Eur Biophys J (1999) 28: 330–337

ARTICLE

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Steady-state diffusion and the cell resting potential

Received: 6 July 1998 / Revised version: 7 November 1998 / Accepted: 10 November 1998

Abstract The steady-state diffusion of ions through separate, selective channels is described according to irreversible thermodynamics. Ion fluxes thus obtained are the same as those in the parallel conductance model. The equivalent electric circuit set up to describe the system has its electromotive forces expressed by the chemical potentials of the diffusing ions. The expression obtained for the potential differs from the Goldman-Hodgkin-Katz formula, and is reputed to be more accurate. In order for the passive diffusion flows to remain steady, active transport mechanisms must pump the ions up their electrochemical potentials. Such pumps have been incorporated into the equivalent circuit. They supply energy lost in the dissipation caused by preexisting passive forces without affecting the potential, which can thus hardly be called passive diffusion potential. Ion pumps can also create an electric potential in excess of that by passive forces, especially when secondary active transport is involved. The same equivalent circuit is, however, able to describe the whole range of seemingly different situations – from passive diffusion of an electrolyte to active extrusion of anions from the living cell. It has been applied to explain the measured plasma membrane potential of cells, especially those whose potential does not behave as the potassium electrode.

Key words Potential generation · Parallel conductance model · Equivalent circuit · Diffusion potential · Pump potential

Introduction

In many instances the resting potential of living cells is found to be equal or near the Nernst equilibrium potential of a certain ion, mostly the potassium ion. Then one would tend to think that the ion in equilibrium has the largest permeability constant and its intracellular and extracellular concentrations determine the resting potential of the cell.

At times the potential could not be accounted for by the ratio of concentrations of a particular ion, but it was found responsive to ion pump inhibitors. Hence it was concluded that electrogenic ion pumps generated the plasma membrane potential of some animal cells (e.g. Bashford and Pasternak 1984).

There are also many attempts to account for the plasma membrane potential of living cells by saying that it is simply a diffusion potential brought about by all the ions permeating through the membrane. There are claims that the potential could be calculated by using the Goldman-Hodgkin-Katz (GHK) equation if only we knew the permeability constants and concentrations of all the ions involved. Alternatively, the same could be achieved by measuring membrane conductances for all the ions and their equilibrium concentration distributions. Here the so-called parallel conductance model is involved. However, that approach has also had its difficulties, and cannot do without invoking contributions from ion pumps.

In general, the approach has been such that an ion is sought whose distribution could account for the membrane potential when using the Nernst formula. If such an ion cannot be found, the potential is said to be created by several ions diffusing down their electrochemical potentials, i.e. by the diffusion potential. More often than not, both the above interpretations have to ascribe a part of the potential, although usually a small one, to the action of an active transport system (Dawson et al. 1989; Gerard et al. 1994).

Taking into account the multitude of experimental and theoretical works that have appeared on the subject, it seems fair to say that the above-mentioned approach has not been successful. Scrutiny of the current electrophysiological literature leaves us with no understanding of the mechanism which creates the cell membrane potential, in particular of the role of the so-called electrogenic pumps,

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the membrane permeability properties toward the respective ions, and their concentration distribution. This does not mean that there is any lack of seemingly plausible models which are able to reproduce experimental data if only fed with a sufficient number of skillfully chosen parameters (Siegenbeek van Heukelom 1994).

In order to have a sound basis, and for completeness of the description, we start with steady-state diffusion of ions using, first, the method of irreversible thermodynamics and then the equivalent circuit method based also on irreversible thermodynamics. The two methods prove to yield the same result, which is the so-called "parallel conductance model", but with a difference with respect to the model known so far. Also, the simple steady-state difffusion situation has been made more realistic by incorporating ion pumps into the circuit, in the form of pump electromotive forces, in order to keep the diffusion steady.

The equivalent circuit proposed here is able to describe the whole range of seemingly different mechanisms of potential generation: from the simple diffusion potential, where chemical potential differences of the diffusing ions cover energy lost on dissipation; via the Nernst equilibrium potential of one of the ions, where the other ion must be pumped up its chemical potential; up to the genuine pump potential set by the EMF of the pump without involvement of ion diffusion, where the energy on dissipation comes from the affinity of a chemical reaction. This unified description indicates a unique mechanism of potential generation, which is a slight charge separation occurring at the membrane (by the distance of the Debye length $\sim 10^{-9}$ m) due to a chemical potential gradient of the ions diffusing (or staying at thermodynamic equilibrium) or caused by a force originating from the affinity of a pump chemical reaction driven by ATP. In the former case the potential is said to be a diffusion potential (or equilibrium potential) and in the latter is a pump potential. This work shows that the physics in both the cases is the same. The only reason for distinguishing between the active and the passive is the way the energy needed to cover dissipation is supplied.

Besides having a sound basis, which is irreversible thermodynamics, being consistent, and universal, the present approach proves to be effective for interpreting the results of electrophysiological experiments, as is shown at the end of this paper.

Steady-state diffusion: irreversible thermodynamics treatment

The most fundamental and safe way of describing steady flows of matter is to assume that any flux, J_k, can be expressed as a linear function of forces, X_k, in the following way: $J_k = \Sigma L_{kl} X_l$, where the phenomenological coefficients L_{kl} are independent of the forces.

Since the cell membrane is known to have separate channels for the respective ions, the description can be simplified even further by dropping the cross coefficients. Thus we have $J_k = L_k X_k$ for the kth ion, where the force $X_k = -\text{grad}\,\boldsymbol{\mu}_k$, with $\boldsymbol{\mu}_k$ being the electrochemical potential of the kth ion. Integrating the flux equations over the membrane thickness, Δx, we get

$$J_k = -\frac{L_k}{\Delta x}\,\Delta\boldsymbol{\mu}_k \tag{1}$$

Now employing the expression $\boldsymbol{\mu}_k = \mu_k^\circ + RT \ln a_k + z_k F \psi$ for the electrochemical potential of an ion of valency z_k and activity a_k, we obtain the expression:

$$J_k = -\frac{L_k}{\Delta x}\left(RT \ln \frac{a_k''}{a_k'} + z_k F \Delta\psi \right) \tag{2}$$

where $\Delta\psi = \psi'' - \psi'$ is the electrical potential difference between the two sides of the membrane. Hence the following expression results for the electric current, $I_k = z_k F J_k$, carried by the ion:

$$I_k = \frac{L_k (F z_k)^2}{\Delta x}\left(-\Delta\psi - \frac{RT}{z_k F} \ln \frac{a_k''}{a_k'} \right) \tag{3}$$

Since the membrane potential E_m is defined as $E_\mathrm{m} = \psi' - \psi'' = -\Delta\psi$, we finally get

$$I_k = g_k (E_\mathrm{m} - E_k) \tag{4}$$

where $g_k = L_k (F z_k)^2 / \Delta x$ is the conductance and $E_k = (RT/z_k F) \ln (a_k''/a_k') = \Delta\mu_k / z_k F$ is the kth ion's chemical potential difference expressed in volts that contributes to or detracts from the flow caused by the membrane potential E_m. From the condition of global electroneutrality, i.e. $\Sigma I_k = 0$, using Eq. (4) we find that $E_\mathrm{m} = \Sigma g_k E_k / \Sigma g_k$, an expression known as Hodgkin-Horowicz equation.

Equation (4) represents the way of describing the passive diffusion of ions across membranes known as the "parallel conductance model", where the passive diffusion of ions across membranes having separate channels for individual ions is modeled as a set of parallel resistors $R_k = 1/g_k$, through which electric currents, I_k, are driven by the difference of electromotive forces, $E_\mathrm{m} - E_k$.

A steady-state operation of such a system is possible only when no electric current is being transferred across the membrane. Otherwise, there would be an accumulation of charges on both sides of the membrane "which is physically and biologically senseless" (Schoffeniels and Margineanu 1990). In order to satisfy this global electroneutrality condition, we have to consider at least two currents of opposite charges flowing in the same direction or of the same-sign charges flowing in opposite directions. Thus, the minimum circuit to consider is the one in Fig. 1 a.

Equivalent circuit treatment

Now, let us make a new attempt at describing the steady diffusion situation, forgetting about the results of the previous description. To begin with, let us take the simplest

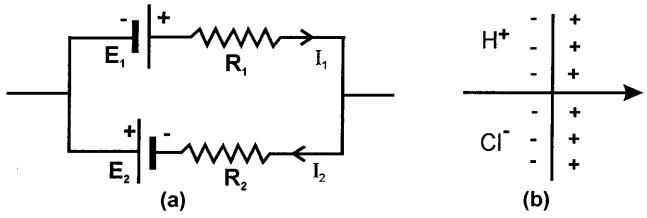

Fig. 1 Equivalent circuit for an electrolyte diffusing downhill its chemical potential (**a**), and the mechanism of potential generation (**b**). Also the equivalent circuit for an electroneutral cation-anion symport, or cation-cation antiport, or anion-anion antiport

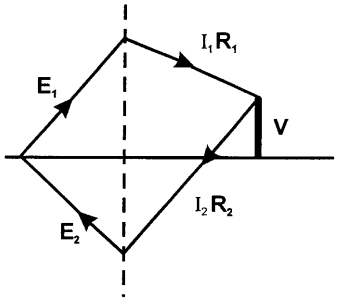

Fig. 2 Electric energy diagram for the equivalent circuit in Fig. 1, with potential difference V across the membrane

situation possible, namely steady-state diffusion of an electrolyte across a membrane permeable to it. If it is an acid, say HCl, protons tend to go ahead of the anions and a potential difference develops as indicated in Fig. 1b. An electrical representation of this situation is the circuit shown in Fig. 1a, where I_1 is the current carried by protons and I_2 that by chloride anions (current of negative charges to the right is the same as current of positive charges to the left).

An energy picture of the circuit is shown in Fig. 2, where V is the voltage that develops on the membrane as a result of the electromotive forces (E_1 and E_2) and potential drops on the resistors. From Fig. 2 we obviously have

$$\begin{aligned} I_1 R_1 = E_1 - V \quad &\Rightarrow \quad I_1 = g_1 (E_1 - V) \\ I_2 R_2 = E_2 + V \quad &\Rightarrow \quad I_2 = g_2 (E_2 + V) \end{aligned} \tag{5}$$

There is, of course, no electric current across the membrane when an electrolyte diffuses across it, so that $I_1 = I_2 = I$, where I is the electric current in the circuit. On substituting expressions (5) into this double equation, we get

$$I = \frac{g_1 g_2}{g_1 + g_2} (E_1 + E_2), \quad V = \frac{g_1 E_1 - g_2 E_2}{g_1 + g_2} \tag{6}$$

Now, we have to identify the electromotive forces E_1 and E_2. For that purpose let us be guided by something tangible and reliable, i.e. the expression for the dissipation function of irreversible thermodynamics: $\Sigma X_k J_k = \sigma T \geq 0$, where $X_k = -\text{grad}\, \mu_k$. This is quite legitimate, since we are describing a passive, spontaneous process, for which the entropy production σ must be positive. For our system in the steady state we consider this means that $(-\Delta\mu_1 J_1 - \Delta\mu_2 J_2) > 0$. Since the two fluxes are positive and downhill the respective electrochemical potentials, both terms in the dissipation function are positive and equal to the work dissipated in each of the resistors:

$$\begin{aligned} -\Delta\mu_1 J_1 &= I_1 (E_1 - V) \\ -\Delta\mu_2 J_2 &= I_2 (E_2 + V) \end{aligned} \tag{7}$$

These equations establish energetic equivalence between ionic diffusion and the electric circuit of Fig. 1a.

Employing now the expression for the electrochemical potential of an ion, $\mu_k = \mu_k^\circ + \mu_k + z_k F \psi$, and putting $F J_1 = I_1$ and $F J_2 = I_2$ into Eqs. (7), we get:

$$\begin{aligned} E_1 &= -\frac{\Delta\mu_1}{F} = \frac{RT}{F} \ln \frac{a'_{\mathrm{H}}}{a''_{\mathrm{H}}} \\ E_2 &= -\frac{\Delta\mu_2}{F} = \frac{RT}{F} \ln \frac{a'_{\mathrm{Cl}}}{a''_{\mathrm{Cl}}} \end{aligned} \tag{8}$$

These expressions together with expressions for the currents [Eqs. (5)] are completely equivalent to Eq. (4) and the respective expressions for E_k, which can be easily verified when noting that $E_{1,2} = -E_k$ and $E_{\mathrm{m}} = -V$.

Comparing with results of the previous section, we see that the straightforward thermodynamic description is fully compatible with that given by the parallel conductance model as represented by the equivalent electric circuit. The two are equivalent both with respect to fluxes and energy. However, this is not the well-known model revisited. An important fact has emerged from our way of treating the subject. Namely, that the electromotive forces E_k are not the Nernst equilibrium potentials of the respective ions, but ion-motive forces established by chemical potential differences of the ions. From the expression $I_k = g_k (E_{\mathrm{m}} - E_k)$ it is seen that $I_k = 0$ when $E_{\mathrm{m}} = E_k$. However, $E_{\mathrm{m}} = -V$, and thus we get $V = -E_k$. This means that the kth ion remains at equilibrium because its motive force E_k is balanced by the membrane potential V, i.e. the two are equal but of opposite sign. Since, by convention, the cell membrane potential $E_{\mathrm{m}} = -V$, the generally practiced identification of the cell equilibrium potential with E_k is technically correct. However, it becomes very awkward and misleading when one tries to understand the physics of membrane potential generation. For this reason it is much better, at least for the purposes of this work, to use the ion-motive forces as defined by Eq. (8).

Besides removing the above-discussed difficulty, the present treatment has the advantage of not making any assumptions (aside of stationary and separate channels for the ions) that could restrict its application scope and/or impair its accuracy.

We can now compare Eq. (6) with the well-known GHK expression for the transmembrane resting potential. For our simple situation of the electrolyte diffusing across a membrane, the GHK equation says that

$$V = \frac{RT}{F} \ln \frac{P_{\mathrm{H}} a'_{\mathrm{H}} + P_{\mathrm{Cl}} a''_{\mathrm{Cl}}}{P_{\mathrm{H}} a''_{\mathrm{H}} + P_{\mathrm{Cl}} a'_{\mathrm{Cl}}} \tag{9}$$

From the parallel conductance model we have [on substituting Eqs. (8) into Eq. (6) for V]:

$$V = \frac{RT}{F} \ln \left[\left(\frac{a'_{\mathrm{H}}}{a''_{\mathrm{H}}} \right)^{\frac{g_1}{g_1+g_2}} \left(\frac{a''_{\mathrm{Cl}}}{a'_{\mathrm{Cl}}} \right)^{\frac{g_2}{g_1+g_2}} \right] \tag{10}$$

The two formulas, (9) and (10), look to be incompatible, and no wonder, for the GHK formula was derived under the assumption of constant electric field and linearity of concentration within the membrane. The two assumptions may impair its accuracy. In fact it was found (Offner 1991) that measured I/V curves depart very far from those predicted by the Goldman equation which was used for deriving the GHK relation. Therefore, the parallel conductance model was considered to be more accurate (Offner 1991), as well as simpler and more representative (Hodgkin and Horowicz 1959; Djamgoz 1987).

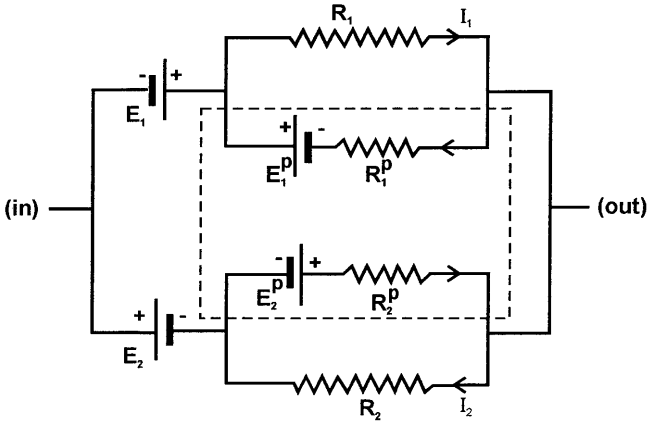

Fig. 3 Equivalent circuit for $Na^+ - K^+$ antiport with electrogenic ion pumps. The elements delimited by the *dashed line* represent the Na,K-ATPase

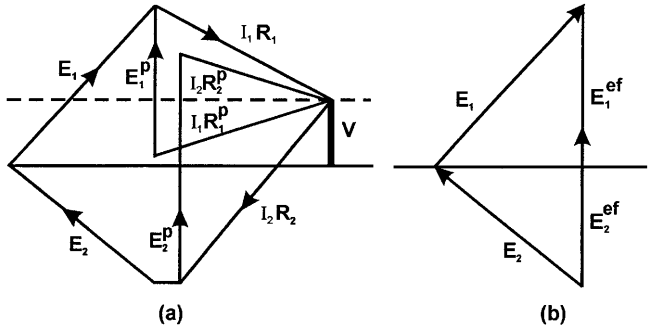

Fig. 4 **a** Electrical energy diagram for the circuit in Fig. 3. The pump EMFs are represented as *vertical jumps* of energy, whereas potential drops in their internal resistors are extended as *inclined segments*. **b** The effective pump EMFs sum up to give the sum of the electromotive forces that drive passive diffusion

Steady-state diffusion with pumps

The equivalent circuit considered so far models electrolyte diffusion, which is always a zero-current flow. In order for it to be applicable to the living cell, the circuit has to represent not only the zero-current but also the zero-substance flow situation. Steady diffusion cannot exist unless there is an agent which restores the ion concentration difference. This can be accomplished either by adding electrolyte to the higher-concentration compartment and removing if from the other compartment, or installing a pump inside the membrane that would return the ions lost by diffusion. Pumps operating at a rate just sufficient to overcome the effect of leakage have been suggested (Djamgoz 1986). So, to be more realistic, we have to include ion pumps, E_1^{p} and E_2^{p}, in the equivalent circuit, as shown in Fig. 3.

It is very helpful to draw the energy profile for the circuit (Fig. 4a), from which it follows that $E_1+E_2=I_1R_1+I_2R_2$, and $E_1^{\mathrm{p}}=I_1(R_1+R_1^{\mathrm{p}})$ and $E_2^{\mathrm{p}}=I_2(R_2+R_2^{\mathrm{p}})$, where E_1^{p} and E_2^{p} are pump EMFs and R_1^{p} and R_2^{p} are internal pump resistors. Because of the resistors, the pump EMFs have to be greater than the effective EMFs, defined as $E_i^{\mathrm{ef}}=E_i^{\mathrm{p}}-I_iR_i^{\mathrm{p}}$, where $i=1$ or 2. In terms of the effective forces, $E_1+E_2=E_1^{\mathrm{ef}}+E_2^{\mathrm{ef}}=E_{\mathrm{p}}^{\mathrm{ef}}$, which is graphically represented in Fig. 4b. The figure and the last equation say that the effective pump EMFs just balance the passive forces E_1 and E_2 set on the membrane by the chemical potential gradients of the diffusing ions. Now, using the last equation and Eqs. (8) for E_1 and E_2, we get for the pump effective EMF:

$$E_{\mathrm{p}}^{\mathrm{ef}} = \frac{RT}{F} \ln \frac{a'_{\mathrm{HCl}}}{a''_{\mathrm{HCl}}} = \frac{-\Delta\mu_{\mathrm{HCl}}}{F} \tag{11}$$

where $a_{\mathrm{HCl}}=a_{\mathrm{H}}a_{\mathrm{Cl}}$ is the activity of the acid. Thus, the effective pump EMF has been expressed by the chemical potential difference of the electrolyte. Hence we conclude that the pump has to work against the chemical potential difference of the electrolyte. In fact, owing to the internal resistance of the pumps, the work done by them is greater than $E_{\mathrm{p}}^{\mathrm{ef}}$ and equal to $E_{\mathrm{p}}=E_{\mathrm{p}}^{\mathrm{ef}}+I_1R_1^{\mathrm{p}}+I_2R_2^{\mathrm{p}}$.

The current I can also be expressed in a consistent way, namely, using Eq. (6) for I we have

$$I = \frac{E_1+E_2}{R_1+R_2} = \frac{E_{\mathrm{p}}^{\mathrm{ef}}}{R_1+R_2} = g_{\mathrm{el}}\,E_{\mathrm{p}}^{\mathrm{ef}} \tag{12}$$

So the current is proportional to the effective pump EMF, with the membrane conductance for electrolyte g_{el} being the proportionality coefficient. This current, however, circulates within the membrane only. The cation and anion fluxes due to passive diffusion of an electrolyte across the membrane are equal to each other for monovalent ions ($J_1=J_2$) and equal to the electrolyte flux $J=I/F$. Using Eq. (12), then Eq. (11), and the relation $g_{\mathrm{el}}=LF^2/\Delta x$ (see above), we obtain

$$J = L\,(-\nabla\mu_{\mathrm{el}}) \tag{13}$$

where L is the thermodynamic phenomenological coefficient and ∇ stands for gradient. Thus the flux of an electrolyte is proportional to the gradient of its chemical potential, as irreversible thermodynamics requires. This flux

is also returned by the pumps, so that effectively the electrolyte does not flow. The steady diffusion potential given by Eq. (10) is, however, created.

Now, let us see whether the presence of pumps affects the membrane potential V given by Eq. (6). To this end we calculate V without using Eq. (6), but operating with the effective pump EMFs: $E_1^{ef}=E_1^p-I_1R_1^p$ and $E_2^{ef}=E_2^p-I_2R_2^p$. Then from Fig. 4a we have $E_1^{ef}=I_1R_1$ and $E_2^{ef}=I_2R_2$. Hence $E_1^{ef}/E_2^{ef}=g_2/g_1$ because $I_1=I_2$. The second equation that we need in order to find that $E_1^{ef}=[g_1/(g_1+g_2)](E_1+E_2)$ is $E_1+E_2=E_1^{ef}+E_2^{ef}$ (see Fig. 4b). E_1^{ef} thus expressed when substituted into the equation $V=E_1-E_1^{ef}$, which follows from Fig. 4a, gives us Eq. (6) for V. Thus, the pumps do not affect the diffusion potential V. Anyway, they have been designed so as to sustain the potential without affecting it.

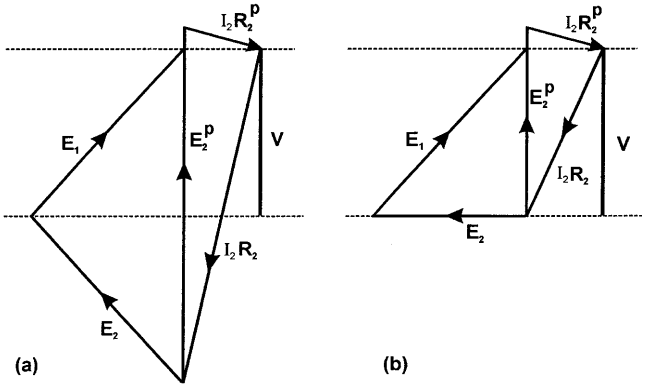

Fig. 5 **a** Electric energy diagram when the first ion is at thermodynamic equilibrium ($E_1=V$) and the second is kept at steady diffusion by a pump's electromotive force E_2^p. **b** When the first ion is at thermodynamic equilibrium and there is no chemical potential difference of the second ion

The Na,K-ATPase

It is worth noting that the equivalent circuit just discussed can model other seemingly different transport situations, such as a redox reaction working in symport with a proton pump, an anion-anion antiport, and a cation-cation antiport.

The last case is well represented by the Na,K-ATPase, known to be responsible for the plasma membrane potential of most animal cells. The current I_1 (see Fig. 3) would then be carried by potassium ions flowing out of a cell, and I_2 would be the current carried by sodium ions flowing into the cell. Both the currents are returned by the pump electromotive forces E_1^p and E_2^p. Thus the part of the circuit delimited by the dashed line in Fig. 3 represents the Na,K-ATPase. Since the currents are returned by the pump, the global electroneutrality condition is automatically satisfied and the condition $I_1=I_2$ can now be dropped, the two currents being coupled by the membrane voltage only. Otherwise they are separate and independent. So the pump stoichiometry may have any value, i.e. also the experimentally found 2:3 value. However, even though 2 potassium ions are exchanged for 3 sodium ions, the pump is not electrogenic in the sense of net charge transfer, because $2K^+$ and $3Na^+$ leak back at the same time. Denoting the pump stoichiometry by r, we have $I_1=rI_2$. Hence, with Eq. (5) for I_1 and I_2, we obtain (Mullins and Noda 1964):

$$V=\frac{g_1E_1-rg_2E_2}{g_1+rg_2} \tag{14}$$

Thus the membrane potential assumes a definite value only when a relation between the two currents, i.e. stoichiometry, is set. The above formula tells us that the potential can have any value from within the interval $V=E_1=E_K$, with $r=0$, and $V=-E_2=-E_{Na}$, with $r=\infty$ ($r=0$ means that potassium is in equilibrium, and $r=\infty$ means that sodium is in equilibrium). This formula gives only a few percent improvement over the one for an electroneutral pump (Eq. 6). So the electrogenity of the pump has little effect on the potential, unless the stoichiometry changes widely. No wonder: because of the leak currents, the electroneutrality of the pump is ensured no matter what the stoichiometry. If it is different from unity, it only means that the turnovers of the two ions are different. We may also note that the potential assumes a zero value when $E_1/E_2=rg_2/g_1$, even though the pump is electrogenic in the sense $r\neq1$.

From the energy profile (Fig. 4a) we see that $E_1-V=I_1R_1$. Hence, $I_1=(E_1-V)/R_1=[-\Delta\mu_1/F-V]/R_1=-\Delta\mu_1/FR_1$; likewise $I_2=-\Delta\mu_2/FR_2$. Thus, the currents are just the passive currents determined by differences (or gradients) of the electrochemical potentials of the respective ions.

It is interesting to see what values the pump EMFs must have in order to keep up the membrane voltage V. Using the energy profile again, we get $E_1^p=E_1-V+I_1R_1^p=-\Delta\mu_1/F-V+I_1R_1^p=-(\Delta\mu_1+FV)/F+I_1R_1^p=-\Delta\mu_1/F+I_1R_1^p$. Likewise, $E_2^p=-\Delta\mu_2/F+I_2R_2^p$. Thus each pump works against the difference of the electrochemical potentials of the ion and the drop of potential on the pump's internal resistance. Combining the above expressions with those for the currents ($I_i=-\Delta\mu_i/FR_i$) we obtain $E_1^p=-I_1(R_1+R_1^p)$ and $E_2^p=-I_2(R_2+R_2^p)$, i.e. the pumps have to supply energy equal to that dissipated on the membrane resistors and their internal resistors, so that they cover dissipation fully, just like the EMFs of galvanic cells.

It is often found that the Na,K-ATPase operates with potassium ions at/or near equilibrium. Let us see how the circuit behaves under such a condition. Putting $\Delta\mu_1=0$, we obtain $V=(RT/F)\ln\{[K^+]^i/[K^+]^o\}=E_K$. The circuit confirms this result. So the membrane potential has to have a definite value, i.e. that given by the Nernst formula for potassium. Obviously, $I_1=\Delta\mu_1/FR_1=0$, and $E_1^p=0$. However, $I_2=-\Delta\mu_2/FR_2=-\Delta\mu_2/FR_2+V/R_2=-\Delta\mu_2/FR_2+E_k/R_2=(E_1+E_2)/R_2$. Hence

$$I_2=\frac{RT}{FR_2}\ln\frac{[Na]^o[K]^i}{[Na]^i[K]^o} \tag{15}$$

Now, substituting the above expression into $E_2^p = I_2(R_2 + R_2^p)$, we obtain

$$E_2^p = \frac{RT}{F} \frac{R_2 + R_2^p}{R_2} \ln \frac{[Na]^o[K]^i}{[Na]^i[K]^o} \quad (16)$$

which, for $R_2^p \ll R_2$, becomes $E_2^p \approx E_{Na} + E_K$. The energy profile then assumes the form in Fig. 5a. Thus, the pump has to work with a definite intensity in order to supply energy dissipated by the sodium ions diffusing down their electrochemical potential, even though the equilibrium distribution of potassium requires no energy to maintain.

The distinction between diffusion potential and pump potential, between passive diffusion and active transport

Under the conditions just described, of equilibrium distribution of potassium, the Na,K-ATPase becomes a sodium pump. It fully covers dissipation on R_2 and R_2^p, which are the membrane resistances for sodium ions. The magnitude of the dissipation ($P = I_2 V \approx V^2/R_2$) depends on the membrane resistance for sodium. For infinitely great resistance the dissipation becomes zero. The pump does not perform work any longer and we get a Donnan equilibrium situation, where one positive ion (potassium) is free to move while another one (sodium) is kept fixed by the membrane. In such a situation a Donnan potential occurs, of magnitude determined by the equation $\Delta\mu_K = 0$, which results in $V = E_1 = \Delta\mu_K/F$ (see Fig. 5a), i.e. the Nernst equilibrium potential for potassium. The transition $R_2 \to \infty$ was accomplished without any change in the membrane potential. Hence the steady state of two ions, one being at equilibrium and the other steadily pumped up its electrochemical potential difference, just to compensate for leakage, is equivalent to the Donnan equilibrium situation as far as membrane potential generation is concerned. This can be generalized for any number of ions kept at steady diffusion by primary active transport mechanisms and one ion left free to traverse the membrane via an open pore channel.

Such a way of generating membrane potentials seems to be common in living cells. Thus, muscle cells and neurons respond to extracellular potassium concentration as a potassium electrode, because no potassium can enter or leave the cell when the fluxes of all the other ions conform to global electroneutrality, i.e. $\Sigma I_i = 0$ except for potassium. An influx or efflux of potassium would make bulk areas of the cell or its surroundings electrically charged, which is, as already indicated, physically and biologically senseless. The tendency of potassium to flow in or out of the cell upon variation of its concentration or channel opening/closing must be balanced by the electric force in the membrane, i.e. changes in the membrane potential. Cells whose potential is not equal to E_k depolarize or hyperpolarize when a potassium ionosphere is applied. A very lucid example of such a behavior can be seen in Bashford and Pasternak (1984), where on addition of valinomycin to a Lettre cell suspension a distinct hyperpolarization is observed.

Figure 5b shows the energy profile for the case of equal concentrations of sodium on either side of the membrane, indicated by the level position of the energy change $E_2 = \Delta\mu_2/F = 0$. The current I_2 that then occurs is solely due to the sodium pump electromotive force E_2^p, although the potential V may be regarded as created by the equilibrium distribution of potassium. Its very existence, however, depends on the operation of the sodium pump, and can thus be called the sodium pump potential. In this case it is evident that three factors participate in the creation of the potential: equilibrium distribution of one cation, electromotive force of a second cation, and its steady-state diffusion. So, the distinction between diffusion potential and pump potential disappears here. No wonder, because the mechanism of electric potential difference creation is always the same, i.e. a slight displacement of positive and negative charges with respect to each other.

Equilibrium distribution of potassium and its concomitant potential, like that of electrons in the case of the contact potential between two metals, cannot sustain any electric current. That is why we may happen to find, especially in textbooks, the assertion that contact potential, or electrode potential, is unmeasurable or even nonexistent.

With a sodium concentration difference present, i.e. $E_2 \neq 0$ (Fig. 5a), the situation is not much different. Only the sodium motive force E_2^p must cover additionally dissipation caused by E_2 and is thus bigger than previously. The potential V is still the sodium pump potential, E_1 and E_2 being then passive elements that play the role of capacitors and voltage dividers only.

When V is smaller than E_1, a flow of potassium develops such that $E_1 - V = I_1 R_1$. That current must be returned by the pump and we have a situation represented by the graph in Fig. 4a and b. The effective $EMF_{eff} = (E_1^p)_{eff} + (E_2^p)_{eff}$, and thus it is now a sodium-potassium pump.

Though the potential can be calculated from formulas applicable to passive diffusion, it can hardly be called diffusion potential because it is maintained by primary active transport. However, this crude way of potential generation seems not to be utilized by the living cell because it is very dissipative.

The active transport of Fig. 5b may be called primary active transport because the sodium motive force ($SMF \equiv E_2^p$) works on sodium dissipation. However, the same scheme can also represent a quite different situation, namely, the SMF can be switched off and E_1, i.e. $\Delta\mu_K/F$, made to work on the transport of sodium ions. This will occur when the permeability of potassium is much bigger than that of sodium ($P_K \gg P_{Na}$). Then potassium will stay at equilibrium and supply energy for sodium ions diffusing down the electric potential V, provided the potassium chemical potential is kept constant by making up for every potassium that has traversed the membrane. The potassium chemical potential difference can of course be replaced by a potassium motive force coupled to a chemical reaction. Then the scheme of Fig. 5b, without E_2^p and R_2^p, represents a galvanic cell that drives positive charges (potassium ions)

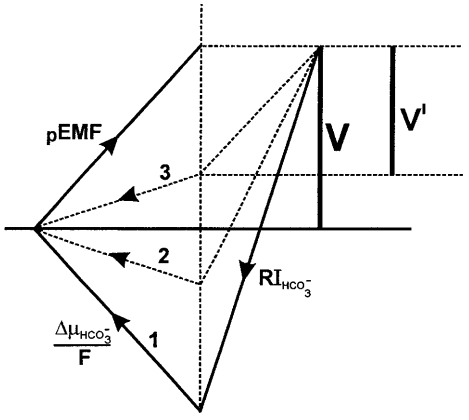

Fig. 6 Energy diagram for secondary active transport of bicarbonate anions, driven by a sodium pump via the proton electromotive force (pEMF = V) and the chemical potential difference of the anions (plots 1 and 2) and solely by pEMF against the chemical potential of the anions (plot 3), with resultant potential V'

up the electric potential and the work thus performed dissipates on the transport of sodium ions. This mode of transport is called secondary active transport, whose main characteristics is that work performed by an active force on one ion is driving the transport of another ion, and net transport of both ions occurs, subject to the electroneutrality condition $I_1 = I_2$.

The assumption $E_2 = 0$, meaning that there is no sodium concentration difference, was made just to make the situation more lucid and in closer semblance to the galvanic cell, and can now be lifted. Higher sodium concentration on the right side favors its transport adding up to E_1^{p}, as seen in the scheme of Fig. 5a. For secondary active transport we thus get the electric energy profile which is the same as that for passive diffusion antiport of ions of the same sign and symport of ions of opposite signs (Figs. 1, 2).

Passive diffusion of an electrolyte may, indeed, realize a secondary active transport. A close look at diffusion of, say, H_2CO_3 should help us accept this apparently wrong statement. As we saw earlier, such diffusion is adequately described by the equivalent circuit of Fig. 1 and the electric energy profile of Fig. 2. The profile shows that the chemical potential difference of protons, $E_1 = \Delta\mu_{\mathrm{H}}/F$, covers dissipation by the diffusing protons ($I_1 R_1$) and partly dissipation by anions (i.e. that part driven by V). The rest of the anion's dissipation is covered by E_2. So this is a case of partial secondary active transport of the anions. Assuming now that the permeability constant $P_{\mathrm{H}} \gg P_{\mathrm{HCO_3^-}}$, we get a situation where protons are at equilibrium (almost) while anions are driven by the potential V created by protons only. However, E_1 can be replaced by a proton EMF and $\Delta\mu_{\mathrm{H}}$ assumed equal zero, while $\Delta\mu_{\mathrm{HCO_3^-}} \neq 0$, provided other ions are present. Such a situation can be represented by the energy diagram of Fig. 6, to be used in the last s ection.

Thus the same membrane potential V can be generated by either passive or active forces, or both occurring simultaneously, and the distinction between diffusion potential and pump potential seems to fade away. The only distinction left is the source of the energy needed to cover dissipation. When dissipation is covered by energy coming from the chemical potential difference of an ion (at equilibrium or not), the potential may be called a diffusion (or equilibrium) potential. It is accompanied by net substance transport and zero electric charge transport. The transport of the counterion may then be, partly or totally, what is called secondary active transport. When the energy needed to move ions up the electric potential (which could already be created by passive forces) comes from a chemical reaction directly, a primary active transport can be accomplished, with zero net flux of the working ion and zero charge flow. The potential thus maintained may be called the pump potential.

The general conclusion that emerges from the aforementioned observations is that there is no difference between the physics of the so-called active and passive phenomena. Membrane potential created in the process of passive diffusion (or at equilibrium) of ions can be identified with the so-called pump potential. Passive diffusion of an electrolyte, or an ion-ion antiport, effects what is a kind of secondary active transport. The only difference between the passive and active is in the way of supplying energy to cover dissipation associated with transport processes.

Application

Before applying the presented description of membrane potential generation for the interpretation of electrophysiological experiments, we have to make allowance for yet another device, besides the primary and secondary active transport, that the living cell operates in order to control its electric potential. That device is the electroneutral ion exchange. As indicated before, the straightforward way of potential generation with the use of the diffusion potential mechanism represented by the circuit in Fig. 3, and its energy diagram in Fig. 4, would be very dissipative. The asymmetrical distribution of sodium and potassium across the plasma membrane, which is necessary to ensure osmotic balance, would be a great burden on the Na,K-ATPase, with small or even zero electric potentials when the permeabilities of the two ions are close to each other. One way out of the problem is to have one ion at equilibrium and the other with very little permeability. Such a solution is indeed commonly found in muscle and nerve cells, and was briefly discussed in the beginning of the previous section. There are, however, other classes of cells (e.g., neutrophils and Lettre cells) where potassium is not at equilibrium, and where a sodium-potassium exchange mechanism is found to operate. This mechanism provides an energetic relief for the pump, allowing the ions pumped to return back without dissipating energy in friction. Thus much of the return current is nondissipative and in our equivalent circuit.

According to the energy diagram in Fig. 4a the SMF has to balance chemical and electrical forces, i.e. SMF=

$E_2^p = E_2 + V = \Delta\mu_{Na}/F + V$ (neglecting the pump's internal resistance R_2^p). This equation allows us to explain the following experimental finding on Lettre cells (Bashford and Pasternak 1984): addition of nigericin, an ionophore that catalyzes 1:1 sodium-potassium exchange in excess of the native one, causes the ions' concentration gradients to level off and even to reverse, and is accompanied by a hyperpolarization that follows the intracellular sodium concentration. Since the SMF, like the EMF of a galvanic cell, is constant, diminishing $\Delta\mu_{Na}$ must result in increasing V according to the equation $SMF = V + \Delta\mu_{Na}/F$.

As already indicated, owing to the native sodium-potassium exchange, the sodium pump can perform electric work only. That work can be spent on a useful purpose, which could be the extrusion of organic anions. In the case of Lettre cells these are bicarbonate and lactate. The transport of these ions can be coupled to sodium transport only electrically. The pump is charging the membrane capacitor while bicarbonate anions are discharging it. Of the 3:2 stoichiometry of the Na,K-ATPase, it is enough to consider the one odd sodium only as doing electric work, since the other two sodiums are balanced by the backflow of two potassiums. The unbalanced sodium ion is driven by the SMF up the electric potential while another sodium is driven in opposite direction by the electric potential, transmitting its energy to a proton in a frictionless $Na^+ - H^+$ exchange. This is as proposed by Bashford and Pasternak (1985) for potential generation in Lettre cells. Thus the electric part of the sodium pump becomes effectively a proton pump, its EMF (pEMF) being equal to $V = SMF - \Delta\mu_{Na}/F$ (see the start of the second paragraph of this section). The sodium-driven proton flux is then coupled to anion transport by the secondary active transport mechanism introduced previously. Its energy diagram is shown in Fig. 6, where $pEMF = V$ and the former $E_2 = \Delta\mu_{HCO_3^-}/F$. This mechanism operates at the thermodynamic equilibrium of the active forces, exactly like in the case of the galvanic cell, hence the equality $pEMF = V$. When the HCO_3^- concentration distribution is like that in the normal diffusion of carbonic acid, i.e. in accord with H^+ concentration, the electric energy profile in Fig. 6 is given by dashed lines 1 and 2, indicating that no change in membrane potential occurs up to when equal concentrations of bicarbonate are on both sides. Going over to reversed bicarbonate concentrations gradients, i.e. extracellular greater than intracellular, we get diminishing potentials (curve 3 and V'). Ultimately, with the opposite bicarbonate gradient just balanced by the electromotive force pEMF, we get zero value for the potential. This prediction is in line with the experimental results (Bashford and Pasternak 1984), where at concentrations above 15 mM both lactate and bicarbonate depolarize cells by 60 mV per decade.

The scheme of Fig. 6 becomes easy to understand if we recall the mechanism of secondary active transport explained in terms of the equivalent circuit in the previous section. The pEMF transmits energy from the SMF of ATPase to bicarbonate ions diffusing out of cell up the membrane electric potential. However, the potential as such cannot perform work on the anion unless the ion is accompanied by a cation pulled up the electric potential by an electromotive force (ultimately by the SMF). As long as the chemical potential gradient of the anion favors its movement (graph 1 in Fig. 6), the electric work covers the anion's dissipation only partially. With $\Delta\mu_{HCO_3^-} = 0$, bicarbonate extrusion is due to electric force only. When a reversed bicarbonate gradient is present, it generates an electric potential of its own, which opposes the pEMF; hence the membrane potential V decreases, becoming zero when the two potentials balance each other. The membrane potential $V' < V = pEMF$ is no longer set by the Na,K-ATPase alone, and this must result in disturbance of the operation of the sodium pump. With constant SMF and decreased V there must be an additional drop of potential on the pump internal resistance, which means increased dissipation inside the pump.

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