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Regulation of Human Red Blood Cell Volume. The Role of Calcium-Activated K^+ Channels

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The role of calcium ions in the mammalian red blood cell volume regulation is investigated using mathematical modelling. It is shown that the effect of Ca^{2+}-activated K^+ channels on the red blood cell volume stabilization is rather strong. Functioning of these channels can provide a 5–10-fold increase in the stabilization volume coefficient. This contribution may be estimated exactly if the ration of maximal K^+ channel permeability to a Ca-binding constant of these channels is known. An experiment design which enables this ratio to be measured for intact red blood cells is suggested.

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Regulation of ionic homeostasis and volume in erythrocytes are dealt with in numerous studies [1–7]. Some works analyze the mathematical models of this regulation [1, 5, 8, 9]. The basic criterion to estimate the operating efficiency of any stabilizer is the stabilization coefficient. For erythrocyte volume this is the ratio of volume increment to the change in membrane permeability for ions (at small changes). As analysis of these models shows, the functioning of Na^+,K^+ pump leads to the stabilization of erythrocyte volume. However, the stabilization coefficient in the models proves to be about 10 times less that in the experiment [10].

Virtually all models of ionic homeostasis are based on the same postulates and systems of equations described the most consistently in the work by Jacobsson [8]. None of the models takes into consideration calcium regulation, in spite of the fact that the effect of calcium on potassium fluxes in erythrocytes is great and has been described a long time ago [11].

Using a mathematical model, this work investigates the role of calcium homeostasis in the regulation of erythrocyte volume.

MATHEMATICAL MODEL

The model is described by a system of 6 equations including 3 nonlinearly differential and 3 algebraic equations:

$$\frac{d\left(X_1 \frac{V}{V_0}\right)}{dt} = KU_1 + G_1 \frac{\Delta\varphi - \frac{F}{RT}\left(X_{1e} - X_1 \exp\left(\Delta\varphi \frac{F}{RT}\right)\right)}{\exp\left(\Delta\varphi \frac{F}{RT}\right) - 1}; \quad (1)$$

$$\frac{d\left(X_2\frac{V}{V_0}\right)}{dt} = -NU_1 + G_2\frac{\Delta\varphi\frac{F}{RT}\left(X_{2e} - X_2\exp\left(\Delta\varphi\frac{F}{RT}\right)\right)}{\exp\left(\Delta\varphi\frac{F}{RT}\right)-1}; \quad (2)$$

$$\frac{d\left(X_3\frac{V}{V_0}\right)}{dt} = -U + G_3\frac{2\Delta\varphi\frac{F}{RT}\left(X_{3e} - X_3\exp\left(2\Delta\varphi\frac{F}{RT}\right)\right)}{\exp\left(\Delta\varphi\frac{F}{RT}\right)-1}; \quad (3)$$

$$X_1 + X_2 - X_4 - Mw = 0; \quad (4)$$

$$X_1 + X_2 + X_4 + w = X_{1e} + X_{2e} + X_{4e} + r = 2L; \quad (5)$$

$$X_4 = X_{4e}\exp\left(\Delta\varphi\frac{F}{RT}\right); \quad (6)$$

where $G_1 = G_1(X_3) = p_1S/V_0$, $G_2 = p_2S/V_0$, $G_3 = p_3S/V_0$; S is the area of the membrane surface; X_1, X_2, X_3, X_4 are intracellular concentrations and $X_{1e}, X_{2e}, X_{3e}, X_{4e}$ are extracellular concentrations of K^+, Na^+, Ca^{2+} ions and permeant anions (Cl^- and $HCO3^-$), respectively; V_1, V_0 are the current and physiological volumes of the cell; $\Delta\varphi$ is the transmembrane potential difference; $p_1 = p_1(X_3)$, p_2, p_3 are the membrane permeability for K^+, Na^+ and Ca^{2+}, respectively; p_{10}, p_{20}, p_{30} are the respective permeabilities in the physiological state; K and N are the number of K^+ and Na^+ ions transferred by Na^+,K^+-ATPase during the hydrolysis of one ATP molecule; w is the intracellular amount of molecules not penetrating through the membrane; M is the mean charge of the nonpenetrating molecules; U_1 is the operation rate of Na^+,K^+-ATPase; U_2 is the operation rate of Ca^{2+}-ATPase; $2L$ is the total concentration of the osmotically active substances in the medium; r is the extracellular concentration of the molecules not penetrating through the membrane.

Differential equations (1, 2) describe the change in the intracellular concentrations of K^+ and Na^+ ions due to the passive leakage of cations by the concentration gradients in accordance with the Goldman approximation [12] as well as owing to the active transport of cations by Na^+,K^+-ATPase (U_1). Splitting one ATP molecule, the ATPase releases N ions of sodium in exchange to K ions of potassium.

Differential equation (3) describes the behaviour of the intracellular concentration of calcium and comprises the terms similar to those of equation (1).

The algebraic equations describe the condition of electroneutrality of the intracellular medium (equation 4). The positive M in this case corresponds to the negative mean charge

of nonpenetrating molecules, to the condition of the osmotic balance between the intra- and extracellular medium (equation 5) as well as to the condition of Donnan equilibrium for anions (equation 6) in the assumption that the membrane is freely penetrable for them.

Equations (4) and (5) do not include the concentration of calcium because it does not exceed a fraction of a percent of the sum of the concentrations both inside and outside the cell. This greatly simplifies the model since calcium in this case affects only membrane permeability for potassium.

To study the model, we select the values of parameters and set the explicit forms of the functions describing the dependences of rates of Na^+,K^+-ATPase (U_1), Ca^{2+}-ATPase (U_2) and membrane permeability for potassium ($G_1 = G_1(X_3)$) on concentrations.

Assuming that the osmocity of the medium is determined by only the extracellular concentrations of permeant ions (i.e., $r = 0$); knowing under physiological conditions the extracellular concentrations of ions ($X_{1e} = 5, X_{2e} = 145, X_{4e} = 160$ mM) [1, 13,14], the intracellular concentrations of ions ($X_1 = 110, X_2 = 10, X_4 = 110$ mM) [1, 13, 14] as well as the operation rate of Na^+,K^+-ATPase under physiological conditions ($U_{10} = 2.1 \cdot 10^{-4}$ mM/s, $K = 2, N = 3$) [19], we can calculate (from equations (1), (2), (4–6) in the steady state) the values of parameters $w = 70$ mM, $M = 0.143$, $G_{10} = 5.1 \cdot 10^{-6}$, $G_{20} = 4.2 \cdot 10^{-6}$ s^{-1}. These magnitudes do not depend on the parameters of calcium regulation.

Comparison of parameters M, w, G_{10}, G_{20} with the literature data [1, 2, 9] shows that the values calculated are within the limits of the existing experimental spread.

The most reliable experimentally determined parameters to affect potassium homeostasis are, in our opinion, only intra- and extracellular concentrations of potassium ($X_{3e} = 1.0, X_{30} = 0.93 \cdot 10^{-4}$ M), the maximal activity of Ca^{2+}-ATPase ($U_{2max} = 2.8 \cdot 10^{-3}$ mM/s) [15, 16] and the maximal permeability of the membrane for potassium $G_{1emax}(X_3) = 2.6 \cdot 10^{-3}$ s^{-1}.

The magnitudes of the other parameters and the form of the functions which determine the operation rates of ATPases and the potassium permeability are to be investigated and shall be considered below.

RESULTS AND DISCUSSION

Dynamic Analysis of the Model. The dynamics of the model was studied with the following assumptions.

1. The rate of Na^+,K^+-ATPase is a monotonously growing function of intracellular concentrations of Na and ATP. It does not depend on other variables. We shall consider only the situations when the extracellular concentration of potassium is constant. Its effect on the operation rate of this ATPase is included into the ATPase activity.

2. The rate of Ca^{2+}-ATPase is a monotonously growing function of the intracellular concentration of Ca [17–19].

3. The permeability of the erythrocyte membrane for potassium is a monotonously growing function of the concentration of intracellular calcium.

Analysis of the model showed that within the range of admissible magnitudes of the volume the system has the only stationary state – a stable node. The dynamics of the model is characterized by three time scales. The fastest variable – intracellular concentration of calcium – changes with the characteristic times on the order of seconds. The concentration of sodium changes 1000 times slower – the characteristic times are tens of minutes. The

changes of erythrocyte volume are still by two orders of magnitude higher – tens of hours.

As the parameters of the model change, the loss of stability usually occurs in an only way: the passive fluxes exceed the maximal rates of pumping the ions, the gradients level up and the volume grows to infinity.

Effect of Ca^{2+}-activated Potassium Channels on the Stabilization of Volume. The presence of the two-gradient system of osmoregulation results in a new way of controlling the cell volume. A high concentration of potassium emerges whose gradient is directed from the cell outwards. If, in response to an external effect which increases the volume, a selective potassium permeability is established in the erythrocyte membrane, K^+ and permeant anions shall flow outwards from the cell. This will lead to a decrease in the volume. It seems that the nature actively uses this regulation.

Besides membrane permeability nonselective for cations (in which case the permeabilities for sodium and potassium ions change synchronously) due to 'defects' in the membrane [5, 20], several mechanisms to change membrane permeability selectively have been described for various cells. These are various systems of transport (at the expense of the concentration gradient, without the loss of energy of ATP hydrolysis). For instance, the Cl^--dependent Na^+,K^+-cotransport system, Cl^--dependent K^+-transport system etc. [21, 22].

In mature mammalian erythrocytes the only device to establish a selective permeability of the membrane are Ca^{2+}-dependent potassium channels. Release of potassium from erythrocytes at the increase of intracellular [Ca^{2+}] had been described in 1958 [11]. Since then, the structure and functioning of the channels responsible for this has been studied in detail [15, 17, 23, 24]; however, the physiological role of this system is not yet clear.

Consider the effect of Ca^{2+}-dependent channels on the regulation of volume. Introduce the functions describing the dependence of membrane permeability for potassium on the concentration of calcium and the dependences of the rates of Na^+,K^+- and Ca^{2+}-ATPases on calcium and other variables.

Permeability of the membrane for potassium can be presented as

$$G_1 = G_{10} + G'_{10} + g_1(X_3), \tag{7}$$

where $G_{10} + G'_{10}$ is the physiological meaning of potassium permeability consisting of the Ca^{2+}-independent leakage due to membrane 'defects' (G_{10}) and the leakage via K^+ channels (G'_{10}) corresponding to the physiological concentration of Ca^{2+}. The calcium activation of K^+ channels is described by the function $g_1(X_3)$, In accordance to the results of [17,23], this function can be represented in the following form:

$$g_1(X_3) = (G_{1\max} \cdot X_3)/(X_3 + k_1), \tag{8}$$

where $G_{1\max}$ is the maximal permeability of K^+ channels and k_1 is the constant of dissociation of Ca^{2+} ions with these channels.

To consider the effects of nonselective changes of membrane permeability on the changes of volume, the value gG_{20} should be added to permeabilities G_1, G_2 and G_3. It should be introduced in such a way that the increment of permeability would be measured in the units of physiologically normal permeability of a cation, for instance, sodium.Thus, $g = 1$ means that the permeability of the membrane increased twofold.

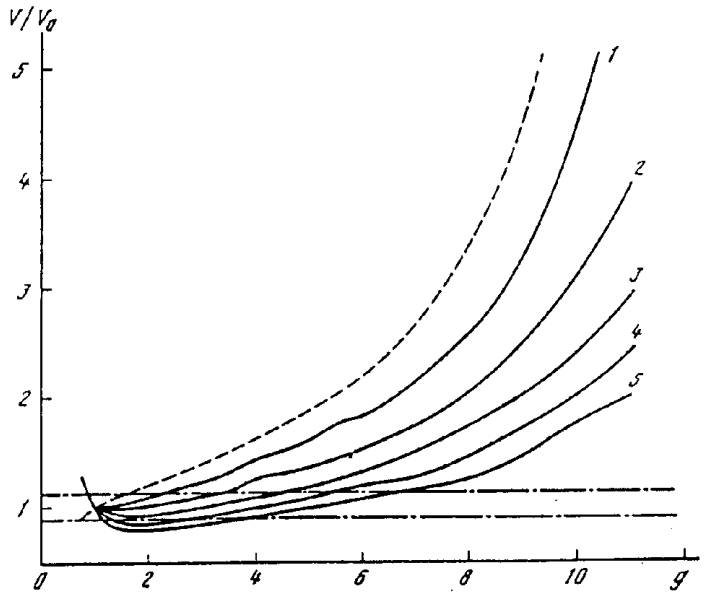

FIGURE 1. Effect of potassium regulation on the stationary dependence of the relative volume of the erythrocyte on the nonselective change in the permeability of the membrane, g, at various parameters of Ca^{2+}-regulated K^+ channels (k_1, μM: 300 (*1*); 150 (*2*); 100 (*3*); 75 (*4*); 60 (*5*). The broken line shows the dependence plotted without taking into account the potassium regulation; dash-dot line, the admissible (10%) range of volume change.

Suppose the rate of Na^+,K^+-ATPase operation depends linearly on the intracellular concentration of Na^+ ions and ATP:

$$U_1 = \alpha_1[\mathrm{ATP}]X_2 \cdot V_0/V, \tag{9}$$

where α_1 is the activity of Na^+,K^+-ATPase and [ATP] is the concentration of ATP.

According to [23], the rate of pumping Ca^{2+} ions from the cell by Ca^{2+}-ATPase can be described by the formula:

$$U_2 = \alpha_1 X_3 (X_3 + k_2)^h, \tag{10}$$

where k_2 is the constant of dissociation of Ca^{2+} ions with their binding site on the intracellular surface of the Ca^{2+} pump; h is the number of independent binding sites.

In accordance with the results of [18, 19] $h = 2$. Under physiological conditions the passive influx of Ca^{2+} is estimated as $U_{20} = 2.8 \cdot 10^{-6}$ mM/s [25]. Under steady-state conditions, from equation (3) it follows that the rate of Ca^{2+} operation is equal to the passive influx of Ca^{2+}. Hence, knowing the concentrations of calcium under physiological conditions, we can assess $k_2 = 2.9 \cdot 10^{-3}$ mM which is consistent with the results of [17, 19]. The magnitude of this influx can be used to find the normal permeability of the membrane for calcium. Substituting it into eq. (3) in a steady state, at a physiological value of the concentration of Ca^{2+} and the transmembrane potential E = 8 mM [9, 10], we find

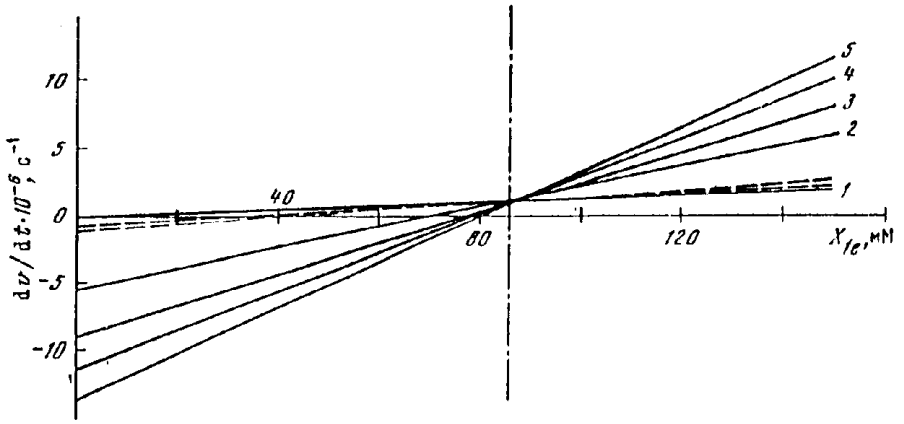

FIGURE 2. Effect of calcium regulation on the dependence of the rate of change in the relative volume of the erythrocyte on the extracellular concentration of potassium, X_{1e}, at various permeabilities of the membrane for calcium: g = 1 (*1*); 5 (*2*); 9 (*3*); 3 (*4*); 17 (*5*). Parameters of Ca^{2+}-regulated K^+ channels: k_1 = 60 μM (solid lines) and 1.2 mM (broken lines).

$G_{20} = 2.0 \cdot 10^{-6}$ s^{-1}.

The experimental magnitudes of the parameters of K^+ channels in erythrocytes and, primarily, their sensitivity to Ca^{2+} (k_1) are observed to vary greatly [17, 23, 24]. The effect of potassium regulation on the steady state dependence of the relative volume of erythrocyte on the nonselective change in membrane permeability for K^+, Na^+, Ca^{2+} at various parameters of Ca^{2+}-regulated K^+ channels is shown in Fig. 1. As seen, the operation of these channels can strongly increase the range of admissible changes of erythrocyte volume, while the effect depends on the sensitivity of the channels to Ca^{2+}.

To qualitatively characterize the stabilization of the volume, let us call the interval of changes in permeability g, in which the volume changes no more than 10%, as the range of volume stabilization.

Thus, the range of volume stabilization shown in Fig. 1 by a dash-dot line changes respectively from 2 to 7 as the constant k_1 changes from 300 to 60 μM (curves *1* and *5*). The respective magnitude in the absence of calcium regulation is 1.5 (broken line). The more sensitive the channels are to Ca^{2+}, the more the volume decreases at small changes in permeability; for curves *4*, *5* it even exceeds the admissible level.

Thus, the major physiological role of Ca^{2+}-regulated K^+ channels in erythrocytes consists in the improved stabilization of the volume at nonspecific changes in membrane permeability.

A Method of Determining the Parameters of Potassium Regulation on Intact Erythrocytes. To assess the contribution of K^+ channels to the stabilization of erythrocyte volume, it is necessary to measure the ratio of the maximal permeability of the channels to the constant of Ca^{2+} dissociation with these channels. It is yet not feasible to measure these parameters on intact erythrocytes. However, the analysis of the model makes it possible to assess this ratio without disturbing the erythrocytes.

At the change in the intracellular concentration of Ca^{2+}, for instance, using the ionophore A23187, the volume of the erythrocyte should change in accordance with Fig. 1. The

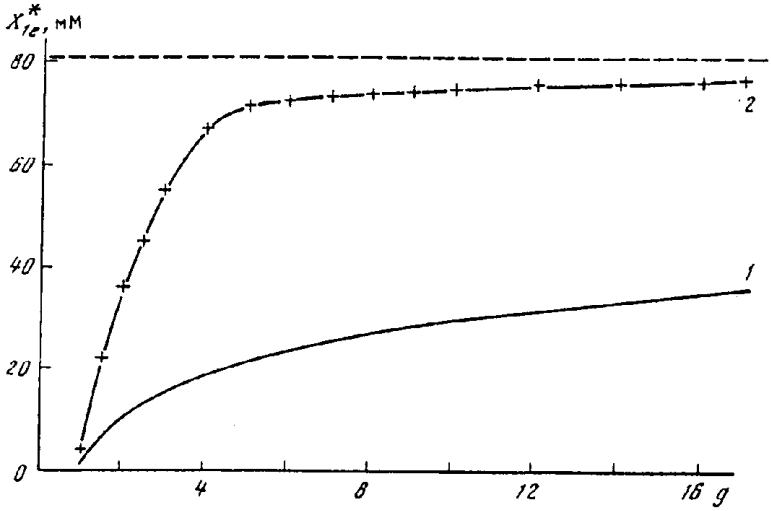

FIGURE 3. Dependence of the extracellular concentration of potassium (X^*_{1e}), corresponding to an invariable volume, on membrane permeability for Ca^{2+} (see the legend to Fig. 2) at various values of the parameter of K^+ channels: k_1 = 1.2 mM (*1*); 60 μM (*2*). The broken line is the ultimate value of X^*_{1e}.

kinetics of erythrocyte change to a new steady state level, as shown by calculations, is such that the first several hours the volume changes almost linearly. One can attempt to compensate the changes of the volume by changing the ratio of the extracellular concentrations of K^+ and Na^+ (leaving their sum constant).

Figure 2 shows the dependence of the rate of erythrocyte volume change (at a linear part of the plot) on the extracellular concentration of potassium. As seen, for each magnitude of membrane permeability for Ca^{2+} there is its extracellular concentration of potassium (X^*_{1e}) at which changes of the volume are completely compensated. This characteristic value of $[K^+]$ at a given permeability depends only on the parameters of K^+ channels: the more sensitive the channels are to Ca^{2+} (i.e., the greater G_{1max}/k_1), the larger this concentration.

Figure 3 shows a dependence of X^*_{1e} on g at k_1 = 1.2 mM (curve *1*) and 60 μM (curve *2*). As seen, the strongest changes are observed under physiological conditions. Therefore, to assess the contribution of Ca^{2+}-regulated K^+ channels to the stabilization of erythrocyte volume, it is sufficient to measure the change of X^*_{1e} at a small increment of potassium permeability of the membrane on intact erythrocytes and compare this magnitude with the one measured on the erythrocytes with blocked K^+ channels (using an inhibitor or in a calcium-free medium).

The experiment proposed shall make it possible not only to assess the value of the ratio of significant molecular constants but, more important, shall enable one to check if the role of the Gardosh effect in the regulation of erythrocyte volume is really great.

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