J. Membrane Biol. 176, 41–52 (2000)
DOI: 10.1007/s00232001074

The Journal of
Membrane
Biology

Electrogenic Properties of the Sodium Pump in a Dynamic Model of Membrane Transport

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Received: 8 September 1999/Revised: 21 March 2000

Abstract. The general purpose of this theoretical work is to contribute to understand the physiological role of the electrogenic properties of the sodium pump, by studying a dynamic model that integrates diverse processes of ionic and water transport across the plasma membrane. For this purpose, we employ a mathematical model that describes the rate of change of the intracellular concentrations of Na^+, K^+ and Cl^-, of the cell volume, and of the plasma membrane potential (V_m). We consider the case of a nonexcitable, nonpolarized cell expressing the sodium pump; Na^+, K^+, Cl^- and water channels, and cotransporters of KCl and NaCl in its plasma membrane. We particularly analyze here the conditions under which the physiological V_m can be generated in a predominantly electrogenic fashion, as a result of the activity of the sodium pump. A major conclusion of this study is that, for the cell model considered, a low potassium permeability is not a sufficient condition for a predominantly electrogenic generation of the V_m by the sodium pump. The presence of an electroneutral exchange of Na^+ and K^+ represents a necessary additional requirement.

Key words: Sodium pump — Electrogenic enzymes — Membrane potential — Mathematical models

Introduction

Electrogenic enzymes contribute to the electrical potential difference across a biological membrane by employing a source of free energy to generate a net ionic current. The majority of the ion ATPases of the plasma membranes of cells belonging to the entire biological world constitute examples of this type of enzyme (Läuger, 1991). As with other dynamic properties of cells, the generation of an electrical potential difference across a biological membrane results from the interaction of several passive and active processes of ionic transport (Schultz, 1980; Jakobsson, 1980; Byrne & Schultz, 1988). These dynamic phenomena are inherently complex, as a consequence of the fact that the transport of ions across the cell membrane affects not only the intracellular concentration of the particular ionic species involved, but also the cell volume (via effects on the intracellular osmolarity) and the membrane electrical potential difference (via the generation of diffusive and/or electrogenic potentials) (Tosteson & Hoffman, 1960; Jakobsson, 1980). Thus, the theoretical study of the mechanisms involved in the generation of the membrane potential requires models that integrate diverse processes of membrane transport. These processes simultaneously affect other cellular variables, like the cell volume and the intracellular ionic concentrations. Under steady-state conditions, the potential difference across the plasma membrane (V_m) can be approximated by formulations of the diffusive and electrogenic components only (Goldman, 1943; Hodgkin & Katz, 1949; Mullins & Noda, 1963; Moreton, 1969; Jacquez, 1971; Jacob et al., 1984; Sjodin, 1984; Hernández, Fischbarg & Liebovitch, 1989; Borst-Pauwels, 1993; Kabakov, 1994). However, the study of the temporal behavior requires systems consisting of several nonlinear differential equations governing the rate of change of the membrane potential, of the cell volume and of some intracellular ionic concentrations. Several dynamic models have considered the direct (electrogenic) and/or indirect (via the generation of electrochemical ionic gradients) participation of ion pumps in the generation of the membrane potential (e.g., Jakob-

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sson, 1980; Scriven, 1981; Lemieux, Roberge & Savard, 1990; Lemieux, Roberge & Joly, 1992; Hernández & Cristina, 1998; Movileanu et al., 1998). In view of their complexity (Jakobsson, 1980; Hernández & Cristina, 1998), the study of the models describing dynamic cellular phenomena dependent on ionic membrane transport is generally restricted to numerical simulations of their transient and/or stationary behavior.

The Na^+-K^+ ATPase of the plasma membrane of animal cells is an electrogenic enzyme, as was demonstrated in diverse cellular types (Thomas, 1972; De Weer & Geduldig, 1978; Sjodin, 1984; Bashford & Pasternak, 1985; Ishida & Chused, 1993; Rakowski et al., 1997*a*). Unlike other ion ATPases exhibiting a strong electrogenic contribution, like the proton pump of *Neurospora crassa* (Slayman, 1987) or the chloride pump of *Acetabularia* (Gradmann, Tittor & Goldfarb, 1982), it has classically been accepted that in most animal cells the direct electrical contribution of the sodium pump to the membrane potential is generally small, representing less than 10% of the total V_m (Sjodin, 1984). By far, in these "typical" animal cells the largest contribution is that of the potassium ion, due to its dominant permeability under resting conditions. However, some cells might be able to create, mainly by means of the electrogenic activity of the sodium pump, a plasma membrane potential similar in magnitude and sign to that resulting from parallel potassium and sodium electrodiffusion (Bashford & Pasternak, 1985; Ishida & Chused, 1993). In general, this type of cell exhibits significantly low potassium permeability. Moreover, it has been suggested that these cells are also characterized by an important activity of co- or countertransport systems determining net electroneutral ionic exchange [e.g., Na^+-H^+ exchange (Bashford & Pasternak, 1986)]. The majority of the models developed to study the electrogenic contribution of the sodium pump assumes the conditions characterizing the typical cell, embodied with a relatively high potassium permeability (Mullins & Noda, 1963; Moreton, 1969; Jacquez, 1971; Jacob et al., 1984; Sjodin, 1984; Hernández et al., 1989; Borst-Pauwels, 1993; Kabakov, 1994). Also, most of these models have employed phenomenological formulations of the sodium pump activity. In previous work (Hernández et al., 1989) we developed a model for the study of the steady-state properties of the plasma membrane potential that incorporated the sodium pump in the form of a kinetic diagram (Chapman, Johnson & Kootsey, 1983). This kinetic scheme has also been employed, for instance, to analyze the role of the enzyme in the repolarization of cardiac cells (Lemieux et al., 1990) and in the regulation of the cell volume (Hernández & Cristina, 1998). The analysis of an integrated dynamic model of membrane transport incorporating explicit kinetic schemes of the sodium pump, the participation of ionic cotransport systems and the condition of a low potassium permeability could represent an important contribution to the theoretical interpretation of the mechanisms of generation of the resting membrane potential.

The general purpose of this work is to contribute to the understanding of the physiological role of the electrogenic properties of the sodium pump, by studying a dynamic model that integrates diverse processes of ionic and water transport across the plasma membrane. Of particular interest is to comprehend the biophysical basis of the generation of a physiological V_m in a predominantly electrogenic fashion, as a result of the activity of the sodium pump. For this purpose, we develop and study here a mathematical model that describes the rate of change of the intracellular concentrations of Na^+, K^+ and Cl^-, of the cell volume and of the electrical potential difference across the plasma membrane. This model and the numerical methodology used here are essentially similar to the ones previously developed by us to analyze the role of the sodium pump in cell volume regulation (Hernández & Cristina, 1998). In essence the model assumes that, in its plasma membrane, a nonexcitable cell expresses the sodium pump; Na^+, K^+, Cl^- and water channels, and systems mediating K:Cl and Na:Cl cotransport processes. The sodium pump is incorporated via an explicit kinetic diagram [a modification of the one analyzed by Chapman et al. (1983)]; the uncoupled ionic fluxes of Na^+, K^+ and Cl^- via classical electrodiffusion expressions (Goldman, 1943; Hodgkin & Katz, 1949). As a major difference with the previous work (Hernández & Cristina, 1998), in this study the K:Cl and Na:Cl cotransporters can only function under basal conditions. We do not consider here the existence of short-term cell volume regulation (Baumgarten & Feher, 1995) mediated, for instance, by the enhanced activity of the abovementioned cotransport processes, triggered by cell volume modifications (Hernández & Cristina, 1998). The inclusion of this or other concurring cell processes would represent an additional source of complexity, not desirable for the basic purpose of this study. One question of physiological interest addressed by this work is whether a cell can maintain similar macroscopic properties (e.g., similar intracellular ionic concentrations, cell volume and V_m) in the presence of different mechanisms of generation of the V_m (e.g., predominantly electrogenic or predominantly electrodiffusional). Among other conclusions, the analysis performed here permits us to ascertain that, for the cell model studied, the simultaneous presence of an electroneutral exchange of Na^+ and K^+ and of a low potassium permeability constitute the basic requirements for a predominantly electrogenic generation of the V_m by the sodium pump.

Mathematical Model

The cell model employed here to derive the mathematical model has similar general characteristics to the ones

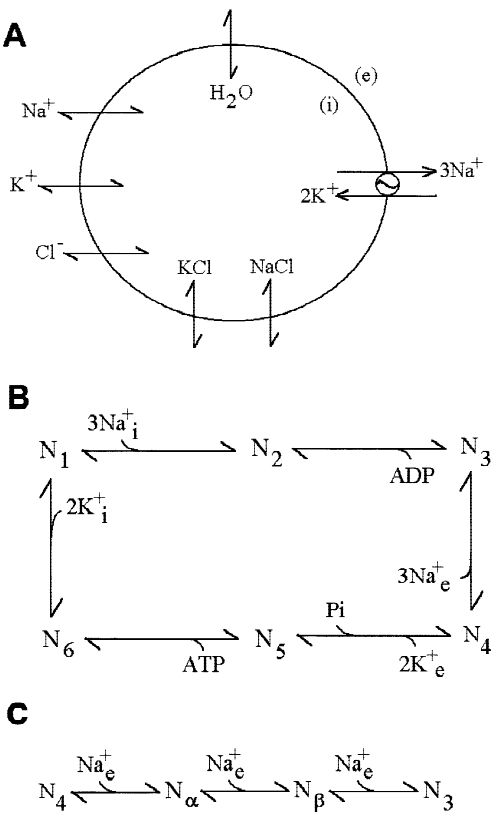

Fig. 1. (*A*) Cell scheme representing the diverse fluxes taking place across the plasma membrane. The scheme includes a NaK ATPase with a fixed $3Na^+/2K^+$ stoichiometric ratio; diffuse paths for Na^+, K^+, Cl^- and water, and NaCl and KCl symport-mediated electroneutral fluxes. (*B*) State diagram of the transport of Na^+ and K^+ mediated by the NaK ATPase [after Chapman et al. (1983)]. $N_1 \ldots N_6$ are the intermediate states of the enzyme. ATP, ADP and P_i represent adenosine triphosphate, adenosine diphosphate and inorganic phosphate, respectively. Na^+_i, K^+_i, Na^+_e and K^+_e are intracellular and extracellular Na^+ and K^+, respectively. (*C*) The elementary steps comprising transition $N_3 \leftrightarrow N_4$, representing the successive binding of the three external sodium ions to the sodium pump. N_α and N_β are arbitrary notations to indicate the intermediate pump states. Following Wuddel & Apell (1995), the rate constants governing the corresponding intermediate steps are g_{3f}, g_{2f}, g_{1f} and g_{1b}, g_{2b}, g_{3b} in the $N_3 \rightarrow N_4$ and $N_4 \rightarrow N_3$ directions, respectively.

assumed in our previous work (Hernández & Cristina, 1998). Basically, we consider a nonpolarized, nonexcitable cell expressing the sodium pump; Na^+, K^+, Cl^- and water channels, and K:Cl and Na:Cl cotransporters in its plasma membrane (*see* Fig. 1*A*; *see also* Introduction). The changes in the cell volume (V_c) are determined by the net water movement between the extracellular and intracellular compartments, as a response to the intracellular osmolarity changes. The total solute concentration of the extracellular compartment remains constant, at an isosmotic value. The cell contains a fixed amount of an impermeant anion (X_i) which, for simplicity, we consider to be monovalent. We assume that the total intracellular osmolarity is given by the sum of the concentrations of Na^+, K^+, Cl^- and of the impermeant species; we also assume ideal osmotic behavior. The sodium pump is represented by the kinetic scheme developed by Chapman et al. (1983) (Fig. 1*B*), in turn a simplification of the classical Post-Albers scheme (Läuger, 1991; Wuddel & Apell, 1995). The dynamic model studied here incorporates the steady-state fluxes of Na^+ and K^+ mediated by the enzyme, as derived from the analysis of the explicit kinetic model shown in Fig. 1*B* (Appendix). For this study, the voltage-dependent step as well as the numerical values employed for the rate constants, have been modified in agreement with more recent evidence (Wuddel & Apell, 1995; Rakowski, Gadsby & De Weer, 1997*b*) (Fig. 1*C*, Appendix). As mentioned, the plasma membrane also contains systems determining net transport of KCl (K^+:Cl^- symport) and of NaCl [a combination of the K^+:Cl^- and of the Na^+:K^+:$2Cl^-$ symports (Baumgarten & Feher, 1995)]. In our previous study (Hernández & Cristina, 1998) these transport processes were considered to remain inactive under basal conditions and were only dramatically triggered to large levels of activation as a result of sudden cell volume changes determined by anisosmotic shocks. As a major difference with that work, in the present study the K:Cl and/or Na:Cl cotransport systems may remain active only at basal values. Also, for this study, the model does not include processes (e.g., the K:Cl and Na:Cl cotransporters, or other mechanisms) mediating short-term cell volume regulation (Baumgarten & Feher, 1995). Finally, we assumed that the total membrane area available for solute and water transport (A_c) remains constant, and independent of the cell volume changes.

Under these assumptions, the following mathematical model governs the rate of change of the cell volume (V_c), of the intracellular amounts (e.g., in moles) of Na^+, K^+ and Cl^- (n_{Na}, n_K and n_{Cl}), and of the electrical potential difference across the plasma membrane (V_m, defined as $V_m = V_{intracellular} - V_{extracellular}$):

$$\begin{aligned} dn_{Na}/dt &= A_c\,(-3J_p + J_{Na} + J_{NaCl}) \\ dn_K/dt &= A_c\,(2J_p + J_K + J_{KCl}) \\ dn_{Cl}/dt &= A_c\,(J_{Cl} + J_{NaCl} + J_{KCl}) \\ dV_c/dt &= (A_c V_w P_w)[(X_i + n_{Na} + n_K + n_{Cl})/V_c - \Pi_e] \end{aligned} \tag{1a}$$

In order to obtain the time dependence of V_m, we employed a stationary solution of the electroneutral condition:

$$-J_p + J_{Na} + J_K - J_{Cl} = 0. \tag{1b}$$

In Eqs. (1a) and (1b), J_p is the cycle flux determined by the pump [Eq. (A1)]; J_{Na}, J_K and J_{Cl} are the corresponding electrodiffusive fluxes, and J_{NaCl} and J_{KCl} are the symport-mediated fluxes of NaCl and KCl respectively. V_w is the partial molar volume of water, the rest of the

Table 1. Glossary of symbols

(I) Variables
- V_c: cell volume
- n_{Na}, n_K, n_{Cl}: intracellular amounts of Na^+, K^+ and Cl^-
- V_m: electrical potential difference across the cell membrane
- t: time

(II) Parameters
- A_c: effective permeant area of the cell surface
- P_{Na}, P_K, P_{Cl}: permeability coefficients of Na^+, K^+ and Cl^-
- P_W: osmotic permeability
- Q_{NaCl}, Q_{KCl}: kinetic parameters of the symport-mediated transports of NaCl and KCl
- θ: time constant of parameter modifications
- $(Na^+)_e$, $(K^+)_e$, $(Cl^-)_e$, $(X)_e$: extracellular concentations of Na^+, K^+, Cl^-
- $(X)_e$: extracellular concentration of impermeant solute
- Π_e: total extracellular solute concentration
- X_i: total amount of intracellular impermeant solute
- N: total NaK ATPase membrane density
- (ATP), (ADP), (P_i): cellular concentrations of ATP, ADP and inorganic phosphate
- $k_{12}, \ldots, k_{61}$: rate constants of transitions 12, . . . , 61
- $k_{16}, \ldots, k_{21}$: rate constants of transitions 16, . . . , 21
- K_{eq}: equilibrium (dissociation) constant of the reaction ATP ↔ ADP + P_i

symbols represent the parameters and variables listed in Table 1.

The electrodiffusive fluxes of N^+, K^+ and Cl^- are given by the modified Goldman expression (Goldman, 1943; Hodgkin & Katz, 1949):

$$J_{Na} = P_{Na}\,\varepsilon_m\,[(Na^+)_e \exp(-u/2) - (n_{Na}/V_c)\exp(u/2)]$$

$$J_K = P_K\,\varepsilon_m\,[(K^+)_e \exp(-u/2) - (n_K/V_c)\exp(u/2)] \quad (2)$$

$$J_{Cl} = P_{Cl}\,\varepsilon_m\,[(Cl^-)_e \exp(u/2) - (n_{Cl}/V_c)\exp(-u/2)],$$

where $u = F\,V_m/(R\,T)$ and $\varepsilon_m = u/[\exp(u/2) - \exp(-u/2)]$, and where F is Faraday's constant, R the gas constant, T the absolute temperature (310 kelvin), and P_i the ionic permeability coefficient ($i = Na^+, K^+, Cl^-$).

We assumed that the symport-mediated fluxes of KCl and NaCl are given by

$$J_{NaCl} = Q_{NaCl}\,[(Na^+)_e\,(Cl^-)_e - (n_{Na}\,n_{Cl}/V_c^{\,2})]$$
$$J_{KCl} = Q_{KCl}\,[(K^+)_e\,(Cl^-)_e - (n_K\,n_{Cl}/V_c^{\,2})], \quad (3)$$

where Q_{NaCl} and Q_{KCl} are characteristic parameters (Table 1).

In agreement with its main specific objective (*see* Introduction), this theoretical study is not intended to be applied to any specific cell type. In the following section we perform some numerical studies of the model, intended to illustrate some basic stationary and dynamic properties of cells following the general design described above (*see* Fig. 1*A*).

Results and Discussion

Numerical Methods

The methods employed in this work are similar to those in our previous work (Hernández & Cristina, 1998), and are summarized here. To perform the simulations, Eqs. (1) were integrated numerically employing the Runge-Kutta fourth order method, except for the determination of V_m. After each integration time step, we determined V_m assuming Eq. (1b), as a solution of the transcendental equation (Hernández et al., 1989; Hernández & Cristina, 1998):

$$V_m = (RT/F)\,ln\,[(\kappa + \lambda)/(\mu + \omega)] \quad (4)$$

In this equation, the functions $\kappa = \kappa(V_m)$, $\lambda = \lambda(V_m)$, $\mu = \mu(V_m)$ and $\omega = \omega(V_m)$ are given by

$$\kappa = [P_{Na}\,(Na^+)_e + P_K\,(K^+)_e + P_{Cl}\,(n_{Cl}/V_c)]\varepsilon_m$$

$$\omega = (N/\Sigma)\,a_{12}\,a_{23}\,a_{34^\circ}\,a_{45^\circ}\,a_{56^\circ}\,a_{61}$$

$$\mu = [P_{Na}\,(n_{Na}/V_c) + P_K\,(n_K/V_c) + P_{Cl}\,(Cl^-)_e]\varepsilon_m$$

$$\lambda = (N/\Sigma)\,a_{21}\,a_{32}\,a_{43^\circ}\,a_{54^\circ}\,a_{65^\circ}\,a_{16}$$

with ϵ_m defined by Eqs. (2) and with Σ and the a_{ij}s defined in the Appendix.

Similarly, the steady-state values of the variables were determined by the iterative procedure employed previously (Hernández & Cristina, 1998).

Within the context of this work we considered that the "electrogenic contribution" of the sodium pump is given by the difference between the actual membrane potential V_m [Eq. (4)] and the corresponding diffusion potential V_m^{diff}:

$$V_m^{diff} = (RT/F)\,ln\,(\kappa/\mu) \quad (5)$$

Equation (5), obtained from Eq. (4) by setting $N = 0$, is actually the Goldman-Hodgkin-Katz (Goldman, 1943; Hodgkin & Katz, 1949) explicit equation for the diffusion potential.

In every run, as a control test, the simulation program checked the simultaneous satisfaction of the conditions of osmotic equilibrium and of macroscopic electroneutrality:

$$(X_i + n_{Na} + n_K + n_{Cl})/V_c = \Pi_e \quad \text{and} \quad X_i + n_{Cl} = n_{Na} + n_K. \quad (6)$$

To perform the dynamic studies, transient responses of the model were produced by perturbing some of the

Table 2. Numerical values of the parameters

A_c:	5×10^{-6} cm^2
P_{Na}, P_K, P_{Cl}:	7×10^{-8}, 7×10^{-7}, 10^{-6} cm sec^{-1} (Cell 0 and Cell 1) 2×10^{-8}, 3×10^{-8}, 2×10^{-8} cm sec^{-1} (Cell 2 and Cell 3)
P_w:	1.5×10^{-2} cm sec^{-1}
Q_{NaCl}, Q_{KCl}:	0, 0 (Cell 0 and Cell 3) 7×10^{-4}, 5×10^{-3} cm^4 mol^{-1} sec^{-1} (Cell 1 and Cell 2)
θ:	14 sec
$(Na^+)_e$, $(K^+)_e$, $(Cl^-)_e$, $(X)_e$:	1.4×10^{-4}, 10^{-5}, 1.4×10^{-4}, 10^{-5} mol cm^{-3}
Π_e:	3×10^{-4} mol cm^{-3}
X_i:	1.3×10^{-13} mol
N:	1.25×10^{-13} mol cm^{-2}
(ATP), (ADP), (P_i):	5×10^{-6}, 6×10^{-8}, 4.95×10^{-6} mol cm^{-3}
k_{12} : 2.5×10^{11} mol^{-3} lt^3 sec^{-1};	k_{21} : 424563 sec^{-1} (*)
k_{23} : 10^4 sec^{-1}	; k_{32} : 10^5 mol^{-1} lt sec^{-1}
$k_{34°}$: 360 sec^{-1}	; $k_{43°}$: 8.5×10^3 mol^{-3} lt^3 sec^{-1}
k_{45} : 1.5×10^7 mol^{-2} lt^2 sec^{-1} ;	k_{54} : 2×10^5 mol^{-1} lt sec^{-1}
k_{56} : 2×10^6 mol^{-1} lt sec^{-1}	; k_{65} : 30 sec^{-1}
k_{61} : 1.15×10^4 sec^{-1}	; k_{16} : 6×10^8 mol^{-2} lt^2 sec^{-1}
K_{eq} : 239000 mol lt^{-1}	

(*) Determined from the detailed balance condition [Eq. (A4)].

characteristic parameters (Table 1). These parameter modifications may or may not have an actual experimental counterpart; as commented above, the purpose is not to mimic particular behaviors but to understand basic properties. We assumed that any modification (activation or inhibition) of the ionic fluxes mediated by the sodium pump and by the cotransporters followed a time course given by

$$Y(t) = Y_\infty[1 - \exp(-t/\theta)] + Y_0 \exp(-t/\theta), \quad (7)$$

where Y_0 and Y_∞ are the initial and final values of the modified parameter respectively, and θ is the time constant. Thus, for the case of the sodium pump, $Y = N$ (pump density); for the case of the Na:Cl and K:Cl cotransporters, $Y = Q_{NaCl}$ and $Y = Q_{KCl}$, respectively.

Throughout this study, the dependent variables were plotted as the absolute values of V_m, V_c and the corresponding ionic concentrations, $(Na^+)_i = n_{Na}/V_c$, $(K^+)_i = n_K/V_c$, and $(Cl^-)_i = n_{Cl}/V_c$.

Reference State

We determined reference states for four different cases (Cells 0, 1, 2 and 3, Table 2). In Cells 0 and 3 the systems mediating the K:Cl and Na:Cl cotransport processes remain inactive (that is, $Q_{NaCl} = Q_{KCl} = 0$), in Cells 1 and 2 they exhibit their basal activities. Cells 0 and 1 exhibit larger ionic permeabilities than Cells 2 and 3. Unless specified, the numerical values of the parameters employed for the simulations were the ones shown in Table 2. They mostly correspond to the ones employed in our previous work (Hernández & Cristina, 1998), except for Q_{NaCl}, Q_{KCl}, the ionic permeabilities (for the cases of Cells 2 and 3), and for X_i, that was estimated here for the case of a smaller cell [e.g., for a cell with an average volume of 10^{-9} cm^3 (Jakobsson, 1980; Hernández & Cristina, 1998)]. The membrane area available for transport (A_c) has also been modified accordingly. The majority of the values listed in Table 2 have an experimental basis (Hernández & Cristina, 1998), others (e.g., Q_{NaCl}, Q_{KCl} and θ) were heuristically determined in order to obtain a plausible behavior of the model. The values for the rate constants of the enzymatic reaction were taken from Chapman et al. (1983) and from Wuddel & Apell (1995) (*see* Appendix).

For the parameter values listed in Table 2, the reference cellular states are determined by the corresponding steady-state values of the variables:

Cell 0: $V_c(0)$: 1.00×10^{-9} cm^3; V_m (0): -5.45×10^{-2} V; V_m^{diff} (0): -5.17×10^{-2} V; $(Na^+)_i$ (0), $(K^+)_i$ (0), $(Cl^-)_i$ (0): 2.2×10^{-5}, 1.27×10^{-4}, 1.8×10^{-5} mol cm^{-3}

Cell 1: V_c (0): 1.04×10^{-9} cm^3; V_m (0): -5.17×10^{-2} V; V_m^{diff} (0): -4.71×10^{-2} V; $(Na^+)_i$ (0), $(K^+)_i$ (0), $(Cl^-)_i$ (0): 3.1×10^{-5}, 1.18×10^{-4}, 2.5×10^{-5} mol cm^{-3}

Cell 2: V_c (0): 1.09×10^{-9} cm^3; V_m (0): -5.40×10^{-2} V; V_m^{diff} (0): -1.70×10^{-2} V; $(Na^+)_i$ (0), $(K^+)_i$ (0), $(Cl^-)_i$ (0): 2.4×10^{-5}, 1.25×10^{-4}, 3.1×10^{-5} mol cm^{-3}

Cell 3: V_c (0): 1.62×10^{-9} cm^3; V_m (0): -1.83×10^{-2} V; V_m^{diff} (0): -1.24×10^{-2} V; $(Na^+)_i$ (0), $(K^+)_i$ (0), $(Cl^-)_i$ (0): 1.2×10^{-5}, 1.37×10^{-4}, 7.0×10^{-5} mol cm^{-3}

As can be seen, Cells 0, 1 and 2 exhibit a basically similar reference state, characterized by comparable values of the membrane potential, cell volume and intracellular ionic concentrations. The major difference between these cells concerns the mechanisms underlying the generation of the membrane potential. As revealed by the particular values of V_m^{diff} (0), while in Cells 0 and 1 the membrane potential is basically determined by electrodiffusion, Cell 2 maintains a similar V_m, albeit the value for V_m^{diff} (0) is significantly lower. Since the ionic permeabilities are substantially smaller, the membrane potential of Cell 2 is mainly determined by the electrogenic activity of the sodium pump. Since Cl^- is only subject to passive transport and since Na^+ already exhibits a negligible permeability in Cell 1, the main difference between Cell 1 and Cell 2, from the point of view of the electrical properties of the plasma membrane, is related to the potassium permeability. For low ionic permeabilities and in the absence of both the K:Cl and Na:Cl cotransport processes (the case of Cell 3), the sodium pump proves to be ineffective in maintaining a membrane potential similar to the ones of the three other referential cells.

The finding of comparable reference states for Cells 0, 1 and 2 reveals the relevant role of electroneutral ionic exchange (for the model analyzed here, net Na^+:K^+ exchange) for the determination of a physiological V_m in a predominantly electrogenic fashion (as in Cell 2). In essence, this is a consequence of the particular electrical and biochemical properties of the sodium pump. The consequences of these noteworthy properties of the sodium pump on the determination of the membrane potential can be interpreted with the aid of Eq. (4). Under conditions of a predominantly electrodiffusional mechanism of generation of the V_m (e.g., for a large potassium permeability), V_m becomes more electronegative with $(K^+)_i$ [Eq. (4)]. In this case (Cells 0 and 1) the enzyme contributes to the physiological V_m in a biochemical fashion, by maintaining a large $(K^+)_i$. However, when the enzymatic terms predominate (e.g., for low ionic permeabilities or relatively large pump activity, the case of Cell 2) V_m becomes more electropositive with $(K^+)_i$ and, correspondingly, with decreasing $Na^+)_i$ [*see* Eq. (4)]. In electrical terms, this results from the fact that the electrogenic current mediated by the sodium pump consists of an outward flow of positive charges. Hence, in this latter case, the effect of an increase in the intracellular concentration of $(K^+)_i$ and of a concomitant decrease in $(Na^+)_i$ (a consequence of the predominance of the pump activity) is a more depolarizing membrane potential. The presence of a net electroneutral Na^+:K^+ exchange (a consequence of simultaneous KCl and NaCl symport-mediated transports, the case of Cell 2) prevents large modifications in the intracellular concentrations of these ions under conditions of predominance of the active ionic fluxes, and thus permits the maintenance of V_m at physiological values. For the case of low ionic permeabilities and absence of both the K:Cl and Na:Cl cotransport processes (the case of Cell 3) the effects produced by the predominant sodium pump-mediated ionic fluxes cannot be counterbalanced. As a consequence, in Cell 3 both $(Na^+)_i$ and $(K^+)_i$ modify to larger extents than in the case of Cell 2 (*see* the reference values). Since the enzymatic terms nevertheless are dominant, a lower $(Na^+)_i$ and a larger $(K^+)_i$ simultaneously contribute to a more depolarizing ("unphysiological") value of V_m [Eq. (4)]. Also for the case of Cell 3, a significant accumulation of intracellular K^+ and Cl^- is responsible for the notoriously larger cell volume exhibited by this cell by comparison with Cell 2.

Since this study mostly has an illustrative character the reference values characterizing cells 0, 1 and 2 do not correspond to any specific cell type. For a roughly spherical cell, a cell volume of 10^{-9} cm^3 would approximately correspond to a cell radius of 6.5 μm. Together with the rest of the values employed for the parameters (Table 2), these reference values could, for instance, approximately be characteristic of some cells of the hematopoietic lines, like small lymphocytes (Segel, Simon & Lichtman, 1979; Severini, et al. 1987; Ishida & Chused, 1993). Transitions between cellular states characterized by different mechanisms of generation of the membrane potential (predominantly electrogenic, as in Cell 2, or predominantly electrodiffusional, as in Cells 0 and 1) have been observed in actual cell types (e.g., in small lymphocytes, Ishida & Chused, 1993). The model studied here may thus provide a formal basis to interpret the transitions experienced by some cellular lines in the course of their development. It may also be suggestive of the basic mechanisms (e.g., modifications in some ionic permeabilities or transport rates) underlying those cellular transitions.

In the following section we show some elementary dynamic and stationary studies of the model for the case of Cell 2, intended to illustrate the basic roles of the individual transport systems (the sodium pump and the K:Cl and Na:Cl cotransport systems), participating in the generation of the membrane potential in an electrogenic fashion.

Effects of the Sodium Pump and of the Ionic Cotransporters on Cell 2

Figure 2 shows the effects produced on Cell 2 by a partial inhibition (Fig. 2*A*) and by an activation (Fig. 2*B*) of the sodium pump. A large inhibition of the enzyme (*not shown*) immediately determines the expected consequences on Cell 2: a decrease in $(K^+)_i$, an increase in $(Na^+)_i$ and $(Cl^-)_i$, an increase in V_c, and membrane depolarization. This agrees with classic experimental evidence (Byrne & Schultz, 1988) and is similar to previous model simulations of the behavior of animal cells embodied with a large potassium permeability (that is, similar to Cell 1) (Jakobsson, 1980; Hernández & Cristina, 1998). In particular, for the case of a complete inhibition of the enzyme, the membrane depolarization consists of two phases: a rapid initial one corresponding to the inhibition of the electrogenic component and a second slow phase associated with the gradual changes of the intracellular ionic concentrations (Hernández & Cristina, 1998). The value reached at the depolarizing peak corresponds to the electrodiffusive membrane potential, and is approximately determined by the initial ionic concentrations [Eq. (5)]. Inhibition to a lower degree (Fig. 2*A*) is compatible with a steady state approximately similar to the referential state of Cell 2. The transient only affects the membrane potential and, similar to the case of a large inhibition, also consists of two phases: a rapid depolarization corresponding to the initial electrogenic inhibition followed by a slower relaxation to the final steady state. The rapid initial depolarization is a consequence of the sudden perturbation of the membrane potential provoked by the abrupt modification in the en-

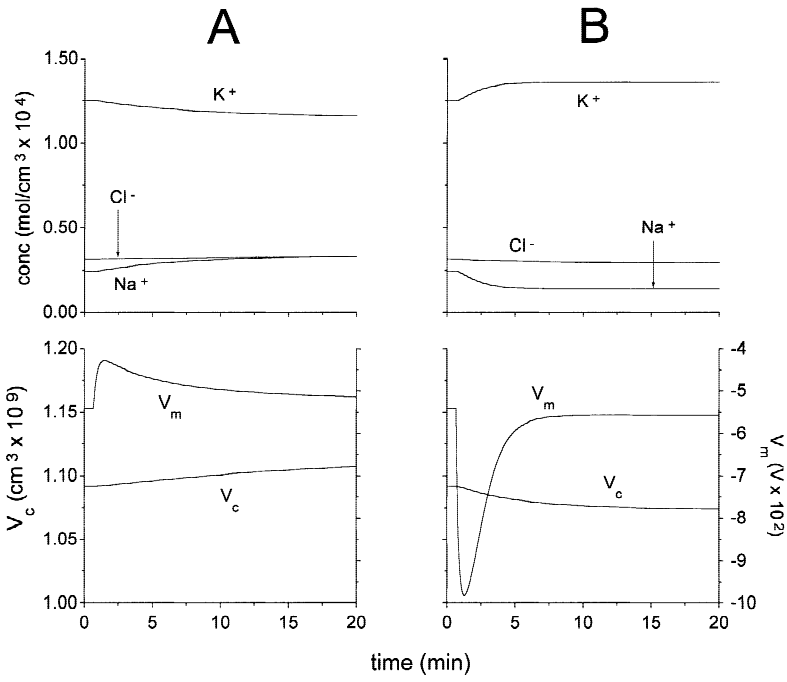

Fig. 2. Dynamic responses of Cell 2 to a partial inhibition (*A*) and activation (*B*) of the sodium pump. At $t = 40$ sec, the enzyme density undergoes a change from its initial value N(0) (as for N in Table 2) toward a final value N_∞ [Eq. (7)]. (*A*) Plots of $(Na^+)_i$, $(K^+)_i$, $(Cl^-)_i$, V_c and V_m as functions of time, for $N_\infty = 0.4 \times N(0)$. *B* Similar to Fig. 2*A*, but for $N_\infty = 4 \times N(0)$.

zyme density. The intracellular ionic concentrations have not undergone any changes yet, therefore they correspond to the initial values. Once the peak is reached, the process undergoes a slower relaxation towards its final steady state. Different from the case of the complete inhibition of the enzyme (*see above*), the slower relaxation exhibited by the partial inhibition consists in a recovery of the resting membrane potential. For the case shown in Fig. 2*A*, the partial enzyme inhibition determines a small increase in $(Na^+)_i$ and a small decrease in $(K^+)_i$, as a consequence the final membrane potential is more electronegative than the depolarizing peak [Eq. (4)] but not significantly different from the initial V_m. The effects of a partial inhibition of the sodium pump, just described for Cell 2, are similar to those encountered from the study of models of cells of the type of Cell 1 (Hernández & Cristina, 1998). This dynamic response to a partial inhibition of the sodium pump, therefore exhibited both by Cells 1 and 2, may provide an alternative explanation of actual experimental findings of spontaneous recovery of the membrane potential after the administration of sodium pump inhibitors (De Weer & Geduldig, 1978; Brismar & Collins, 1993).

Figure 2*B* shows the effects produced on Cell 2 by a fourfold activation of the sodium pump. The activation of the enzyme produces the expected consequences on the intracellular ionic concentrations: an increase in $(K^+)_i$, a decrease in $(Na^+)_i$ and almost no modifications in $(Cl^-)_i$. V_c also experiences an insignificant decrease, as a consequence of a larger extrusion of osmotic particles by the sodium pump. Again, the intermediate transient characterizing the time course of V_m can be interpreted in similar terms as previously: the abrupt hyperpolarizing peak reveals the activation of the electrogenic component under initial conditions, while the subsequent slow depolarizing relaxation is a consequence of the slower gradual changes produced in $(K^+)_i$ and $(Na^+)_i$ by the enzyme activation [Eq. (4)]. It must be noticed that the rapid initial electrical response is basically a consequence of the relatively small value assumed for the time constant (θ) of the enzyme modifications [Table 2, Eq. (7)], the effect of larger values of θ on the dynamic response has not been explored here. Figure 2*B* therefore reveals that the final steady-state value achieved for V_m is not significantly different from the initial one. This is a consequence of the modifications produced on the ionic concentrations. Under the electrogenic mode of generation of V_m, the increase in $(K^+)_i$ and the decrease in $(Na^+)_i$ produced by the activation of the sodium pump compensate the initial hyperpolarizing tendency [Eq. (4)]. Analogous to the case of a partial inhibition (*see above*), the effects produced on Cell 2 by activation of the sodium pump within physiological values are also similar to those exhibited by cell models of the type of Cell 1 (Hernández & Cristina, 1998).

The results shown in Fig. 2 therefore permit us to conclude that the individual modifications of the sodium pump determine changes on Cell 2 that are basically similar to those produced on cells of the type of Cell 1. A partial inhibition and an activation of the enzyme may be compatible with the maintenance of nearly physiological cellular steady states. In particular, no significant modifications on the final steady-state membrane potential were produced by modifications of the enzyme

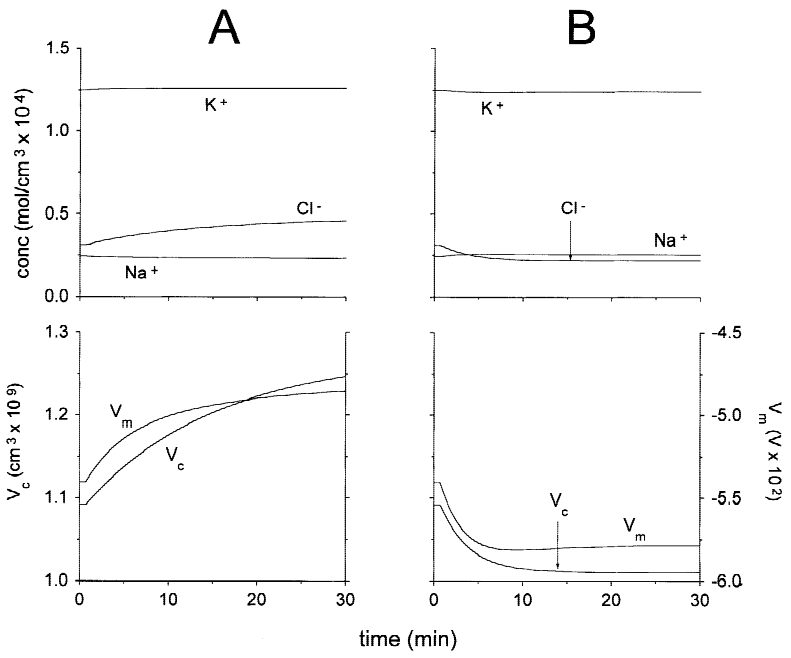

Fig. 3. Dynamic response of Cell 2 to a modification in the K:Cl cotransporter activity. At $t = 40$ sec, Q_{KCl} undergoes a change from its initial value Q_{KCl} (0) (as for Q_{KCl} in Table 2) toward a final value $Q_{KCl\infty}$ [Eq. (7)]. (*A*) Plots of $(Na^+)_i$, $(K^+)_i$, $(Cl^-)_i$, V_c and V_m as functions of time, for the case of a 50% inhibition [$Q_{KCl\infty} = 0.5 \times Q_{KCl}$ (0)]. (*B*) Similar to Fig. 3*A*, but for a twofold activation [$Q_{KCl\infty} = 2 \times Q_{KCl}$ (0)].

activity within a physiological range. However, this type of modification determined a characteristic transient response, in the form of a depolarizing (for the case of partial inhibition) or a hyperpolarizing (for pump activation) electrical signal, both for a cell with the characteristics of Cell 1 (Hernández & Cristina, 1998) and for Cell 2 (this work). From these results we may conclude that, even for a cell generating its membrane potential in an electrogenic fashion, the electrogenic properties of the sodium pump may mostly be revealed by the characteristic short-duration electrical signals induced by modifications in the enzyme activity within physiological values.

Figure 3*A* shows the effects of a partial inhibition of the K:Cl cotransporter (to one half of the basal value of Q_{KCl}) on Cell 2. The immediate consequence of the inhibition is an increase in the intracellular mass of Cl^-. The increase in $(Cl^-)_i$ results in membrane depolarization [Eq. (4)]. V_c increases, thus compensating for the increase in $(Cl^-)_i$ by diluting the impermeant intracellular anion. Figure 3*B* shows the effects of a twofold activation of the K:Cl cotransporter. The effects produced are the opposite than in the case of inhibition: the activation determines a net decrease in $(Cl^-)_i$, membrane hyperpolarization and a compensatory decrease in the cell volume. A partial inhibition of the Na:Cl cotransporter (to one half of the basal value of Q_{NaCl}, Fig. 4*A*) produces a decrease in the intracellular mass of Na^+ and Cl^-. Electroneutrality imposes a reduction in V_c, in order to compensate for the decrease in $(Cl^-)_i$ by concentrating the impermeant intracellular anion. The consequent increase in $(K^+)_i$ and the simultaneous decrease in $(Na^+)_i$ result, under the electrogenic mode, in a more depolarized V_m [Eq. (4)]. Figure 4*B* shows the effects produced on Cell 2 by a twofold activation of the Na:Cl cotransporter. This activation results in opposite effects to the case of inhibition: an increment in V_c, in order to compensate for the increase in $(Cl^-)_i$, plus a decrease in $(K^+)_i$ and a simultaneous increase in $(Na^+)_i$ that determine, under the electrogenic mode, a more hyperpolarized V_m [Eq. (4)]. It is interesting to note that, due to the alterations produced on $(K^+)_i$ and $(Na^+)_i$, the modifications on the Na:Cl cotransport process determine larger changes on V_m than similar relative modifications on the K:Cl cotransporter. It is also noteworthy that modifications on each one of the ionic cotransport processes determine significantly larger changes on the cell volume than relatively large modifications on the sodium pump activity (compare Figs. 3 and 4 with Fig. 2).

The results obtained so far permit us to conclude that modifications induced on some of the individual transport systems of Cell 2 mostly produce either nonsignificant effects on the final V_m (e.g., partial inhibition or activation of the sodium pump) or relatively important changes on the membrane potential accompanied by significant modifications on other variables, like the cell volume (e.g., inhibition or activation of the individual Na:Cl and K:Cl cotransport processes). We now illustrate the effects of modifications in the rate of net electroneutral Na^+:K^+ exchange. As mentioned above, this exchange is the result of the simultaneous activity of both the Na:Cl and K:Cl cotransport processes.

Figure 5 shows the effects produced on Cell 2 by a simultaneous partial inhibition (to one half of the basal value of Q_{KCl} and of Q_{NaCl}, respectively, Fig. 5*A*) and by a simultaneous twofold activation (Fig. 5*B*) of both the

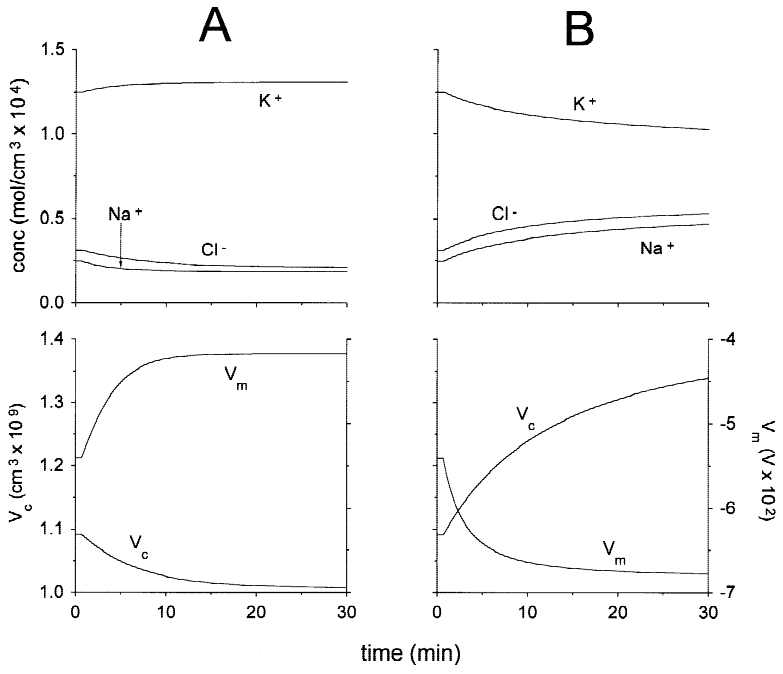

Fig. 4. Dynamic response of Cell 2 to a modification in the Na:Cl cotransporter activity. At $t = 40$ sec, Q_{NaCl} undergoes a change from its initial value Q_{NaCl} (0) (as for Q_{NaCl} in Table 2) toward a final value $Q_{NaCl\infty}$ [Eq. (7)]. (*A*) Plots of $(Na^+)_i$, $(K^+)_i$, $(Cl^-)_i$, V_c and V_m as functions of time, for the case of a 50% inhibition [$Q_{NaCl\infty} = 0.5 \times Q_{NaCl}$ (0)]. (*B*) Similar to Fig. 4*A*, but for a twofold activation [$Q_{NaCl_\infty} = 2 \times Q_{NaCl}$ (0)].

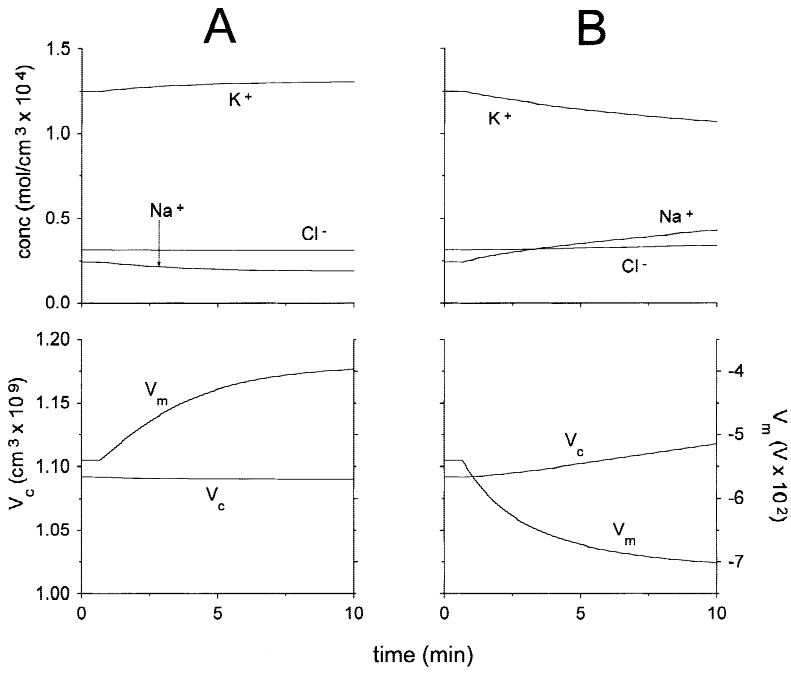

Fig. 5. Dynamic responses of Cell 2 to a simultaneous partial inhibition (*A*) and simultaneous activation (*B*) of the K:Cl and Na:Cl cotransport processes. At $t = 40$ sec Q_{KCl} and Q_{NaCl} undergo changes from their initial values Q_{KCl} (0) and Q_{NaCl} (0) (as for Q_{KCl} and Q_{NaCl} in Table 2) toward final values $Q_{KCl\infty} = 0.5\ Q_{KCl}$ (0) and $Q_{NaCl\infty} = 0.5 \times Q_{NaCl}$ (0) (*A*) and final values $Q_{KCl\infty} = 2 \times Q_{KCl}$ (0) and $Q_{NaCl\infty} = 2 \times Q_{NaCl}$ (0) (*B*) [Eq. (7)]. The figure shows plots of $(Na^+)_i$, $(K^+)_i$, $(Cl^-)_i$, V_c and V_m as functions of time.

K:Cl and Na:Cl cotransport processes. The inhibition of the ionic cotransport processes (Fig. 5*A*) determines the expected increase in $(K^+)_i$ and simultaneous decrease in $(Na^+)_i$ (upper panel). These modifications result in a more depolarizing membrane potential (Fig. 5*A*, lower panel), as a consequence of the predominance of the enzymatic terms in the determination of V_m [Eq. (4)]. For the case of Cell 1, characterized by a predominantly electrodiffusional mechanism of generation of the membrane potential, an analogous perturbation in the cotransport processes does not determine changes in V_m (*not shown*). It is noteworthy that the simultaneous inhibition of the cotransporters does not determine significant modifications in the cell volume, either for the case of Cell 1 (*not shown*) or Cell 2 (Fig. 5*A*, lower panel).

Both in Cell 1 (*not shown*) and in Cell 2 (Fig. 5*B*) the

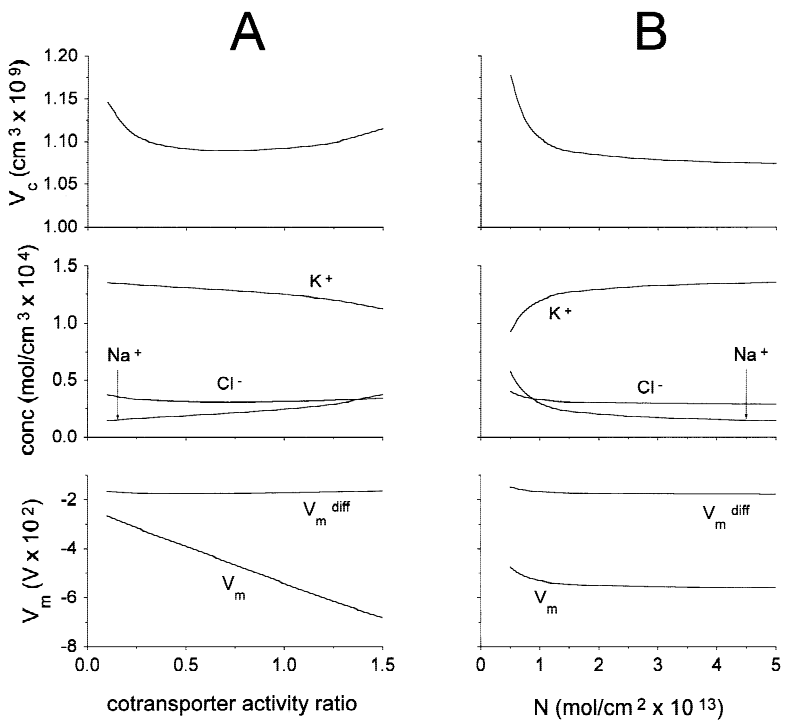

Fig. 6. Plots of the steady-state values of $(Na^+)_i$, $(K^+)_i$, $(Cl^-)_i$, V_c, V_m, and V_m^{diff} as functions of the cotransporter activity ratio (*A*) and the sodium pump density (*B*), for the case of Cell 2. The cotransporter activity ratio (r) is defined as: $r = Q_{KCl\infty}/Q_{KCl}\,(0) = Q_{NaCl\infty}/Q_{NaCl}\,(0)$, where $= Q_{KCl\infty}$ and $Q_{NaCl\infty}$ are the modified values and where $Q_{KCl}\,(0)$ and $Q_{NaCl}\,(0)$ are the initial values (as in Table 2) of Q_{KCl} and Q_{NaCl}, respectively.

simultaneous activation of the ionic cotransporters determines a decrease in $(K^+)_i$ and a simultaneous increase in $(Na^+)_i$. Also, both cells experience a very small increase in the cell volume associated with a slight increase in $(Cl^-)_i$, in turn a consequence of a somewhat larger rate of Na:Cl cotransport. In the case of Cell 1 the membrane potential only experiences a negligible depolarization (*not shown*). Under the electrogenic mode of generation of the membrane potential, the changes in the intracellular concentrations of K^+ and Na^+ experienced by Cell 2 (Fig. 5*B*) determine a significant hyperpolarization [Eq. (4)]. This result is consistent with those obtained by Jacob et al. (1984) from the analysis of a steady-state model generalizing the classic Mullins-Noda phenomenological approach (Mullins & Noda, 1963).

From the above we may conclude that the simultaneous modifications in both ionic cotransporters produce larger changes on the membrane potential of Cell 2 than similar independent modifications in each one of the cotransport processes (compare Fig. 5 with Figs. 3 and 4). Also, the conservation of the osmotic balance implicit in the net K^+:Na^+ exchange determines that the changes produced on the cell volume are significantly smaller. Therefore, the modification in the rate of net K^+:Na^+ exchange represents a good candidate for the modulation of the membrane potential under the electrogenic mode of generation. However, for the case of a cell embodied with a large potassium permeability (e.g., as Cell 1), the modifications in the rate of K^+:Na^+ exchange do not produce significant changes in the membrane potential (*see* discussion above). As shown above, for the case of Cell 2, the modifications of the sodium pump activity within the physiological range do not determine significant final changes in the membrane potential (*see* Fig. 2). Figure 6 summarizes these ideas, by comparing the final steady-state values achieved by Cell 2 as a function of the rate of K^+:Na^+ exchange (Fig. 6*A*) and of the sodium pump activity (Fig. 6*B*). As can be seen, the main difference refers to the membrane potential: while V_m hyperpolarizes linearly to large extents with the rate of ionic exchange, it experiences almost no modification with the pump density within physiological values. Taken together (*see also* Reference States), these results are consistent with the idea suggested by some authors (Bashford & Pasternak, 1986) that electroneutral ionic exchange may play a relevant role in the determination of the membrane potential in an electrogenic fashion (*see* Introduction).

Conclusions

In summary, the results of this study permit us to conclude that, for the cell model considered, the maintenance of the plasma membrane potential at nearly physiological values by means of the electrogenic contribution of the sodium pump requires the simultaneous existence of a relatively large rate of electroneutral Na^+:K^+ exchange and of a low potassium permeability. As mentioned, these results are consistent with experimental findings about this type of cell (Bashford & Pasternak, 1986; Ishida & Chused, 1993). From the analysis per-

formed here we may conclude that the main reason for these requirements is that the Na^+:K^+ exchange prevents large modifications in the intracellular concentrations of these ions when the enzyme-mediated ionic fluxes become prevailing. In this way, a cell can maintain similar macroscopic properties (membrane potential, cell volume, intracellular ionic concentrations) in the presence of different mechanisms of generation of the membrane potential (predominantly electrogenic or predominantly electrodiffusional). The numerical studies of the model also show that, under the electrogenic mode of generation of the membrane potential, modifications in the sodium pump activity within physiological values do not determine significant changes in the steady-state membrane potential. The electrogenic properties of the enzyme are specially revealed in the characteristic short-duration electrical responses in the transient behavior. For the particular case of a partial inhibition of the sodium pump, the biphasic curve conforming the transient consists in a rapid depolarization followed by a slower recovery of the resting membrane potential. This behavior is similar to that occasionally encountered in some actual experimental systems, where a depolarization induced by an inhibition of the enzyme is followed by a spontaneous recovery of the membrane potential (De Weer & Geduldig, 1978; Brismar & Collins, 1993). Finally, our theoretical study also suggests that, for the case of nonexcitable cells maintaining a predominantly electrogenic membrane potential, the most efficient mechanism for regulating the steady-state membrane potential may consist in modifications in the rate of Na^+:K^+ exchange. For the model studied here, small modifications in this rate determine relatively larger changes in the membrane potential and less significant changes in the cell volume than similar relative modifications in the individual transport systems.

This work was supported by grants from the Programa para el Desarrollo de las Ciencias Básicas (PEDECIBA) and from the Comisión Sectorial de Investigación Científica (CSIC) de la Universidad de la República, Uruguay.

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Appendix

Steady-state Analysis of the NaK ATPase Kinetic Model

The transport of Na^+ and K^+ mediated by the NaK ATPase is described by the diagram shown in Fig. 1*B*. For this diagram, $k_{12}, \ldots, k_{61}$ ($k_{16}, \ldots, k_{21}$) are the rate constants governing the corresponding transitions in the clockwise (counterclockwise) direction. The analysis of this diagram has been performed previously (Chapman, Johnson & Kootsey, 1983; Hernández et al., 1989; Lemieux, Roberge & Savard, 1990), we summarize the main results here. In the steady state the cycle flux J_p (considered positive in the clockwise direction) can be expressed, employing the diagram method (Hill, 1977), as

$$J_p = (1/\Sigma)\,(\alpha - \beta) \tag{A1}$$

In this equation α and β are defined by

$$\alpha = N\, a_{12}\, a_{23}\, a_{34}\, a_{45}\, a_{56}\, a_{61} \text{ and } \beta = N\, a_{21}\, a_{32}\, a_{43}\, a_{54}\, a_{65}\, a_{16} \tag{A2a}$$

with

$$\begin{aligned} a_{12} &= k_{12}\,(n_{Na}/V_c)^3 & a_{21} &= k_{21} \\ a_{23} &= k_{23} & a_{32} &= k_{32}\,(\mathrm{ADP}) \\ a_{34} &= k_{34} & a_{43} &= k_{43}\,(\mathrm{Na}^+)_e^{\,3} \\ a_{45} &= k_{45}\,(K^+)_e^{\,2} & a_{54} &= k_{54}\,(P_i) \\ a_{56} &= k_{56}\,(\mathrm{ATP}) & a_{65} &= k_{65} \\ a_{61} &= k_{61} & a_{16} &= k_{16}\,(n_K/V_c)^2 \end{aligned} \tag{A2b}$$

Σ is the sum of all the directional diagrams of the model, and is therefore a function of all the rate constants and ligand concentrations [*see* Hernández et al. (1989) for explicit expression].

In their original work, Chapman et al. (1983) performed the numerical studies assuming that step $N_5 \leftrightarrow N_6$ was voltage-dependent. More recent evidence suggests that the main electrogenic steps correspond to the successive bindings of the extracellular sodium ions (Wuddel & Apell, 1995; Rakowski et al., 1997*b*). Since the extracellular sodium concentration is a parameter of the model studied here (Table 1), these successive steps (Fig. 1*C*) can be reduced to a single step (e.g., to transition $N_3 \leftrightarrow N_4$), governed by pseudo-first order rate constants (Hill, 1977). Thus, if states N_α and N_β (Fig. 1*C*) are transient intermediates (Hill, 1977), the (reduced) rate constants k_{34} and k_{43} become, in terms of the basic constants considered by Wuddell & Apell (1995),

$$\begin{aligned} &k_{34} = g_{3f} g_{2f} g_{1f}/G \text{ and } k_{43} = g_{3b}\, g_{2b}\, g_{1b}\, (Na^+)e^3/G, \\ &\text{with } G = g_{3b}\, g_{2b}\, (Na^+)_e^{\,2} + g_{1f} g_{3b}\, (Na^+)_e + g_{2f} g_{1f}. \end{aligned} \tag{A2c}$$

The rate constants k_{34} and k_{43} are assumed here to depend on V_m according to

$$k_{34} = k_{34^\circ} \exp\,[FV_m/(2RT)];\; k_{43} = k_{43^\circ} \exp\,[-FV_m/(2RT)] \tag{A3}$$

where k_{34° and k_{43° are independent of V_m.

For zero voltage, the parameter values employed by Wuddell & Apell (1995) were

g_{3f} : 1400 sec^{-1} ; g_{3b} : 14000 mol^{-1} lt sec^{-1}

g_{2f} : 700 sec^{-1} ; g_{2b} : 467 mol^{-1} lt sec^{-1}

g_{1f} : 4000 sec^{-1} ; g_{1b} : 14000 mol^{-1} lt sec^{-1}

Substitution of these values in Eqs. (A2*c*) yields the values for k_{34° amd k_{43° shown in Table 2.

The detailed balance condition imposes the following restriction:

$$K_{eq} = k_{12}\, k_{23}\, k_{34^\circ}\, k_{45}\, k_{56}\, k_{61}/(k_{65}\, k_{54}\, k_{43^\circ}\, k_{32}\, k_{21} k_{16}) \tag{A4}$$

where K_{eq} is the dissociation constant of the reaction ATP + $H_2O \leftrightarrow$ ADP + P_i.

The active fluxes of Na^+ and K^+ (positive in the inward direction) are respectively equal to $-3J_p$ and $2J_p$.