area  $A$ , by the relation

$$i = ze\phi A = \text{const.} \quad (20)$$

We insert  $\phi$  from this equation in the  $x$  component of Eq. (3), and multiply the resulting equation by  $\exp(ze(U - U_0)/kT)$ , introducing a constant voltage  $U_0$  chosen such that

$$U\left(-\frac{d}{2}\right) = U_0 + \frac{v}{2}, \quad U\left(\frac{d}{2}\right) = U_0 - \frac{v}{2} \quad (21)$$

Then we find that

$$\begin{aligned} \frac{i}{A} \exp\left(\frac{ze(U - U_0)}{kT}\right) \\ = -zeukT \frac{d}{dx} \left[ [S] \exp\left(\frac{ze(U - U_0)}{kT}\right) \right] \end{aligned} \quad (22)$$

Here  $U$ ,  $[S]$  and  $A$  are functions of  $x$ , while all other quantities are constant (note, however, that the mobility  $u$  may be reduced in a very narrow channel; one possible way to take into account such an  $x$  dependence of  $u$  is to replace the area  $A$  by an effective area  $A_{\text{eff}}$  which is smaller than  $A$ ). Integrating from the inside  $x = -d/2$  to the outside  $x = d/2$  we obtain

$$i = -\frac{zeukT}{I} \left[ [S]_e \exp\left(-\frac{zev}{2kT}\right) - [S]_i \exp\left(\frac{zev}{2kT}\right) \right] \quad (23)$$

where

$$I = \int_{-d/2}^{d/2} \frac{1}{A} \exp\left(\frac{ze(U - U_0)}{kT}\right) dx \quad (24)$$

The concentrations are  $[S]_i$  on the inside and  $[S]_e$  on the outside. If we extract a factor  $\sqrt{[S]_e[S]_i}$ , and write the ratio of the concentrations in terms of the Nernst potential defined in Eq. (4), Eq. (23) can be written in the following way:

$$\begin{aligned} i &= \frac{zeukT}{I} \sqrt{[S]_e[S]_i} \\ &\times \left[ \sqrt{\frac{[S]_i}{[S]_e}} \exp\left(\frac{zev}{2kT}\right) - \sqrt{\frac{[S]_e}{[S]_i}} \exp\left(-\frac{zev}{2kT}\right) \right] \\ &= \frac{2zeukT}{I} \sqrt{[S]_e[S]_i} \sinh\left(\frac{ze(v - v_s)}{2kT}\right) \end{aligned} \quad (25)$$

Equation (25) is our general result that follows from the combined Ohm's and Fick's law.

Now the integral  $I$  depends upon both the voltage  $U = U(x)$  and the cross section  $A = A(x)$ . To determine the  $x$  dependence of  $U(x)$  one would need Poisson's equation for the electrostatic potential, taking into account the net charge distribution in the membrane, including both positive and negative ions. However, this charge distribution will depend upon detailed properties of membranes and their channels that have been little known so far. Thus it seems a reasonable approach to make certain assumptions directly about  $U(x)$ .

A commonly used assumption is that  $U(x)$  is linear, i.e. that the electric field  $-dU/dx$  is constant, and that the cross section is constant,  $A(x) = A_0$ . Then Eq. (25) takes the form

$$i = (ze)^2 u \sqrt{[S]_e [S]_i} \frac{A_0 v \sinh\left(\frac{ze(v-v_S)}{2kT}\right)}{d \sinh\left(\frac{zev}{2kT}\right)} \quad (26)$$

As should be expected, this relation simplifies to the usual Ohm's law in the special case  $[S]_i = [S]_e$ , since then  $v_S = 0$  by Eq. (4). Equation (26) is known as the Goldman constant field approximation (Goldman 1943).

A more general case, perhaps somewhat more realistic, where the integral  $I$  can still be calculated exactly, is that of an ion channel having a constant area  $A_0$ , except for a short and narrow constriction or pore in its middle, with an area  $A_p$  much smaller than  $A_0$ . An example is a cylindrical pore of radius 3 Å and length 5 Å, which is typical for ionic channels. If we furthermore assume a constant electric field everywhere in the channel, and if the length of the pore is  $\epsilon d$ , then we have that

$$I = \frac{2dkT}{zev} \left[ \frac{1}{A_0} \sinh\left(\frac{zev}{2kT}\right) + \left(\frac{1}{A_p} - \frac{1}{A_0}\right) \sinh\left(\frac{\epsilon zev}{2kT}\right) \right] \quad (27)$$

The limit of this as  $v \rightarrow 0$  is

$$I_0 = d \left[ \frac{1-\epsilon}{A_0} + \frac{\epsilon}{A_p} \right] \approx \frac{\epsilon d}{A_p} \quad (28)$$

The last approximation holds when the contribution from the pore dominates the integral, which will be the case, for example, when the ratio of areas,  $A_p/A_0$ , is of the order  $\epsilon^2$ . For  $\epsilon v$  small but nonzero the  $v$  dependence of  $I$  is only of second order in  $\epsilon v$ ; thus it will be a good approximation in a finite voltage range to take  $I$  to be constant, equal to  $I_0$ . The approximation  $I = \text{constant}$  is also valid under more general conditions than those assumed in the above oversimplified derivation, and it gives

$$i = k_S \sinh\left(\frac{ze(v-v_S)}{2kT}\right) \quad (29)$$

Here  $k_S$  is independent of  $v$ , e.g. in the case considered above

$$k_S = 2zeukT \sqrt{[S]_e [S]_i} \frac{A_p}{\epsilon d} \quad (30)$$

For Na and K ions it is a good approximation to consider the square root of the concentrations  $\sqrt{[S]_e [S]_i}$  to be constant, while for Ca ions the relative change in concentration is more significant during one action potential. In the present work we used  $k_S = \text{constant}$  in all three cases, for simplicity. We have checked that this does not affect our numerical results significantly.

There is reason to ask whether the linear voltage profile  $U(x)$  can be a reasonable approximation in the presence of a pore. Indeed, it might seem natural to conclude that most of the voltage drop must be concentrated at the pore owing to its large resistance. However, with the combined Ohm's and Fick's law, the current is driven by gradients in both voltage and concentration, as follows from Eq. (3). A large current may be due to a large voltage drop over the pore, or it may be due to a large change in concentration, without any large voltage difference. Thus, in general one has to take into account the detailed properties of the channel in order to see which one of the gradients is the dominant driving force in a given situation.

In a recent investigation by Nonner and Eisenberg (1998), Poisson's equation relating the net charge density and electrostatic potential was included in a more extensive analysis for a specific model for a channel with a narrow pore. In their analysis they indeed find that only part of the voltage drop is across the pore (something like half of it). In their numerical simulations the voltage in the pore is dominated by the presence of charged carboxyl groups, and thus almost independent of the transmembrane voltage. This lends support to the approximation used here, that the integral  $I$ , Eq. (24), can be regarded as being constant.

Thus our simple result of Eq. (29) has the characteristic features of the current-voltage relationships obtained by Nonner and Eisenberg in their more extensive investigation. One characteristic feature is that Eq. (29) shows inward rectification for large values of  $[S]_e/[S]_i$ , i.e. increased conductance for large negative potentials. Indeed, the curves in Fig. 3A in Nonner and Eisenberg (1998) have the shapes of a hyperbolic sine. Such a behavior is not predicted by the Goldman (1943) equation, Eq. (26), but is seen in many excitable cells (Hille 1992). This is another reason to base our computations on Eq. (29) in order to see the consequences of its application.

#### Potassium channels

If the flux of ions is given by Eq. (29) and regulated by the fraction of open channels  $x$ , the membrane current through potassium channels is

$$i_K = k_K x \sinh\left(\frac{e(v-v_K)}{2kT}\right)$$

$$\frac{dx}{dt} = \frac{1}{\tau_K} \cosh\left(\frac{2e(v - v_K)}{kT}\right) \times \left\{ \frac{1}{2} \left[ 1 + \tanh\left(\frac{2e(v - v_K)}{kT}\right) \right] - x \right\} \quad (31)$$

where  $\tau_K = 1/2\lambda$  is the maximum value of the relaxation time,  $k_K$  is the conductance parameter of Eq. (30),  $v_K$  is given by Eq. (6), and the time dependence of  $x$  is given by Eq. (10) with Eqs. (16) and (19) for  $x_\infty$  and  $\tau$ , respectively. Here we have used the identity

$$\frac{1}{2}(1 + \tanh \phi) = \frac{1}{1 + \exp(-2\phi)} \quad (32)$$

### Calcium and sodium channels

The calcium and sodium channels have an inactivation mechanism in addition to the above activation mechanism. We can view these mechanisms as two independent Markov processes, or equivalently two independent sliding doors, which are both affected by voltage. An ion can only go through if both sliding doors are at least slightly open. Here the activation mechanism is very fast, with a time constant of only a few milliseconds, so we use the steady state fraction of open channels, Eq. (16), for this. The maximum time constant of inactivation for calcium and sodium channels are of the same order of magnitude as the maximum time constant of the activation of the potassium channel (typically a few hundred milliseconds); thus

$$\begin{aligned} i_{Ca} &= k_{Ca} f d_\infty \sinh\left(\frac{e(v - v_{Ca})}{kT}\right) \\ d_\infty &= \frac{1}{2} \left[ 1 + \tanh\left(\frac{2e(v - v_d)}{kT}\right) \right] \\ \frac{df}{dt} &= \frac{1}{\tau_{Ca}} \cosh\left(\frac{2e(v - v_f)}{kT}\right) \\ &\times \left\{ \frac{1}{2} \left[ 1 - \tanh\left(\frac{2e(v - v_f)}{kT}\right) \right] - f \right\} \end{aligned} \quad (33)$$

and

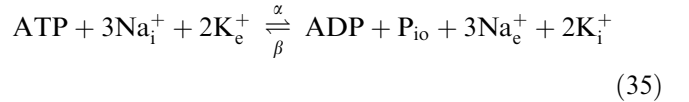
$$\begin{aligned} i_{Na} &= k_{Na} h m_\infty \sinh\left(\frac{e(v - v_{Na})}{2kT}\right) \\ m_\infty &= \frac{1}{2} \left[ 1 + \tanh\left(\frac{2e(v - v_m)}{kT}\right) \right] \\ \frac{dh}{dt} &= \frac{1}{\tau_{Na}} \cosh\left(\frac{2e(v - v_h)}{kT}\right) \\ &\times \left\{ \frac{1}{2} \left[ 1 - \tanh\left(\frac{2e(v - v_h)}{kT}\right) \right] - h \right\} \end{aligned} \quad (34)$$

where  $k_{Ca}$  and  $k_{Na}$  are the conductance parameters of the calcium and sodium currents, respectively,  $v_{Ca}$  and  $v_{Na}$  are given by Eqs. (7) and (8),  $v_d$  and  $v_m$  are the half-activation potentials, and  $v_f$  and  $v_h$  are the half-inactivation potentials.

Note that the activation and inactivation mechanisms work in the same way, and differ in two respects only. Firstly, the time constants differ experimentally by two orders of magnitude; and secondly, the gating charge  $q$ , Eq. (13), is  $+4e$  in one case  $-4e$  in the other case.

### Na<sup>+</sup>,K<sup>+</sup> pump

Na,K-ATPase is found in the plasma membrane of virtually all animal cells and is responsible for active transport of sodium and potassium. Low sodium concentration and high potassium concentration in the cytosol are essential for basic cellular functions such as excitability, secondary active transport and volume regulation. In our model, the Na<sup>+</sup>,K<sup>+</sup> pump is the only energy source. We shall assume that the following equation is a complete macroscopic description of the pump reaction (Chapman 1978):



where ATP, ADP and P<sub>io</sub> are adenosine triphosphate, adenosine diphosphate and inorganic phosphate, while  $\alpha$  and  $\beta$  are the rates for the forward and backward reactions. The energy involved in the movement of  $3\text{Na}^+$  and  $2\text{K}^+$  ions against their electrochemical gradients is

$$\Delta G_{Na} = -3e(v - v_{Na}) \quad (36)$$

$$\Delta G_K = +2e(v - v_K) \quad (37)$$

where  $v_K$  and  $v_{Na}$  are given by Eqs. (6) and (8). This result is independent of the detailed interaction between ions, molecules and the ATPase enzyme. Therefore, the total change in Gibbs free energy is

$$\begin{aligned} \Delta G &= \Delta G_{ATP} + \Delta G_{Na} + \Delta G_K \\ &= e(v_{ATP} + 3v_{Na} - 2v_K - v) \end{aligned} \quad (38)$$

where  $\Delta G_{ATP}$  is the free energy associated with the breakdown of ATP, and  $v_{ATP} = \Delta G_{ATP}/e$ . Note that  $\Delta G$  has to be negative, at least when averaged over time, but the sum  $\Delta G_{Na} + \Delta G_K$  may very well be positive, since  $\Delta G_{ATP}$  is large and negative. Thus, part of the energy from ATP breakdown goes into increasing the free energy of potassium and sodium ions.

In practice, such a pump or motorized swing door will quickly reach saturation. We therefore choose the sum of the forward and backward rates to be constant, resembling the maximum possible speed of the swing door in the forward and backward directions:

$$\alpha + \beta = \lambda \quad (39)$$

where  $\lambda$  is a constant. At equilibrium, the forward reaction must occur just as frequently as the reverse reaction, giving

$$\frac{\alpha}{\beta} = \exp\left(-\frac{\Delta G}{kT}\right) \quad (40)$$

Solving Eqs. (39) and (40) for  $\alpha$  and  $\beta$  gives

$$\alpha = \frac{\lambda \exp\left(-\frac{\Delta G}{kT}\right)}{1 + \exp\left(-\frac{\Delta G}{kT}\right)} \quad (41)$$

$$\beta = \frac{\lambda}{1 + \exp\left(-\frac{\Delta G}{kT}\right)} \quad (42)$$

The difference

$$\alpha - \beta = \lambda \frac{\exp\left(-\frac{\Delta G}{2kT}\right) - \exp\left(\frac{\Delta G}{2kT}\right)}{\exp\left(-\frac{\Delta G}{2kT}\right) + \exp\left(\frac{\Delta G}{2kT}\right)} = \lambda \tanh\left(-\frac{\Delta G}{2kT}\right) \quad (43)$$

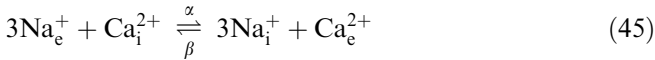
gives the net pump current for a cell with  $M$  pumps as

$$\begin{aligned} i_{\text{NaK}} &= Me(\alpha - \beta) \\ &= k_{\text{NaK}} \tanh\left(\frac{e(v + 2v_K - 3v_{\text{Na}} - v_{\text{ATP}})}{2kT}\right) \end{aligned} \quad (44)$$

where  $k_{\text{NaK}} = Me\lambda$ .

#### $\text{Na}^+, \text{Ca}^{2+}$ exchanger

To maintain a steady state for the intracellular calcium concentration in heart cells, for example, the amount of calcium that enters the cell via ionic channels must be extruded. The  $\text{Na}^+, \text{Ca}^{2+}$  exchanger is the major mechanism responsible for achieving a balance between calcium entry and extrusion in oscillating cells. We assume that the rates for the forward ( $\alpha$ ) and backward ( $\beta$ ) exchange reactions given by (Mullins 1977)



are governed largely by the electrochemical gradients for sodium and calcium, together with the membrane potential. In other words, the energy produced when three extracellular sodium ions take the elevator down into the cytosol is used to elevate one calcium ion up from the cytosol into the extracellular space:

$$\Delta G_{\text{Na}} = +3e(v - v_{\text{Na}}) \quad (46)$$

$$\Delta G_{\text{Ca}} = -2e(v - v_{\text{Ca}}) \quad (47)$$

where  $v_{\text{Ca}}$  and  $v_{\text{Na}}$  are given by Eqs. (7) and (8). The total work done in reaction (45) is

$$\Delta G = \Delta G_{\text{Na}} + \Delta G_{\text{Ca}} = e(v - 3v_{\text{Na}} + 2v_{\text{Ca}}) \quad (48)$$

The ratio of  $\alpha$  to  $\beta$  in Eq. (45) is again determined by  $\Delta G$  like in Eq. (40). However, in the present case, saturation effects are not expected and furthermore  $\Delta G$  will vary around zero, so we put according to Mullins (1981):

$$\alpha = \lambda [\text{Na}]_e^3 [\text{Ca}]_i \exp\left(-\frac{ev}{2kT}\right) \quad (49)$$

$$\beta = \lambda [\text{Na}]_i^3 [\text{Ca}]_e \exp\left(\frac{ev}{2kT}\right) \quad (50)$$

For a cell with  $N$  exchangers the net current is then

$$\begin{aligned} i_{\text{NaCa}} &= -Ne(\alpha - \beta) \\ &= Ne\lambda \left[ [\text{Na}]_i^3 [\text{Ca}]_e \exp\left(\frac{ev}{2kT}\right) \right. \\ &\quad \left. - [\text{Na}]_e^3 [\text{Ca}]_i \exp\left(-\frac{ev}{2kT}\right) \right] \\ &= k_{\text{NaCa}} \sinh\left(\frac{e(v - 3v_{\text{Na}} + 2v_{\text{Ca}})}{2kT}\right) \end{aligned} \quad (51)$$

For simplicity we make the assumption that

$$k_{\text{NaCa}} = 2Ne\lambda \sqrt{[\text{Na}]_e^3 [\text{Na}]_i^3 [\text{Ca}]_e [\text{Ca}]_i}$$

is a constant (Mullins 1981), and we have checked that this assumption had negligible influence on the numerical results.

#### Membrane voltage

Imagine that the electrical activity of a cell is described by the five currents discussed above, and that all the other currents (Boyett et al. 1996) are of minor importance. The standard differential equations for the voltage, and the conservation laws for intracellular ionic concentrations are then

$$\frac{dv}{dt} = -\frac{1}{C} (i_K + i_{\text{Ca}} + i_{\text{Na}} + i_{\text{NaCa}} + i_{\text{NaK}}) \quad (52)$$

$$\frac{d}{dt} [\text{K}]_i = \frac{2i_{\text{NaK}} - i_K}{FV} \quad (53)$$

$$\frac{d}{dt} [\text{Ca}]_i = \frac{2i_{\text{NaCa}} - i_{\text{Ca}}}{2FV} \quad (54)$$

$$\frac{d}{dt} [\text{Na}]_i = \frac{-i_{\text{Na}} - 3i_{\text{NaK}} - 3i_{\text{NaCa}}}{FV} \quad (55)$$

where  $C$  is cell capacitance,  $F$  is Faraday's constant and we assume that the cell volume  $V$  is constant. Now Eqs. (53), (54) and (55) can be solved for  $i_K$ ,  $i_{\text{Ca}}$  and  $i_{\text{Na}}$ , and we obtain

$$i_K = -FV \frac{d}{dt} [\text{K}]_i + 2i_{\text{NaK}} \quad (56)$$

$$i_{\text{Ca}} = -2FV \frac{d}{dt} [\text{Ca}]_i + 2i_{\text{NaCa}} \quad (57)$$

$$i_{\text{Na}} = -FV \frac{d}{dt} [\text{Na}]_i - 3i_{\text{NaK}} - 3i_{\text{NaCa}} \quad (58)$$

Inserting this into Eq. (52) yields

$$\frac{dv}{dt} = \frac{FV}{C} \frac{d}{dt} ([\text{K}]_i + 2[\text{Ca}]_i + [\text{Na}]_i) \quad (59)$$

since the remaining currents cancel. This equation can also be written as

$$\frac{d}{dt} \left( v - \frac{FV}{C} \{ [\text{K}]_i + 2[\text{Ca}]_i + [\text{Na}]_i \} \right) = 0 \quad (60)$$

This integrated gives

$$v - \frac{FV}{C} ([K]_i + 2[Ca]_i + [Na]_i) = v_0 \quad (61)$$

where the integration constant  $v_0$  has to be determined. Given that the voltage across a capacitor is zero when the net charge difference is zero, we must choose the integration constant

$$v_0 = -\frac{FV}{C} ([K]_e + 2[Ca]_e + [Na]_e) \quad (62)$$

which gives

$$v = \frac{FV}{C} \{ [K]_i - [K]_e + 2([Ca]_i - [Ca]_e) + [Na]_i - [Na]_e \} \quad (63)$$

This choice of  $v_0$  depends on the assumption that all other ions have the same concentrations on both sides, consistent with Eq. (52) where it is assumed that they do not contribute to the current. [To be more precise, the constant  $v_0$  in Eq. (62) can be uncertain since the inside and the outside of the cell after all is different, but this kind of uncertainty can be neglected in Eq. (63) as anything like  $\pm 100$  mV has a tiny influence upon concentrations as the prefactor  $FV/C$  is very large. This we also verified by a simulation.]

Above we have assumed that all currents result in free ions in the cell, i.e. intracellular binding like the one of Ca with calmodulin that buffers the free Ca concentration is neglected. This will influence Nernst potentials, but not the voltage directly, only via modified currents, and thus we do not consider this crucial for the present model.

Equation (63) is nothing but the relation between electric potential and charge of a capacitor, which is actually the origin of Eq. (52). Thus it is completely general and independent of the number of membrane currents in a model. It means that:

*The voltage across the membrane of a cell is caused by, and is directly proportional to, the surplus of charge inside the cell.*

Since Eq. (63) is the explicit integral of Eq. (52), it can be used instead of Eq. (52) [or the equivalent of Eq. (52)] in any model. The differential equation, Eq. (52), is needed only in models where the intracellular ionic concentrations are not tracked individually [for example, the Hodgkin–Huxley equations (1952)].

A variant of Eq. (63) has recently been derived by Varghese and Sell (1997). However, they did not identify the integration constant  $v_0$ , which is related to the initial ionic concentrations and represents the initial net charge via the electric capacitance of the cell as shown in Eq. (63).

There is reason to ask whether it is a reasonable approximation to omit the anions in Eq. (63). This can be justified if the total concentration of cations is approximately the same on both sides. Indeed, this property is seen in most ionic models, like for instance in Wilders (1993), where the cation concentrations are

$$\begin{aligned} [K]_e &= 5.4 \text{ mM} & [Ca]_e &= 2 \text{ mM} & [Na]_e &= 140 \text{ mM} \\ [K]_i &= 140 \text{ mM} & [Ca]_i &= 0.0000804 \text{ mM} \\ [Na]_i &= 7.5 \text{ mM} \end{aligned} \quad (64)$$

Finally, in this part we may comment upon our equations in view of the well-known Goldman equation (1943). This equation follows when ions of the same magnitude ( $|z| = \text{const}$ ) flow in a channel, such that each current is given by Eq. (26), and it yields the potential  $v$  in a stationary state with zero net current. The Goldman equation is obtained by adding currents for various ions such that the terms on the right-hand side of Eq. (26) add up to zero, and then solving with respect to  $v$ .

However, for the situation considered in the present work this is modified, but Eq. (26) can still be regarded as the general basis for the currents through various channels in the cell membrane. Firstly, the Ca ions have twice the charge of Na and K ions, and we have a  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  exchanger which yields a net current involving two Nernst potentials. Further, in the present case, ionic channels are active in the sense of having a closing and opening mechanism which is needed to obtain spontaneous oscillations. Effectively this means that the coefficient  $u$  in Eq. (26) depends heavily upon the cell voltage  $v$  with time delays incorporated. Finally, the  $\text{Na}^+$ ,  $\text{K}^+$  pump is the driving mechanism or energy input to build up the voltage of the cell or “load the battery”, which is then regulated by the various ionic currents in channels. This pumping involves the corresponding chemical potential of the ATP too which itself, however, does not contribute to the current. Adding all currents we will obtain a generalized form of the Goldman equation for the stationary state [Eq. (52) with  $dv/dt = 0$ ]. However, for a suitable choice of parameters the stationary state of this highly non-linear equation becomes unstable, and we find the oscillating behavior for our coupled set of equations.

Energy balance and osmotic pressure

The current  $i$  in Eq. (20) may be written as

$$i = ze \frac{dn}{dt} \quad (65)$$

where  $n$  is the number of ions transferred from the inside to the outside of the membrane, and  $dn/dt$  is the rate of transfer. The change in free energy when one ion is transferred is  $\Delta G = -ze(v - v_s)$ , and the total change in free energy over a time interval is

$$\Delta G = - \int_0^n ze(v - v_s)dn = - \int_0^t i(v - v_s)dt \quad (66)$$

It follows from Eq. (29) that the current  $i$  has the same sign as the voltage  $v - v_s$  as required in general to have thermodynamic stability, so that the integrand in Eq. (66) is positive (strictly speaking, non-negative), and therefore  $\Delta G < 0$  (or  $\Delta G \leq 0$ ).

By similar reasoning, taking into account all the reversal potentials and the free energy associated with the breakdown of ATP, we find that the total change in free energy due to the five currents in our model is

$$\Delta G = - \int_0^t [i_K(v - v_K) + i_{Ca}(v - v_{Ca}) + i_{Na}(v - v_{Na}) + i_{NaCa}(v - 3v_{Na} + 2v_{Ca}) + i_{NaK}(v + 2v_K - 3v_{Na} - v_{ATP})] dt \quad (67)$$

Each of the five terms in the integrand is positive (non-negative), since each current has the same sign as the corresponding voltage. In other words, energy is dissipated all the time by all the five currents, implying that  $\Delta G \leq 0$ .

The main contribution to the negative  $\Delta G$  is the ATP term

$$\Delta G_{ATP} = \int_0^t i_{NaK} v_{ATP} dt \quad (68)$$

which in practice is negative all the time, and furthermore is large in magnitude compared to the other terms. It should be noted that this term is the source of useful energy that is dissipated to maintain the activity of the cell and keep it away from equilibrium. Keeping this term apart, we may calculate the change in free energy of the ionic system

$$\begin{aligned} \Delta G_{ions} &= \Delta G - \Delta G_{ATP} \\ &= - \int_0^t [i_K(v - v_K) + i_{Ca}(v - v_{Ca}) + i_{Na}(v - v_{Na}) + i_{NaCa}(v - 3v_{Na} + 2v_{Ca}) + i_{NaK}(v + 2v_K - 3v_{Na})] dt \end{aligned} \quad (69)$$

Using Eqs. (52), (56), (57) and (58) to eliminate the currents, and assuming the capacitance  $C$  and the volume  $V$  to be constant, we find that

$$\begin{aligned} \Delta G_{ions} &= C \int_0^v v dv - FV \int_{[K]_e}^{[K]_i} v_K d([K]_i) \\ &\quad - 2FV \int_{[Ca]_e}^{[Ca]_i} v_{Ca} d([Ca]_i) - FV \int_{[Na]_e}^{[Na]_i} v_{Na} d([Na]_i) \end{aligned} \quad (70)$$

where the reversal potentials  $v_K$ ,  $v_{Ca}$  and  $v_{Na}$  depend on the integration variables  $[K]_i$ ,  $[Ca]_i$  and  $[Na]_i$  according to Eqs. (6), (7) and (8). Integrating from the equilibrium state  $v = 0$ ,  $[K]_i = [K]_e$ ,  $[Ca]_i = [Ca]_e$  and  $[Na]_i = [Na]_e$ , using the indefinite integral

$$\int \ln \phi d\phi = \phi \ln \phi - \phi \quad (71)$$

we find

$$\begin{aligned} \Delta G_{ions} &= \frac{1}{2} C v^2 + RTV \left\{ [K]_i \ln \left( \frac{[K]_i}{[K]_e} \right) + [Ca]_i \ln \left( \frac{[Ca]_i}{[Ca]_e} \right) \right. \\ &\quad \left. + [Na]_i \ln \left( \frac{[Na]_i}{[Na]_e} \right) \right\} \\ &\quad - RTV ([K]_i - [K]_e + [Na]_i - [Na]_e + [Ca]_i - [Ca]_e) \end{aligned} \quad (72)$$

In passing it may be noted that in the present case the equilibrium state is simply the one with equal concentrations of cations on both sides, as follows from our assumption of having the same concentration of anions or negative charge on both sides of the membrane. More generally, equilibria for ionic systems are described by the Donnan (1911) equilibrium that can yield different concentrations on both sides of the membrane.

Since  $\Delta G_{ions}$  is a function only of the state of the cell and is independent of the process by which the state is reached, it represents a potential energy for the cell, which we will call  $P$ . Note that  $P = 0$  in the equilibrium state, whereas  $P > 0$  in all other states.  $P$  is the minimum energy needed to bring a thermal system away from equilibrium with its surroundings, or equivalently, the maximum work that can be performed by the system when returning to equilibrium.

The potential energy  $P$ , as defined in Eq. (72), contains three terms, each of which can be given a more direct physical interpretation. The first term is simply the electrostatic energy of a capacitor, while the two temperature-dependent terms are related to thermal properties. In fact, since we assume ideal dilute solutions, the change in entropy due to changes of ion concentrations away from their equilibrium values is

$$\begin{aligned} s &= RV \left\{ [K]_i \ln \left( \frac{[K]_e}{[K]_i} \right) + [Ca]_i \ln \left( \frac{[Ca]_e}{[Ca]_i} \right) \right. \\ &\quad \left. + [Na]_i \ln \left( \frac{[Na]_e}{[Na]_i} \right) \right\} \end{aligned} \quad (73)$$

Under the same changes, the change in osmotic pressure inside the cell is equal to the difference in osmotic pressure across the membrane, which is, for ideal solutions

$$\pi = RT([K]_i - [K]_e + [Na]_i - [Na]_e + [Ca]_i - [Ca]_e) \quad (74)$$

Note that for a fixed volume the anions will not contribute to the difference in osmotic pressure, but they will contribute if the volume is changed and the membrane is impermeable to them. In terms of the change in transmembrane voltage,  $v$ , the change in entropy,  $s$ , and the change in transmembrane osmotic pressure,  $\pi$ , as compared to the equilibrium state, we may write

$$P = \frac{1}{2} C v^2 - Ts - V\pi \quad (75)$$



Equation (74) is the van't Hoff equation (1887) for the osmotic pressure across a solute impermeable barrier separating two ideal dilute solutions. In 1887, van't Hoff noticed that the behavior of solutes in dilute solutions resembles the behavior of a perfect gas (Jones 1899).

Rewriting Eq. (72), using Eqs. (69) and (75), we may summarize the energy balance in the following way:

$$\begin{aligned}
 & - \int_0^t i_{\text{NaK}}(v + 2v_K - 3v_{\text{Na}})dt \\
 & = \frac{1}{2}Cv^2 - sT - \pi V + \int_0^t [i_K(v - v_K) + i_{\text{Ca}}(v - v_{\text{Ca}}) \\
 & \quad + i_{\text{Na}}(v - v_{\text{Na}}) + i_{\text{NaCa}}(v - 3v_{\text{Na}} + 2v_{\text{Ca}})]dt \quad (76)
 \end{aligned}$$

The left-hand side of this equation is the useful work performed upon the cell by the  $\text{Na}^+$ ,  $\text{K}^+$  pumps, moving  $\text{Na}^+$  and  $\text{K}^+$  ions against their potential gradients. The energy supplied by the pumping of ions produces the following effects that either change the potential energy of the cell or cause energy loss by dissipation:

1. A transmembrane voltage difference,  $v$ .
2. A change in entropy,  $s$ .
3. A transmembrane osmotic pressure difference,  $\pi$ .
4. Downhill ionic currents through the exchangers and channels,  $i_K$ ,  $i_{\text{Ca}}$ ,  $i_{\text{Na}}$  and  $i_{\text{NaCa}}$ .

In an oscillating cell, as described by the present model, the following two inequalities will hold, over a sufficiently long time interval:

$$\begin{aligned}
 -\Delta G_{\text{ATP}} &= - \int_0^t i_{\text{NaK}} v_{\text{ATP}} dt > \\
 & - \int_0^t i_{\text{NaK}}(v + 2v_K - 3v_{\text{Na}})dt > 0 \quad (77)
 \end{aligned}$$

The first inequality is simply the inequality  $i_{\text{NaK}}(v + 2v_K - 3v_{\text{Na}} - v_{\text{ATP}}) > 0$ , which follows from Eq. (44). It means that the energy released by breakdown of ATP is larger than the useful work performed by the pumps, as required from general principles; in other words, that energy is dissipated by the current  $i_{\text{NaK}}$  produced by the pumps. The second inequality must hold due to Eq. (76), where the right-hand side consists of three oscillating potential energy terms plus four positive terms that describe energy dissipation. This inequality shows that the useful work performed by the pumps is positive, as required to maintain the dissipation due to the other currents of a working cell away from thermal equilibrium.

We may remark that the laws of Ohm and Fick, Eqs. (1) and (2), are consistent with the use of ideal solutions and osmotic pressure that assume independent (non-interacting) particles (Arrhenius 1902).

In the model considered the osmotic pressure  $\pi$  has not been involved in the dynamics. However, in an extended model with variable volume  $V$  it will be more important as it will determine the solute flux through the membrane.

## A model for cardiac pacemaker cells

In the above, a mathematical model of the membrane potential has been derived where Eqs. (6), (7) and (8) represent the equilibrium potentials, Eqs. (31), (33) and (34) the ionic currents, Eqs. (51) and (44) the exchanger and the pump currents, Eqs. (53), (54) and (55) the ionic concentrations, Eq. (63) the membrane voltage and, finally, Eq. (75) the osmotic pressure across the cell membrane. The model has six time-dependent variables,  $x, f, h, [\text{K}]_i, [\text{Ca}]_i$  and  $[\text{Na}]_i$ , and the equations are summarized in Appendix A.

### Ionic mechanisms in the cardiac pacemaker

Noma published in 1996 an excellent review of the ionic mechanisms of the cardiac pacemaker potential (Noma 1996). In this short paper, Noma investigated the mechanisms that produce spontaneous activity in sinoatrial node cells, and introduced the following overview of the relevant ionic currents.

#### *Channel gating which drives membrane depolarization during diastole*

1. Deactivation of  $i_K(i_{\text{Kr}})$ .
2. Removal of inactivation of  $i_{\text{Ca,L}}$  and  $i_{\text{st}}$ .
3. Activation of the hyperpolarization-activated current ( $i_f$ ).
4. Activation of L-type  $\text{Ca}^{2+}$  current ( $i_{\text{Ca,L}}$ ).
5. Activation of T-type  $\text{Ca}^{2+}$  current ( $i_{\text{Ca,T}}$ ).

#### *Background conductance*

1.  $i_{\text{b,Na}}$ : a cation current with reversal potential of about  $-20$  mV.
2.  $i_{\text{K,ACh}}$ : spontaneous openings of the  $\text{K}^+$  channels.
3.  $i_{\text{NaK}}$ : Na/K pump current.
4.  $i_{\text{NaCa}}$ : Na/Ca exchange current.
5.  $i_{\text{K,ATP}}$ : ATP-sensitive  $\text{K}^+$  channels.

We will not try to determine the relative amplitude of the above current components here, but instead demonstrate that only five membrane currents are sufficient to ensure stable intracellular ionic concentrations. These are  $i_K, i_{\text{Ca}}, i_{\text{NaK}}, i_{\text{NaCa}}$  and  $i_{\text{Na}}$ . In our model we assume that  $i_{\text{Ca,T}}$  and  $i_{\text{Ks}}$  are of minor importance, i.e. when we talk about  $i_{\text{Ca}}$  we mean  $i_{\text{Ca,L}}$ , and when we talk about  $i_K$  we mean  $i_{\text{Kr}}$ .

## Model parameters

The various parameters play different roles in the model, and we list them in different tables to distinguish between fundamental physical constants (Table 1), experimentally observed constants (Table 2), adjustable parameters (Table 3) and initial conditions (Table 4) in the model. One parameter not listed is the gating charge  $q$ , Eq. (13), which is the origin of the factor  $2e/kT$  in Eqs. (31), (33) and (34). This corresponds to a slope factor for the activation and inactivation curves of

**Table 1** Fundamental physical constants

Name	Value	Unit
$k$	$1.38065812 \times 10^{-20}$	mJ K <sup>-1</sup>
$e$	$1.60217733 \times 10^{-19}$	C
$F$	96485.30929	C mol <sup>-1</sup>
$R = kF/e$	8314.511935	J kmol <sup>-1</sup> K <sup>-1</sup>

**Table 2** Observed constants

Name	Value	Unit
$T$	310.15	K
$[K]_e$	5.4	mM
$[Ca]_e$	2	mM
$[Na]_e$	140	mM
$V$	10	$10^3 \mu\text{m}^3$
$C$	47	pF
$v_x$	-25.1	mV
$v_d$	-6.6	mV
$v_f$	-25.0	mV
$v_m$	-41.4	mV
$v_h$	-91.0	mV
$v_{ATP}$	-450	mV
$\tau = \tau_K = \tau_{Ca} = \tau_{Na}$	200	ms

**Table 3** Adjustable parameters

Name	Value	Unit
$k_{Ca}$	26.2	pA
$k_{b,Ca}$	0.01645	pA
$k_{Na}$	112.7	pA
$k_K$	32.9	pA
$k_{NaCa}$	1400.0	pA
$k_{NaK}$	11.46	pA

**Table 4** Initial conditions

Name	Value	Unit
$x_0$	0.1	—
$f_0 = 1 - x_0$	0.9	—
$h_0$	0.008	—
$[K]_{i0}$	130.66	mM
$[Ca]_{i0}$	0.0006	mM
$[Na]_{i0}$	18.7362	mM

$kT/4e \approx 6.68$  mV at 37 °C. The observed slope factors are 7.4 mV for activation of  $i_K$  (Shibasaki 1987), 6.6 mV for activation of  $i_{Ca}$  (Hagiwara et al. 1988), 6.0 mV for inactivation of  $i_{Ca}$  (Hagiwara et al. 1988), 6.0 mV for activation of  $i_{Na}$  (Muramatsu et al. 1996) and, finally, 6.4 mV for inactivation of  $i_{Na}$  (Muramatsu et al. 1996). Hence, we see that  $kT/4e$ , corresponding to a gating charge of  $q \approx \pm 4e$ , is a good approximation.

The half-activation and inactivation potentials in the model ( $v_x, v_d, v_f, v_m$  and  $v_h$ ) are based on the experiments of Shibasaki (1987), Hagiwara et al. (1988) and Muramatsu et al. (1996), and we use a value of  $v_{ATP}$  that gives a reversal potential for the sodium pump in good agreement with the experiments of Sakai et al. (1996). The maximum time constants in these experiments were 203 ms for activation of  $i_K$  (Shibasaki 1987), 225 ms for inactivation of  $i_{Ca}$  (Hagiwara et al. 1988) and 174 ms for inactivation of  $i_{Na}$  (Muramatsu et al. 1996). In the model, however, we combine these and use a maximum time constant of 200 ms for  $\tau_K, \tau_{Ca}$  and  $\tau_{Na}$ . Finally, we use typical values for cell volume, cell capacitance and extracellular ionic concentrations.

Two of the differential equations in the model have almost identical but opposite dynamics. If we modify the half-inactivation potentials of calcium from  $v_f = -25.0$  mV to  $v_f = v_x = -25.1$  mV, it is possible to relate the inactivation gating of calcium to the activation gating of potassium by the equation

$$x + f = 1 \quad (78)$$

since the time constants for these two processes are equal. We have thus reduced the number of differential equations in the model by one.

This computational saving will be irrelevant for the computation of one action potential, but is important in an extended model with thousands of coupled cells, or in a long-time integration of the single cell model.

## Pacemaker current

The relative amplitude of the ionic currents that drive membrane depolarization during diastole is still a matter of debate. DiFrancesco (1993) argues that the hyperpolarization activated current ( $i_f$ ) is the only current that can generate and control the slow depolarization of pacemaker cells. The  $i_f$  current is normally carried by  $Na^+$  and  $K^+$ . Guo et al. (1995a, b, 1996) reported another current, called the sustained inward current  $i_{st}$ , where the major charge carrier is believed to be  $Na^+$ . Also a  $Ca^{2+}$  “window” current has been observed in rabbit sinoatrial node cells (Denyer and Brown 1990). It is possible that any one of these currents, or a combination of them, is responsible for membrane depolarization during diastole. However, the estimates of the net membrane current during diastole is so imprecise (Zaza et al. 1997) that we would not form a judgement on the question.

During diastole the electrochemical driving forces produce outward  $K^+$  currents and inward  $Na^+$  and

$\text{Ca}^{2+}$  currents, and the driving force for  $\text{Ca}^{2+}$  is much larger than that for  $\text{Na}^+$ . These findings imply that a significant background influx of  $\text{Ca}^{2+}$  is possible during diastole, and that this current might be responsible for the pacemaker activity in sinoatrial node cells. We denote the conductance for this current  $k_{b,\text{Ca}}$ , and modify Eq. (33) combined with Eq. (78) to read

$$i_{\text{Ca}} = [k_{\text{Ca}}(1-x)d_{\infty} + k_{b,\text{Ca}}] \sinh\left(\frac{v - v_{\text{Ca}}}{\frac{kT}{e}}\right) \quad (79)$$

In our model,  $k_{b,\text{Ca}}$  is responsible for the slow diastolic depolarization, and  $i_{b,\text{Ca}}$  is thus our pacemaker current. However, as suggested by Guo et al. (1995b), it is indeed possible that the sustained inward current  $i_{\text{st}}$  may largely replace the role of the  $\text{Ca}^{2+}$  currents, assumed here and in previous studies (Wilders 1993).

### Adjustable parameters

The density of ionic channels, exchangers and pumps (i.e.  $k_{\text{Ca}}$ ,  $k_{b,\text{Ca}}$ ,  $k_{\text{Na}}$ ,  $k_{\text{K}}$ ,  $k_{\text{NaK}}$  and  $k_{\text{NaCa}}$ ) can vary significantly from cell to cell. In order to reproduce recorded action potentials [fig. 7A in Baruscotti et al. (1996)], we fit the adjustable parameters (Table 3) and the initial conditions (Table 4) numerically. More details of the method are given in Endresen (1997a). Many different combinations of  $k_{\text{Ca}}$ ,  $k_{b,\text{Ca}}$ ,  $k_{\text{Na}}$ ,  $k_{\text{K}}$ ,  $k_{\text{NaK}}$  and  $k_{\text{NaCa}}$  resulted in good approximations to the experimentally recorded waveform, from which we conclude that different cells can produce the same action potential although they have different mixtures of ionic channels, exchangers and pumps.

### Simulation results

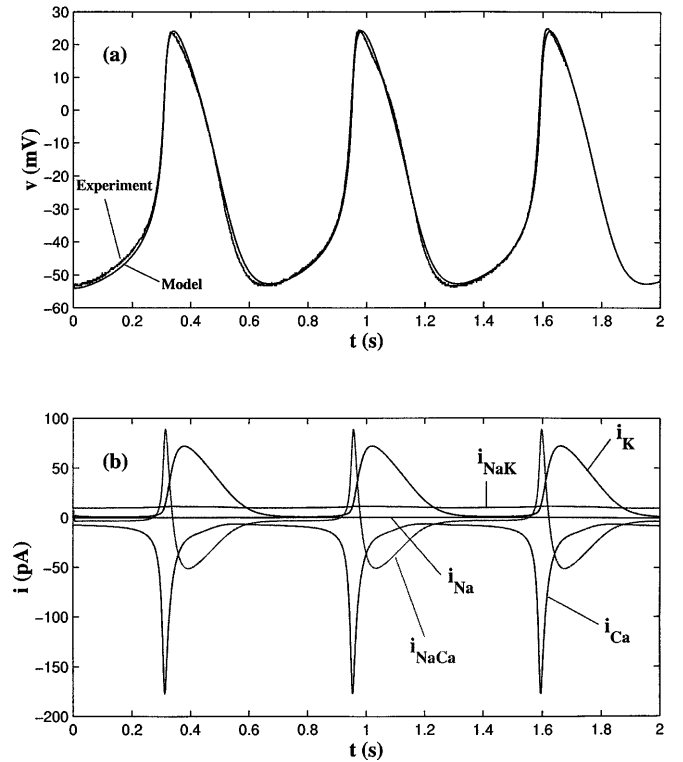
The five differential equations in the model were solved numerically using a fifth-order Runge-Kutta method with variable steplength. More details are given in Endresen (1997b). We computed the work  $W$ , defined as minus the integral on the right-hand side of Eq. (69), to check that the equation  $W + P = 0$  was satisfied numerically. This could also be used for varying the steplength in an efficient way (Marthinsen et al. 1997), since the solution of our differential equations must satisfy this constraint. These “checksum equations” are shown in Appendix B.

In Fig. 1a the modeled action potential is shown together with the experimental curve of Baruscotti et al. (1996). The curves are identical in shape, but we adjusted the modeled curve somewhat (we multiplied the voltage amplitude by a factor 1.25, without changing the minimum value) to obtain the same voltage amplitudes. At present it is not clear to us which mechanism is needed in the model in order to avoid this factor. Figure 1b shows the five membrane currents in the model,  $i_{\text{Ca}}$ ,  $i_{\text{Na}}$ ,  $i_{\text{K}}$ ,  $i_{\text{NaK}}$  and  $i_{\text{NaCa}}$ .

Figure 2 shows the spontaneous action potentials together with the intracellular ionic concentrations and the osmotic pressure across the cell membrane. These computations used the initial conditions stated in Table 4. Cells must generate their membrane potential by actively transporting ions against the respective concentration gradients. To examine this process in our model, we ran a simulation starting with equal intracellular and extracellular ionic concentrations:  $[\text{K}]_i = [\text{K}]_e = 5.4 \text{ mM}$ ,  $[\text{Ca}]_i = [\text{Ca}]_e = 2 \text{ mM}$  and  $[\text{Na}]_i = [\text{Na}]_e = 140 \text{ mM}$ . The results are presented in Fig. 3, that shows the voltage and Nernst potentials (a) and the energies (b) in a long time simulation. After approximately 750 s (12.5 min) the system reaches oscillations identical to the original oscillations shown in Figs. 1 and 2 (this cannot be seen from Fig. 3 since the time scale is very different). This long time simulation is a numerical indication that the oscillations in Figs. 2 and 3 indeed correspond to a stable limit cycle.

## Discussion

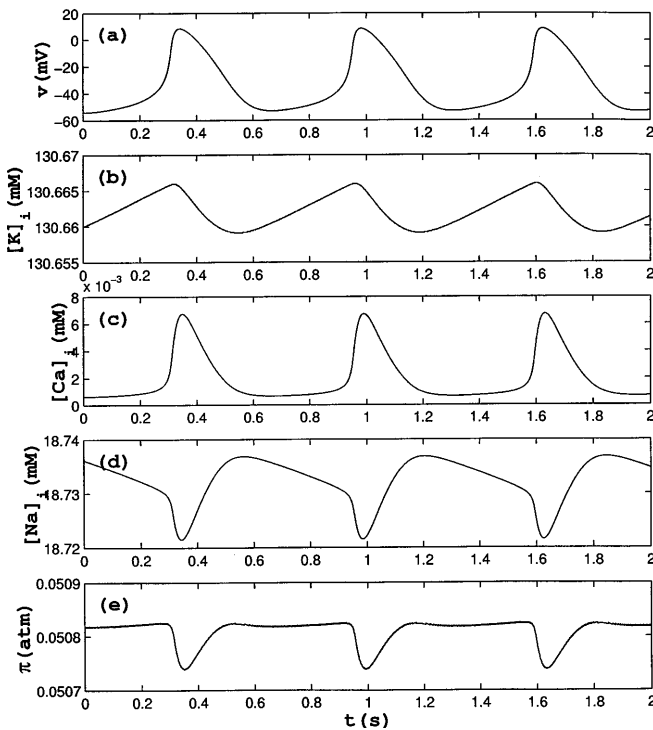
We have presented a simple model for the cells of the rabbit sinoatrial node. Our model involves only  $\text{Na}^+$ ,  $\text{K}^+$



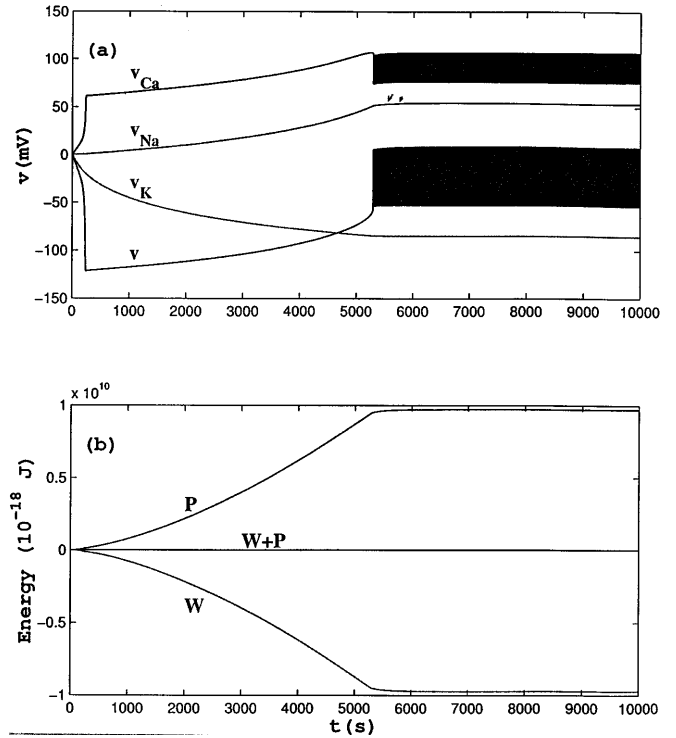
**Fig. 1a, b** Action potential and currents. **a** Experimentally recorded and scaled (by a factor of 1.25) model-generated rabbit sinoatrial action potential waveform. **b** The outward delayed rectifying potassium current ( $i_{\text{K}}$ ), the inward calcium current ( $i_{\text{Ca}}$ ), the inward sodium current ( $i_{\text{Na}}$ ), the sodium calcium exchange current ( $i_{\text{NaCa}}$ ) and the sodium potassium pump current ( $i_{\text{NaK}}$ ). These computations used the initial conditions in Table 4

and  $\text{Ca}^{2+}$  ions, their respective channels, the  $\text{Na}^+, \text{Ca}^{2+}$  exchanger and the  $\text{Na}^+, \text{K}^+$  pump. The equations were derived using basic physical principles and conservation laws. Because of this strategy, all the equations for the membrane currents have the desired property that the relationship between membrane current and voltage is linear close to equilibrium [ $\sinh(x)$  and  $\tanh(x)$  reduce to  $x$  when  $x$  is small]. This property is not always seen in other models. Furthermore, since the only source of energy in our model is the sodium-potassium pump, we can easily track the flow of energy, and show that the pump works to generate a transmembrane voltage, osmotic pressure difference and an entropy. Our equations also account for the energy lost due to downhill ionic fluxes through the exchanger and channels. A prediction of osmotic pressure variations is a novel result of our energy analysis.

The intracellular ionic concentrations are dynamic variables in our model, governed by the conservation of Eqs. (53), (54) and (55). This allows us to replace the standard differential equation for the voltage [Eq. (52)] with the algebraic Eq. (63). Although a number of other ionic models also keep track of intracellular ionic concentrations [see Wilders (1993)], we are unaware of any other model using an algebraic equation for the membrane potential. Models that use the standard voltage differential [Eq. (52)] have a phase space with one



**Fig. 2a-e** Membrane potential (not scaled), intracellular ionic concentrations and osmotic pressure of a rabbit sinoatrial node cell. **a** Model-generated action potential waveform, **b** potassium concentration  $[\text{K}]_i$ , **c** calcium concentration  $[\text{Ca}]_i$ , **d** sodium concentration  $[\text{Na}]_i$  and **e** the osmotic pressure  $\pi$  across the cell membrane. These computations used the initial conditions in Table 4



**Fig. 3a, b** Long time simulation showing the membrane potential, Nernst potentials and energies starting with equal intracellular and extracellular concentrations:  $[\text{K}]_i = [\text{K}]_e = 5.4$  mM,  $[\text{Ca}]_i = [\text{Ca}]_e = 2$  mM and  $[\text{Na}]_i = [\text{Na}]_e = 140$  mM. **a** Nernst potential for calcium ( $v_{\text{Ca}}$ ), potassium ( $v_{\text{K}}$ ), sodium ( $v_{\text{Na}}$ ) and membrane potential  $v$ . **b** Work ( $W$ ), potential energy ( $P$ ) and total energy balance ( $W + P$ )

superfluous extra dimension. The initial condition for this extra differential equation cannot be chosen independently of the initial conditions for the conservation [Eqs. (53), (54) and (55)]; if it is, the computed membrane potential will be erroneous. For these reasons, we suggest that our algebraic expression for the membrane potential should replace the standard voltage differential equation in models where intracellular ionic concentrations are dynamic variables.

Our model does not include the funny current ( $i_f$ ), ATP sensitive channels, stretch-activated channels or other ion channels that may be important (Boyett et al. 1996). We also ignored the effect of calcium uptake and release from the sarcoplasmic reticulum, which would affect the Nernst potential of calcium, but not the membrane potential. We have assumed that the ionic channels are governed by a Markov process, that the maximum of the activation/inactivation time constant occurs at the same voltage as the inflection point of the sigmoidal steady state activation/inactivation curve, and that the steady state activation/inactivation curves were temperature independent. Also, we have assumed that the cell volume is constant. While such assumptions reduce the number of parameters in the model, they may also result in discrepancies with experiment.

Finally, we would like to point out that our model is based on experiments where some were conducted at

room temperature (22–24 °C) (Baruscotti et al. 1996; Muramatsu et al. 1996), while others were performed at 37 °C (Shibasaki 1987; Hagiwara et al. 1988; Sakai et al. 1996). It is not clear what effect varying the temperature has in our model as this was not checked out numerically.

The values of the parameters  $k_{Ca}$ ,  $k_{Na}$ ,  $k_K$ ,  $k_{NaK}$  and  $k_{NaCa}$ , given in Table 3, are only an estimate of the actual physiological parameters. We did not systematically study the dynamics of the model for different values of the parameters, but we hope that future experiments will help to discriminate between different parameter sets that all do reproduce the experimentally recorded action potentials. However, interested readers can download the source code for the model from the following internet site: <http://www.phys.ntnu.no/~endresen/san.html>.

## Appendix A: equations of motion

$$v = \frac{FV}{C} \{ [K]_i - [K]_e + 2([Ca]_i - [Ca]_e) + [Na]_i - [Na]_e \} \quad (A1)$$

$$v_K = \frac{kT}{e} \ln \frac{[K]_e}{[K]_i}, \quad v_{Ca} = \frac{kT}{2e} \ln \frac{[Ca]_e}{[Ca]_i}, \quad v_{Na} = \frac{kT}{e} \ln \frac{[Na]_e}{[Na]_i} \quad (A2)$$

$$i_K = k_K x \sinh \left( \frac{v - v_K}{\frac{2kT}{e}} \right) \quad (A3)$$

$$i_{Ca} = [k_{Ca}(1-x)d_\infty + k_{b,Ca}] \sinh \left( \frac{v - v_{Ca}}{\frac{kT}{e}} \right), \quad d_\infty = \frac{1}{2} \left\{ 1 + \tanh \left( \frac{v - v_d}{\frac{kT}{2e}} \right) \right\} \quad (A4)$$

$$i_{Na} = k_{Na} h m_\infty \sinh \left( \frac{v - v_{Na}}{\frac{2kT}{e}} \right), \quad m_\infty = \frac{1}{2} \left\{ 1 + \tanh \left( \frac{v - v_m}{\frac{kT}{2e}} \right) \right\} \quad (A5)$$

$$i_{NaK} = k_{NaK} \tanh \left( \frac{v + 2v_K - 3v_{Na} - v_{ATP}}{\frac{2kT}{e}} \right) \quad (A6)$$

$$i_{NaCa} = k_{NaCa} \sinh \left( \frac{v - 3v_{Na} + 2v_{Ca}}{\frac{2kT}{e}} \right) \quad (A7)$$

$$\frac{d}{dt} [K]_i = \frac{2i_{NaK} - i_K}{FV} \quad (A8)$$

$$\frac{d}{dt} [Ca]_i = \frac{2i_{NaCa} - i_{Ca}}{2FV} \quad (A9)$$

$$\frac{d}{dt} [Na]_i = \frac{-i_{Na} - 3i_{NaK} - 3i_{NaCa}}{FV} \quad (A10)$$

$$\frac{dx}{dt} = \frac{1}{\tau_K} \cosh \left( \frac{v - v_x}{\frac{kT}{2e}} \right) \left\{ \frac{1}{2} \left[ 1 + \tanh \left( \frac{v - v_x}{\frac{kT}{2e}} \right) \right] - x \right\} \quad (A11)$$

$$\frac{dh}{dt} = \frac{1}{\tau_{Na}} \cosh \left( \frac{v - v_h}{\frac{kT}{2e}} \right) \left\{ \frac{1}{2} \left[ 1 - \tanh \left( \frac{v - v_h}{\frac{kT}{2e}} \right) \right] - h \right\} \quad (A12)$$

## Appendix B: checksum equation: $W + P = 0$

$$\frac{dW}{dt} = i_K(v - v_K) + i_{Ca}(v - v_{Ca}) + i_{Na}(v - v_{Na}) + i_{NaCa}(v - 3v_{Na} + 2v_{Ca}) + i_{NaK}(v + 2v_K - 3v_{Na}) \quad (B1)$$

$$P = \frac{1}{2} C v^2 - sT - \pi V \quad (B2)$$

$$s = RV \left\{ [K]_i \ln \left( \frac{[K]_e}{[K]_i} \right) + [Ca]_i \ln \left( \frac{[Ca]_e}{[Ca]_i} \right) + [Na]_i \ln \left( \frac{[Na]_e}{[Na]_i} \right) \right\} \quad (B3)$$

$$\pi = RT \{ [K]_i - [K]_e + [Na]_i - [Na]_e + [Ca]_i - [Ca]_e \} \quad (B4)$$

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