

Original Paper

Computation of Pump-Leak Flux Balance in Animal Cells

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Key Words

Cell ion balance • Cell water balance • Ion transport • Sodium pump • Cotransport • Channels • Ion fluxes

Abstract

Background/Aims: Many vital processes in animal cells depend on monovalent ion transport across the plasma membrane via specific pathways. Their operation is described by a set of nonlinear and transcendental equations that cannot be solved analytically. Previous computations had been optimized for certain cell types and included parameters whose experimental determination can be challenging. **Methods:** We have developed a simpler and a more universal computational approach by using fewer kinetic parameters derived from the data related to cell balanced state. A file is provided for calculating unidirectional Na^+ , K^+ , and Cl^- fluxes via all major pathways (i.e. the Na/K pump, Na^+ , K^+ , Cl^- channels, and NKCC, KC and NC cotransporters) under a balanced state and during transient processes. **Results:** The data on the Na^+ , K^+ , and Cl^- distribution and the pump flux of K^+ (Rb^+) are obtained on U937 cells before and after inhibiting the pump with ouabain. There was a good match between the results of calculations and the experimentally measured dynamics of ion redistribution caused by blocking the pump. **Conclusion:** The presented approach can serve as an effective tool for analyzing monovalent ion transport in the whole cell, determination of the rate coefficients for ion transfer via major pathways and studying their alteration under various conditions.

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Introduction

Electrochemical gradients of monovalent ions across the plasma membrane are indispensable for normal functioning of any cell. They are determined by charged osmotically active material sequestered in the cell and by the active and passive transport of ions mediated

by various carriers and channels. The overall ion traffic is described by a set of nonlinear and transcendental equations, which cannot be solved analytically. Numerical solutions of this problem have been developed in a series of studies [1–10]. Some of the published codes and executable files are available in a form that allows modification of kinetic parameters to some extent [7, 10]. Nevertheless, further study in this direction seems to be necessary because the reported detailed computations have been optimized only for certain cell types, such as human red blood cells and reticulocytes [3, 10], guinea-pig cardiomyocytes [7], or frog skeletal muscle [4, 8]. However, erythrocytes and muscle cells are characterized by rather extreme values of membrane potential (MP), on the order of -10 mV and -90 mV, respectively. For our study we chose the proliferating human lymphoid U937 cell line, which better represents the typical animal cell owing to its faster metabolism and intermediate (-40÷-60 mV) MP. The monovalent ion flux balance in U937 cells has been comprehensively characterized in our laboratory [11–14]. In the present paper we have developed an improved computational model which, compared to the previous models, uses phenomenological parameters derived from the balanced state. We show how the Na/K pump, Na⁺, K⁺, Cl⁻ ion channels, and the three major types of cotransporters, Na⁺-K⁺-2Cl⁻ (NKCC), K⁺-Cl⁻ (KC) and Na⁺-Cl⁻ (NC), should affect ion fluxes and MP in cells of different types, i.e. high-potassium cells with high MP (excitable nerve and skeletal muscle cells), high-potassium cells with low MP (human erythrocytes), and low-potassium cells with low MP (erythrocytes of some carnivores and ruminants). Due to a large number of variables it is impossible to demonstrate all possible outcomes. Therefore, we present an executable file which allows modeling of the behavior of any cell type either under conditions of balanced transmembrane ion distribution or during transition processes caused by alteration of ion pathways and/or of the cell environment. For input parameters, we use intracellular [Na]_i, [K]_i, [Cl]_i concentrations and Rb⁺ ouabain-inhibitable and -resistant influxes, which are assumed to be known from the experiment. It was found that modeling based on parameters derived from balanced ion distribution achieves quantitatively accurate description of transient processes caused by blocking the pump with ouabain. We consider this outcome as an indication that our mathematical model is well suited to describe real cellular processes.

Materials and Methods

Human histiocytic lymphoma U937 cells were obtained from the Russian Cell Culture Collection (cat. number 160B2). The cells were maintained in RPMI 1640 medium (Biolot, Russia) with 10% fetal calf serum (HyClone, Standard, United State) at 37°C and 5% CO₂. Ouabain was purchased from Sigma-Aldrich (Steinheim, Germany). Percoll was from Pharmacia (Sweden). The isotope ³⁶Cl⁻ was from Isotope (Russia). Salts were of analytical grade and were from Reaches (Russia). The experimental methods used in this work have been described in detail earlier [11–15]. Briefly, intracellular K⁺, Na⁺ and Rb⁺ content was analyzed by flame emission spectrometry, Cl⁻ content was measured using ³⁶Cl⁻ isotope. Rb⁺ influx via Na⁺/K⁺ pump was evaluated from the difference between Rb⁺ uptake in the presence and absence of 0.1 mM ouabain. The water content was evaluated by cell buoyant density in Percoll density gradient. The results were statistically treated using Student t-test.

Results

Equations

The mandatory conditions of macroscopic electroneutrality and osmotic balance can be written as [6, 9]:

$$[Na]_i + [K]_i - [Cl]_i + \frac{zA}{V} = 0 \quad (1)$$

$$[Na]_o + [K]_o + [Cl]_o + [B]_o = [Na]_i + [K]_i + [Cl]_i + \frac{A}{V} \quad (2)$$

The subscripts *i* and *o* indicate the intracellular and extracellular concentrations in mM, respectively; *V* is the volume occupied by intracellular water (ml), which is assumed to be approximately equal to the cell volume; *A* is the total amount (in mmol) of membrane-impermeant osmolytes with mean valence *z*; $[B]_o$ is the external concentration of the membrane-impermeant solute (see Table 1 for symbols and definitions).

From Eqs. 1, 2 it follows that

$$z = ([Cl]_i - [Na]_i - [K]_i) / ([Na]_o + [K]_o + [Cl]_o + [B]_o - [Cl]_i - [Na]_i - [K]_i) \quad (3)$$

$$V/A = 1 / ([Na]_o + [K]_o + [Cl]_o + [B]_o - [Cl]_i - [Na]_i - [K]_i) \quad (4)$$

$$V = zA / ([Cl]_i - [Na]_i - [K]_i) \quad (5)$$

Dependences of ion fluxes via electroconductive channels and NKCC, KC, NC cotransporters on the ion concentration gradients and MP were formulated in the traditional way [3, 7–10]. The equation for Na⁺ and K⁺ fluxes via the pump was used in a simple linear form [1].

To express the fluxes, the following equations were used:

$$\frac{d([Na]_i V)}{dt} = V \{ (p_{Na} u ([Na]_i \exp(u) - [Na]_o) / g - \beta [Na]_i + J_{NC} + J_{NKCC} \} \quad (6)$$

$$\frac{d([K]_i V)}{dt} = V \{ (p_K u ([K]_i \exp(u) - [K]_o) / g + \beta [Na]_i / \gamma + J_{NKCC} + J_{KC} \} \quad (7)$$

$$\frac{d([Cl]_i V)}{dt} = V \{ (p_{Cl} u ([Cl]_o \exp(u) - [Cl]_i) / g + J_{NC} + J_{KC} + 2J_{NKCC} \} \quad (8)$$

Here *u* is the dimensionless MP related to absolute values *U* (mV) as $U = uRT/F = 26.7u$ for 37°C and $g = 1 - \exp(u)$

The left-hand sides of Eqs. 6–8 represent the rates of change in the cell ion content. The right-hand sides express fluxes via channels, the Na/K pump, and cotransporters. The rate coefficients p_{Na} , p_K , p_{Cl} characterizing channel ion transfer are similar to the Goldman's coefficients. Fluxes J_{NC} , J_{KC} , J_{NKCC} are related to internal and external ion concentrations as

$$\begin{aligned} J_{NC} &= i_{NC} ([Na]_o [Cl]_o - [Na]_i [Cl]_i) \\ J_{KC} &= i_{KC} ([K]_o [Cl]_o - [K]_i [Cl]_i) \\ J_{NKCC} &= i_{NKCC} ([Na]_o [K]_o [Cl]_o [Cl]_o - [Na]_i [K]_i [Cl]_i [Cl]_i) \end{aligned} \quad (9)$$

Here i_{NC} , i_{KC} , and i_{NKCC} are the rate coefficients for cotransporters.

By multiplying Eq. 1 by *V* and taking a time derivative, we obtain the following relationship:

$$\frac{d([Na]_i V)}{dt} + \frac{d([K]_i V)}{dt} - \frac{d([Cl]_i V)}{dt} = 0, \quad (10)$$

From Eqs. 6–8 and taking Eq. 10 into account, a transcendental equation for the potential *u* follows:

$$F(u) = \exp(u) - \frac{p_{Na} [Na]_o + p_K [K]_o + p_{Cl} [Cl]_i + \beta [Na]_i (1 - 1/\gamma) / u}{p_{Na} [Na]_i + p_K [K]_i + p_{Cl} [Cl]_o + \beta [Na]_i (1 - 1/\gamma) / u} = 0 \quad (11)$$

Algorithm

Modeling of the flux balance in a cell requires a numerical solution of a Cauchy problem for the set of Eqs. 6–8 [16]. The values of z and V compatible with the conditions of electroneutrality and osmotic balance have to be computed at the outset. Cell volume V and potential u are found at every step of numerical integration. The transcendental Eq. 11 is solved in two stages. First, one finds the values of u that ensure that $F(u)$ crosses zero

Table 1. Symbols and definitions

Definitions	In text, Figures and Equations	In files DATA.txt, RES.txt	Units
Ion species	$\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{Rb}^+$	$\text{Na}, \text{K}, \text{Cl}$	
Types of cotransport	$\text{NC}, \text{NKCC}, \text{KC}$		
Concentration of ions in cell water or external medium	$[\text{Na}]_i, [\text{K}]_i, [\text{Cl}]_i, [\text{Na}]_o, [\text{K}]_o, [\text{Cl}]_o$	$na, k, cl, na0, k0, cl0$	mM
External concentration of membrane-impermeant osmolytes	$[\text{B}]_o$	$B0$	mM
Intracellular content of membrane-impermeant osmolytes	A		mmol, may be related to g cell protein or cell number etc.
Cell water volume	V		ml, may be related to g cell protein or cell number etc.
Membrane-impermeant osmolyte concentration in cell water	A/V	A/V	mM
Cell water content per unit of A	V/A	V/A	ml·mmol ⁻¹
Mean valence of membrane-impermeant osmolytes, A	z	z	dimensionless
Permeability coefficients	$p_{\text{Na}}, p_{\text{K}}, p_{\text{Cl}}$	pna, pk, pcl	min ⁻¹
Pump rate coefficient	β	$beta$	min ⁻¹
Na/K pump fluxes stoichiometry	γ	$gamma$	dimensionless
Membrane potential, MP	U	U	mV
Dimensionless membrane potential	$u, U = uRT/F$	u	dimensionless
Net fluxes mediated by cotransport NC, KC, NKCC	$J_{\text{NC}}, J_{\text{NKCC}}, J_{\text{KC}}$	$NetNC, NetKC, NetNKCC$	μmol·min ⁻¹ ·(ml cell water) ⁻¹
Na efflux via the pump	$-\beta[\text{Na}]_i$	$PUMP(\text{Na})$	μmol·min ⁻¹ ·(ml cell water) ⁻¹
K influx via the pump	$\beta[\text{Na}]_i/\gamma$	$PUMP(\text{K})$	μmol·min ⁻¹ ·(ml cell water) ⁻¹
Net fluxes mediated by channels		$NetCh$	μmol·min ⁻¹ ·(ml cell water) ⁻¹
Unidirectional influxes of Na, K or Cl via channels, or cotransporters NC, KC or NKCC		$InCh, InNC, InKC, InNKCC$	μmol·min ⁻¹ ·(ml cell water) ⁻¹
Time derivatives of concentrations		$prna, prk, prcl$	mM·min ⁻¹
Cotransport rate coefficients	$i_{\text{NC}}, i_{\text{KC}}, i_{\text{NKCC}}, S_{oO}, S_{oN}$	$inc, ikc, inkcc$	ml·μmol ⁻¹ ·min ⁻¹ ml ³ ·μmol ⁻³ ·min ⁻¹ mM
“Old” and “new” external concentration of all osmolytes at anisotonic shift			
S_{oN}/S_{oO} ratio	kv	kv	dimensionless
Number of time points between output of results		hp	dimensionless
Transmembrane electrochemical potential difference for Na ⁺ , K ⁺ , and Cl ⁻	$\Delta\mu_{\text{Na}}, \Delta\mu_{\text{K}}, \Delta\mu_{\text{Cl}}$	$mun, muk, mucl$	mV
Ratio of ouabain-sensitive to ouabain-resistant Rb ⁺ (K ⁺) influx	$OSOR$	$OSOR$	dimensionless

at physically meaningful values of u . Next, the process of root refining ZEROIN from the calculation package FMM [17] is applied. Numerical solution of Eqs. 6–8 was accomplished by the explicit Euler method. To monitor the approach to stationary state, time derivatives of sodium, potassium and chloride concentrations are displayed.

This system of equations does not have a physically meaningful solution for every value of parameters or initial concentrations. This was taken into account in program development, and the following diagnostic tools were provided to assist the user:

1. In studying the dependence of the process on external ion concentrations, one enters new concentrations along with parameter $kv = S_{oN}/S_{oO}$, where the second subscripts stand for the old (O) and new (N) values.

$$S_{oO} = [\text{Na}]_{oO} + [\text{K}]_{oO} + [\text{Cl}]_{oO} + [\text{B}]_{oO}$$
$$S_{oN} = [\text{Na}]_{oN} + [\text{K}]_{oN} + [\text{Cl}]_{oN} + [\text{B}]_{oN}$$

If the parameter kv or the initial concentrations do not meet the requirements of electric or osmotic equilibrium, a message “BAD INITIAL DATA” is generated.

2. If the program fails to compute the dimensionless potential u in the physically justified range (which may happen if one enters unrealistic parameters or initial concentrations) the program reports an error, “RANGE NOT FOUND”.

Table 2. The layout of the file DATA.txt. The parameters *na0*, *k0*, *cl0* and *B0* are extracellular concentrations (mM); *na*, *k* and *cl* are intracellular concentrations; *kv* is the ratio of the initial external to internal osmolarity; *beta* is the pump rate coefficient (min^{-1}); *gamma* is the pump Na/K stoichiometric coefficient; *pna*, *pk*, *pcl* are channel permeability coefficients (min^{-1}); *inc*, *ikc*, *inkcc* are the rate coefficients for the NC, KC cotransporters ($\text{ml} \cdot \mu\text{mol}^{-1} \cdot \text{min}^{-1}$) and for cotransporter NKCC ($\text{ml}^2 \cdot \mu\text{mol}^{-3} \cdot \text{min}^{-1}$). The parameter *hp* determines the number of integration steps between data output. If one wishes to follow the initial stages of the process, *hp* should be made small (5-50), and if the process is slow and one is interested in the balanced state, *hp* can be increased to 1000-5000

<i>na0</i>	<i>k0</i>	<i>cl0</i>	<i>B0</i>	<i>kv</i>	<i>na</i>	<i>k</i>	<i>cl</i>	<i>beta</i>	<i>gamma</i>	<i>pna</i>	<i>pk</i>	<i>pcl</i>	<i>inc</i>	<i>ikc</i>	<i>inkcc</i>	<i>hp</i>
140	5.8	116	48.2	1	58	136	63	0.039	1.5	0.00317	0.023	0.00354	0.00003	0.0	0.0	250

Table 3. Transition of the system to the balanced state as displayed in the file RES.txt: The values typical of U937 cells were chosen for this example of transition to the balanced state. In addition to the initial parameters already contained in Table 1, the parameters and variables here include the following: current intracellular ion concentrations *na*, *k*, *cl* (mM); membrane potential *U* (mV); cell water content *V/A* (ml per mmol of A); transmembrane electrochemical potential differences, *mun*, *muk*, *mucl* (mV) for Na^+ , K^+ , and Cl^- , respectively, calculated as $\text{mun} = 26.7 \cdot \ln(\text{na}/\text{na0}) + U$, $\text{muk} = 26.7 \cdot \ln(\text{k}/\text{k0}) + U$, $\text{mucl} = 26.7 \cdot \ln(\text{cl}/\text{cl0}) - U$; derivatives *prna*, *prk*, *prcl*, characterizing the rate of change in $[\text{Na}]_i$, $[\text{K}]_i$, and $[\text{Cl}]_i$; net fluxes of Na^+ , K^+ , and Cl^- via separate ion pathways ($\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$); *A/V* (mM) and the mean valence of membrane-impermeant intracellular osmolytes, *z*. The displayed values of fluxes, as well as *OSOR*, the ratio of ouabain-sensitive to ouabain-resistant K^+ influx, are calculated only in the bottom line, i.e. for the latest time point. The fluxes at any time can be found by varying *hp*. The values of fluxes per g of cell protein can be found by multiplying the displayed values by cell water content per g of cell protein, which is close to $6.5 \text{ ml} \cdot \text{g}^{-1}$ in U937 cells under normal conditions

<i>t</i>	<i>U</i>	<i>na</i>	<i>k</i>	<i>cl</i>	<i>V/A</i>	<i>mun</i>	<i>muk</i>	<i>mucl</i>	<i>prna</i>	<i>prk</i>	<i>prcl</i>
0	-54.7	58	136	63	18.87	-78.2	29.5	38.4	0	0	0
25	-52.9	43.8	150.2	63.1	18.88	-83.9	34	36.6	-0.3353	0.3299	0.0129
50	-52.1	38.5	155.3	63.5	18.97	-86.6	35.6	36.1	-0.1249	0.1162	0.0206
75	-51.8	36.5	157.1	64	19.08	-87.7	36.3	35.9	-0.0468	0.0376	0.0218
Omitted lines											
225	-51.1	35.2	157.3	66.7	19.65	-88	37	36.4	-9E-4	-0.0046	0.0129
250	-51.1	35.2	157.1	67	19.72	-88	37	36.4	-7.5E-4	-0.0042	0.0117
*	<i>na0</i>	<i>k0</i>	<i>cl0</i>	<i>B0</i>	<i>kv</i>	<i>beta</i>	<i>gamma</i>	<i>z</i>			
*	140	5.8	116	48.2	1	0.039	1.5	-2.47			
*	<i>pna</i>	<i>pk</i>	<i>pcl</i>	<i>inc</i>	<i>ikc</i>	<i>inkcc</i>	<i>A/V</i>	<i>hp</i>			
*	0.00317	0.023	0.00354	3E-5	0	0	50.72	250			
*	Fluxes	<i>PUMP</i>	<i>NetCh</i>	<i>NetNC</i>	<i>NetKC</i>	<i>NetNKCC</i>	<i>InCh</i>	<i>InNC</i>	<i>InKC</i>	<i>InNKCC</i>	
*	Na	-1.372	0.9592	0.4165	0	0	0.9962	0.487	0	0	
*	K	0.9146	-0.8979	0	0	0	0.2994	0	0	0	
*	Cl	0	-0.396	0.4165	0	0	0.136	0.487	0	0	
*	<i>OSOR</i>	3.05									

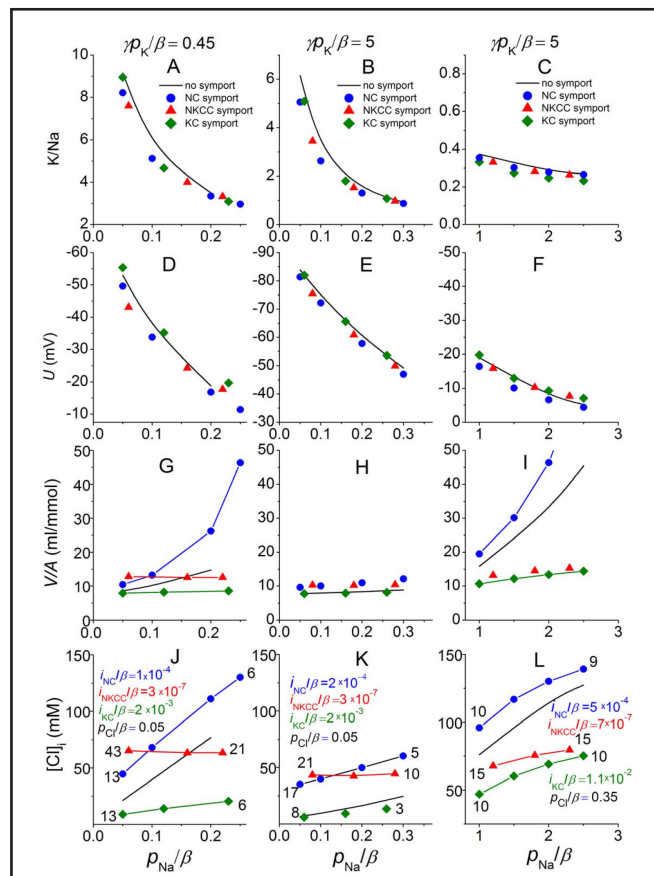
3. If in the process of computation the internal sodium concentration drops below 0.1, a message "LOW SODIUM" appears, and the computation stops.

The executable file BEZ01.exe should be used on Win32 systems together with the file DATA.txt placed in the same directory. The results of computation appear in the file RES.txt. The executable file and DATA.txt file should be obtained by e-mail: verenino@gmail.com or via1938@mail.ru or v.yurinskaya@mail.ru.

Examples of using the executable file

Finding a balanced state of a system with arbitrary kinetic parameters. The program can be used to find intracellular concentrations $[\text{Na}]_i$, $[\text{K}]_i$, $[\text{Cl}]_i$, the MP and cell water content corresponding to balanced ion distribution, i.e. the state when the total influx of each ion species is equal to its efflux and the derivatives are zero (within specified limits). The kinetic parameters, initial intracellular ion concentrations and concentrations of ions and other osmolytes in the external solution are entered into the file DATA.txt (Table 2). One should

Fig. 1. Dependence of cell ion balance on the ratios p_{Na}/β and $\gamma p_K/\beta$ in the presence of various cotransporters. The data were obtained by using a procedure similar to that illustrated in Table 3 but with initial ion concentrations corresponding to $z = -1.30$; the external concentrations were $[Na]_o = 150$ mM, $[K]_o = 5$ mM, $[Cl]_o = 155$ mM. In the presence of cotransport, the curves were computed for the indicated values of kinetic parameters inc/β , $inkcc/\beta$, ikc/β , p_{Cl}/β . The numbers by the curves in panels J, K, and L show the ratios (in %) of cotransport influxes to the Na^+ pump efflux (for NC) or to the K^+ pump influx (for NKCC, KC).



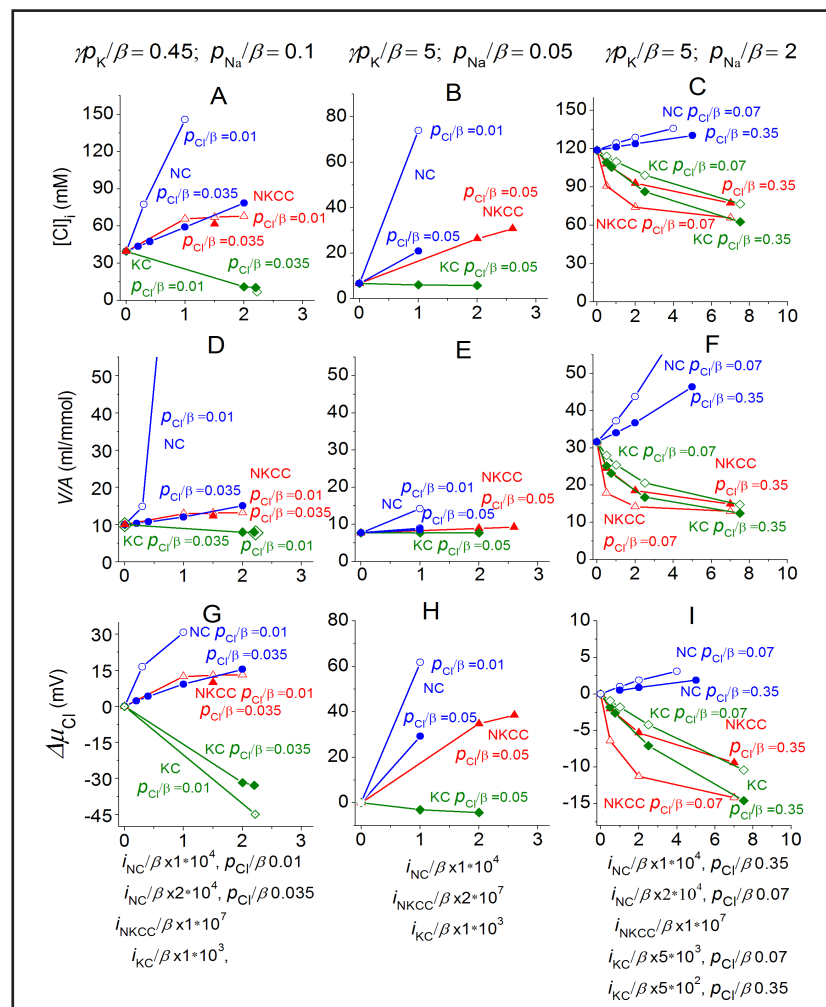
keep in mind that intracellular concentrations $[Na]_i$, $[K]_i$, $[Cl]_i$ and the total extracellular concentration of osmolytes determine z (see Eq. 3). Therefore, the balanced states calculated for different initial $[Na]_i$, $[K]_i$, $[Cl]_i$ may be different even when the composition of the medium and all kinetic parameters are identical.

When running BEZ01.exe, the results appear after some delay in the file RES.txt (Table 3). To illustrate the process, we ran the program for unbalanced initial concentrations $[Na]_i = 58$ mM, $[K]_i = 136$ mM, $[Cl]_i = 63$ mM. The parameters were chosen such as to ensure the balanced concentrations similar to those in U937 cells. One can see that at $t = 250$ min the total Cl^- influx/efflux ratio differs from unity by 5.2 % ($0.4165/0.3960 = 1.052$). This corresponds to $prcl = 0.0117$. Practically, this means that a balanced distribution of Cl^- is achieved at 4 h. The distribution of Na^+ and K^+ at 250 min is even closer to the balanced state. The values $prna$, prk , and $prcl$ in the last columns enable validation of the balance at each time moment.

Balanced state as a function of kinetic parameters. The executable file allows derivation of the dependence of balanced values of $[Na]_i$, $[K]_i$, $[Cl]_i$, MP and cell water content on kinetic parameters characterizing the pump, channels and cotransporters. Importantly, balanced concentrations are determined not by the absolute values of parameters but by their ratios. Indeed, if the left sides of Eqs. 6-8 are set to zero, the equalities remain true if divided by a constant. We prefer to relate the kinetic parameters to the pump rate constant β because it can be obtained directly from the experiment. Only the rate of approaching the balanced state depends on the absolute values of kinetic parameters.

Fig. 1 shows how the Na/K pump activity, Na^+ , K^+ , Cl^- ion channel permeabilities, and the three major types of cotransport should affect ion and water balance and the MP. It can be seen that the type of cells (high-potassium - high MP, high-potassium - low MP or low-potassium - low MP) is determined mainly by the two ratios, $\gamma p_K/\beta$ and p_{Na}/β . In the given examples, the combination of $\gamma p_K/\beta = 0.45$ and $p_{Na}/\beta = 0.05 \div 0.3$ will produce cells with

Fig. 2. Effect of cotransporters on $[Cl]_i$, cell water (V/A) and $\Delta\mu_{Cl}$ for various cell types and p_{Cl^-} . NKCC - triangles, KC - diamonds, NC - circles. The X axis units for cotransporters are shown at the bottom of the Figure. The data were computed as for Fig. 1.



relatively high MP and high K/Na ratios. If $\gamma p_K/\beta$ is increased to 5, the K/Na will drop and the MP will increase further. Increasing the p_{Na}/β ratio while keeping $\gamma p_K/\beta = 5$ will yield low-potassium, low MP cells. Conversely, the values of K/Na, U , V/A and $[Cl]_i$ determine the magnitude of $\gamma p_K/\beta$ and p_{Na}/β . This fact can be used to find the range of variation of these important parameters.

The effect of the cotransporters is the strongest on $[Cl]_i$ and V/A , while the K/Na ratio and MP are only slightly affected. Importantly, the effects of p_K , β and p_{Na} on $[Cl]_i$ and V/A depend on whether cotransporters are present in the system. The NC cotransport enhances, and NKCC or KC cotransport reduces the effect. The relationships between cotransport fluxes and other fluxes are shown in Fig. 1J-1L. Under some circumstances, the NC cotransport can substantially affect cell ion and water even if its share in the overall flux is only 5-10%. Such small fluxes can easily escape detection by inhibitors, and calculations would be the most effective way to study them.

Fig. 2 shows the dependence of $[Cl]_i$, V/A and $\Delta\mu_{Cl}$ on cotransport rate coefficients under different p_{Cl^-} . The effect of the NC cotransport is to increase V/A , $[Cl]_i$ and $\Delta\mu_{Cl}$ (inside positive). Nonzero $\Delta\mu_{Cl}$ at the balanced state means that there is a steady net flux through Cl^- channels that depends on p_{Cl^-} . Therefore the whole ion and water balance becomes dependent on p_{Cl^-} . The KC transport has the opposite effect. The sign of NKCC-induced changes depends on the cell type.

The main conclusion is that the relationships in this multivariable system are quite complex, and a variety of outcomes are possible. The presented executable file enables one to estimate the effects of each species of transporters and channels, as well as their

Fig. 3. Ion balance disturbance caused by turning off the pump at various p_{Cl} . A model without cotransporters was used. The parameters were chosen as follows: $na0$ 140, $k0$ 5.8, $cl0$ 116, $B0$ 48.2, kv 1, na 52.6, k 144.2, cl 11.9, β 0 (at the initial balanced value of 0.039), γ 1.5, p_{Na} 0.006, p_K 0.06, p_{Cl} 0.1 (triangles), or 0.001 (circles), or 0.0001 (solid lines), hp 10000, $inc = ikc = inkcc = 0$.

combination, in every cell type and in every particular case.

Inverse problem

It is relatively straightforward to experimentally measure $[Na]_i$, $[K]_i$, $[Cl]_i$, β and the ratio of the ouabain-sensitive to ouabain-resistant Rb^+ influxes (OSOR) at the balanced state. The other question is how to calculate p_{Na} , p_K , p_{Cl} , i_{NC} , i_{NKCC} , i_{KC} from experimental data. This inverse problem is much harder than finding concentrations from known parameters, and we approach it by trial and error. In the case of U937 cells, $[Na]_i$, $[K]_i$, $[Cl]_i$, cell water content per unit of A , the pump coefficient β and OSOR were measured as described previously [11-15]. These data proved sufficient to find a unique set of parameters corresponding to the balanced state for a model that includes the pump, Na^+ , K^+ , Cl^- channels and a single type of cotransport. One can verify by using BEZ01.exe that different sets of p_{Na} , p_K , p_{Cl} and i_{NC} can yield the same $[Na]_i$, $[K]_i$, $[Cl]_i$, and the cell water content V/A for a given β . However, the ratio OSOR in each case will be different. Because OSOR is easily determined in the experiment, a unique solution to the problem can be found.

When more than one type of cotransport is present, additional information is needed to arrive at a unique solution. Such information can be obtained, for example, by using cotransport blockers. In doing so, one needs to keep in mind that: (1) as we showed earlier, small fluxes that may easily escape detection by inhibitors can strongly influence the system; (2) many cells have significant one-to-one exchange with a zero net flux. This exchange seriously complicates the study of minor components by means of inhibitors. The analysis of flux balance using BEZ01.exe enables one to tell when a minor flux can be detected using inhibitors and when this would be impractical.

The reason that only the NC symport was assumed in modeling of U937 cells was that the transmembrane Cl^- electrochemical potential difference in these cells ($\Delta\mu_{Cl}$ in Fig. 2, mu_{Cl} in Table 3) is internally positive, whereas KC would create a negative $\Delta\mu_{Cl}$ (Fig. 2) and NKCC should be ineffective because of the nearly-zero sum $mun+muk+mu_{Cl}$ (Table 3). The parameters found in this way were taken as inherent for U937 cells in examples presented in Tables 1 and 2.

Errors in the determination of the parameters may result from errors in the measurement of cell ion and water content or may be due to the fact that the state that was assumed to be balanced was still in transition. Errors of the first type can be estimated by analysis of the relationships like those in Fig. 1 and 2 or by using BEZ01.exe. Errors of the second type will be discussed below.

Dynamics of ion balance caused by blocking the pump: prediction and experiment

It is well known that without the pump, a balanced ion distribution across the cell membrane cannot exist, and the cells would be swelling infinitely [6, 9, 18]. Nevertheless, real cells following pump inhibition by ouabain often do not swell for a long time. Low

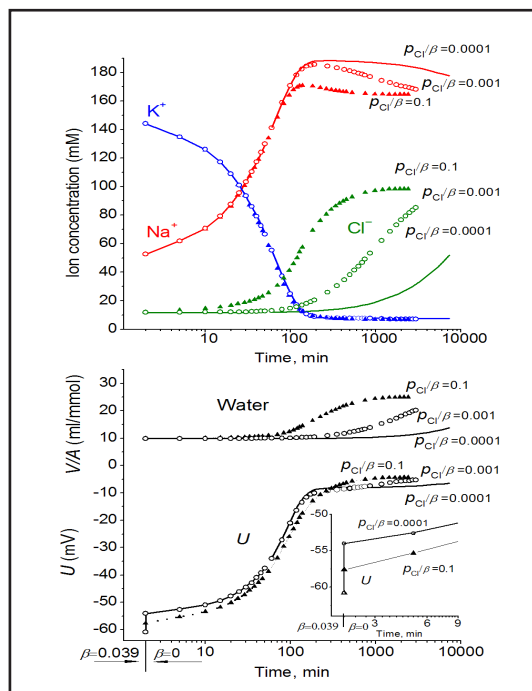
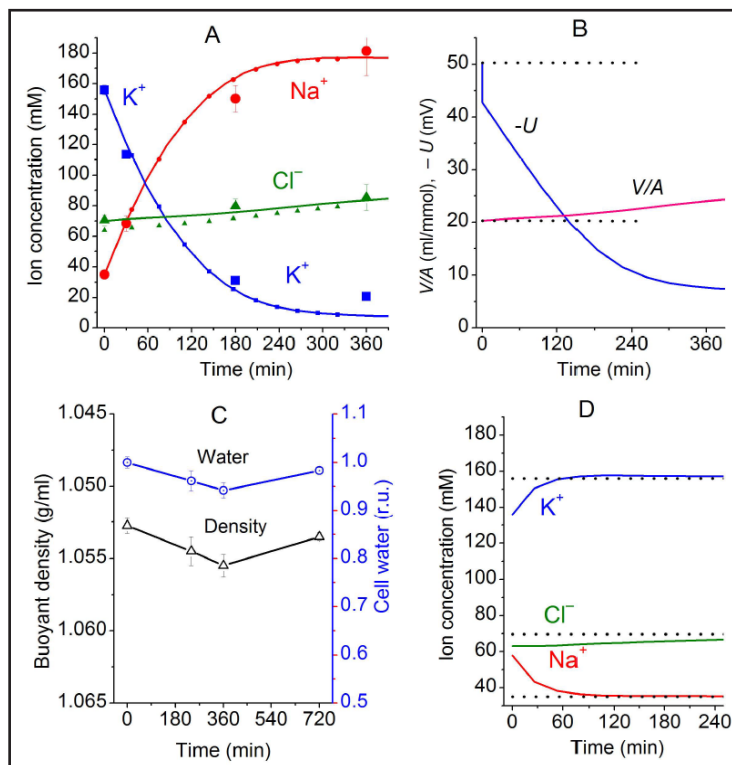


Fig. 4. Predicted and observed changes in U937 cells following pump inhibition with ouabain. (A) Time course of the changes in $[Na]_i$, $[K]_i$, and $[Cl]_i$. Large symbols - experimental data (means $\pm$ SE, $n=4$). Solid lines - data calculated for the following parameters: $na0$ 140, $k0$ 5.8, $cl0$ 116, $B0$ 48.2, kv 1, na 35, k 156, cl 70, β 0, pna 0.00317, pk 0.023, pcl 0.00354, inc $3E-5$, $ikc = inkcc = 0$, hp 400, (before blocking the pump β was 0.039), $OSOR$ 3.06, γ 1.5. Small symbols - data calculated for parameters: na 37.4, k 156.3, cl 63.7, pna 0.00367, pk 0.0247, pcl 0.0035, inc $2.6E-5$. (B) Time course of the changes in MP ($-U$, mV) and cell water content (V/A , ml/mmol) calculated for the same conditions as for solid lines in (A). Initial levels are shown by the dashed line. (C) Time course of changes in cell buoyant density ($g \cdot ml^{-1}$) and cell water content (relative units, means $\pm$ SE, $n=8$, 2, 3, 3 for $t=0, 240, 360, 720$ min, respectively). Cell water was calculated by density as described [13, 16]. (D) Changes in $[Na]_i$, $[K]_i$, and $[Cl]_i$ in the vicinity of the balanced state starting from $na0$ 140, $k0$ 5.8, $cl0$ 116, $B0$ 48.2, kv 1, na 58, k 136, cl 63 and calculated for the parameters: β 0.039, pna 0.00317, pk 0.023, pcl 0.00354, inc $3E-5$, $ikc = inkcc = 0$.



permeability of Cl^- channels as well as the involvement of the Ca-ATPase pump, Na-Ca antiporter and NKCC cotransport have been listed as possible reasons for the delay in swelling [19–21]. Fig. 3 illustrates the development of a disturbance caused by stopping the pump in the simplest cell model with Na^+ , K^+ and Cl^- channels and no symports. One can see that substantial water accumulation starts only when the K^+/Na^+ inversion is almost complete. Thus, the simple model, which only assumes low permeability for Cl^- , is sufficient to explain the slow swelling caused by ouabain. No significant changes in the rate of K^+-Na^+ redistribution occur when p_{Cl} is varied by as much as three orders of magnitude, and only water and Cl^- dynamics are affected. It should be noted that the prompt shift in the MP due to pump blocking, i.e. the “electrogenic” effect of the pump, is sensitive to p_{Cl} (see insert in Fig. 3). This is because the contribution of the pump to the total transmembrane current depends on all ion channels, including those for Cl^- .

Fig. 4A–4C compares the observed and predicted time courses of ion and water changes caused by ouabain. The parameters used for calculations were derived from the analysis of the balanced state of untreated U937 cells. One could expect that the real properties of the ion transport machinery might change during a transition accompanied by such large variations in intracellular Na^+ and K^+ and in the MP. Nevertheless, the simplest model developed for the balanced state turned out to be quite effective in quantitative prediction of the complex interplay of ion fluxes after pump inhibition. This strongly implies that the rate coefficients for ion transfer via channels and NC cotransport, as well as the intracellular content of impermeant osmolytes A and their charge z , remained stable during the transition. This indicates also that the flux equations used in the model are right.

The question may arise as to the magnitude of an error in calculating the dynamics of transition if the parameters were based on an incompletely balanced state, e.g. after equilibration of cells for 1 h instead of 4 h. Fig. 4D shows the changes in $[Na]_i$, $[K]_i$ and $[Cl]_i$ in the vicinity of the balanced state. Only $[Cl]_i$ changes more or less appreciably (from 63.7 to 66.8 mM) between 1 and 4 h. The kinetics of transition calculated using parameters based on an incomplete one-hour equilibration is shown in Fig. 4A by small symbols. Evidently, the introduced inaccuracy did not lead to significant errors. Thus, we conclude that the agreement between the observed and predicted dynamics is sufficiently good and holds in spite of possible errors in the parameters.

Discussion

Monovalent ions, cell volume and MP form a self-contained physiological “circuit”. The ability to analyze and predict its behavior is essential for understanding the action of membrane-active drugs, apoptotic vs. oncotic cell death or for calibration of intracellular ion measurements. Unfortunately, all too often the complexity of the system makes it tempting to bypass the rigorous analysis, resulting in hasty and erroneous conclusions.

The main result of the present study is the executable file for calculation and analysis of monovalent ion fluxes across the plasma membrane mediated by the Na/K pump, ion channels, and the three types of cotransporters for any type of animal cell. Fluxes of Na^+ , K^+ , and Cl^- across the plasma membrane in a whole cell are highly interdependent first and foremost due to the conditions of macroscopic electroneutrality and osmotic balance. These are all well-established principles that are automatically satisfied by our model, enabling one to focus on specific changes in channels and transporters and on the effects of various conditions. Each ion pathway is characterized by a single parameter - the proportionality coefficient between the driving force and the ion flux via that particular pathway. For the driving force we used the traditional definition as the electrochemical potential difference for a single ion in case of its moving through the electroconductive channels and the sum of the electrochemical potential differences in case of the coupled ion transfer via co- and counter-transporters. To simplify the model, subtle dependencies of the rate coefficients on concentrations of other ions, as well as on the MP and other factors, were neglected. The Na^+ and K^+ fluxes mediated by the pump were expressed as a linear function of intracellular Na^+ concentration with a fixed Na/K flux ratio of 1.5 [1]. These simplifications made the model more general and easier to use.

The reported data illustrate several of important general principles. First, the cell type (high-potassium with high MP, high-potassium or low-potassium, with low MP) is determined primarily by the two ratios, $\gamma p_K/\beta$ and p_{Na}/β . Importantly, the balanced state depends not on the absolute values of the coefficients but on their ratios. The pump rate coefficient would be most appropriate as a reference because it can be obtained directly by measuring the intracellular Na^+ and ouabain-sensitive Rb^+ or K^+ influxes. Only the rate of approaching the balanced state depends on the absolute values of kinetic parameters. Second, the cotransporters NC, NKCC and KC should strongly affect the intracellular chloride and water content but to a much lesser extent the cell K/Na ratio and MP. Their effects would vary significantly with the cell type and other conditions and depend on the permeability of Cl^- channels, i.e. on p_{Cl} . The most one can say is that the NC cotransport tends to increase intracellular chloride and water content, whereas NKCC, and to a certain extent, KC cotransport stabilize water balance against changes in the Na/K pump and channels. Under some circumstances, the NC cotransport can substantially affect cell ion and water balance even if its share in the overall flux is only 5-10 %. Such small fluxes can easily escape detection by inhibitors, and calculations would be the most effective way to study them.

The presented approach was applied to analyzing the time course of ion and water disturbance in U937 cells caused by stopping the sodium pump with ouabain. It turned out, rather unexpectedly, that using the parameters found for the balanced state gave a near-

perfect match between the observed and calculated time courses. One should remember that our mathematical model, as well as those by all previous authors, is based on the assumptions that: (1) monovalent ion fluxes are determined by their concentration gradients and MP according to the indicated original equations; (2) the intracellular content of impermeant osmolytes and their average charge remain constant during transient process; (3) all the kinetic parameters remain constant. Thus, three different types of causes could lead to discrepancy between prediction and reality. Data obtained on U937 cells showed that in the considered case the original flux equations were adequate and changes in the kinetic parameters, as well as in the intracellular content of impermeant osmolytes and their charge, were insignificant in spite of inversion of the intracellular K^+/Na^+ ratio and a large decrease in MP. We believe that computational modeling using our executable file could be a promising tool for revealing possible changes in the machinery regulating MP and monovalent ion gradients.

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