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PHYSIOLOGY

A Mathematical Model of the Cell Volume Regulation in a Hypotonic Medium

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Epithelial cells contact the environment where osmotic pressure may change in a wide range. Efficient mechanisms providing for cell volume regulation are necessary for maintaining osmotic balance, especially in a hypotonic medium. In this work, we have experimentally studied the changes in the volume of the principal cells of the renal collecting ducts in a hypoosmotic medium and proposed a mathematical model describing the regulatory volume decrease (RVD).

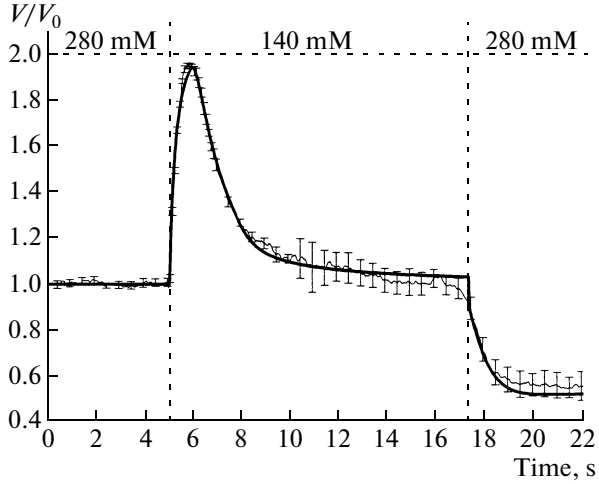
Hypotonic shock was experimentally studied using the principal cells of microdissected rat renal collecting ducts as described earlier [13], using fragments of the outer medullar collecting ducts (OMCDs) of 60-day-old Wistar rats of both sexes. In the experiment, the osmolarity of the external medium was halved during approximately 0.1 s to keep a cell in a hypotonic medium for 12 s; then, the normal osmolarity was restored. The changes in cell volume were recorded by a fluorescence technique and expressed as relative values [12]. All the experimental records were obtained in six independent experiments ($n = 6$), and the data were expressed as $M \pm SE$. As evident from the figure, the hypoosmotic shock was accompanied by a drastic increase in cell volume followed by a regulatory volume decrease. The volume decrease in a hypotonic medium was determined by that the cell lost part of its osmolytes: after the shock, the equilibrium volume in an isotonic medium was about 60% of the initial value.

The mathematical model of a cell is a system of ordinary differential equations describing the changes in cell volume V and intracellular amounts of the ions K^+, Na^+, and Cl^- and the organic anions X (n_{Na}, n_K, n_{Cl}, and n_X) caused by the transmembrane fluxes of water and ions via water and ion channels, Na/K pump, and KCC and NKCC cotransporters according to the data of [8, 9, 11, 15]. The area of the membrane surface, A, was assumed to be constant. The transmembrane electric potential, E_m, was calculated from the current total intracellular charge using the value of the membrane specific electric capacity, C_m [1]. Approximation of the cell volume profile using the mathematical model gave quantitative estimates and time characteristics of the fluxes of the main osmolytes and plasma membrane permeabilities during cell volume regulation in a hypotonic medium. The main equations of this model are

$$\frac{dn_{Na}}{dt} = A\left[-3J_P + J_{Na} + J_{NKCC}\right],$$

$$\frac{dn_K}{dt} = A\left[2J_P + J_K + J_{KCC} + J_{NKCC}\right],$$

$$\frac{dn_{Cl}}{dt} = A\left[J_{Cl} + 2J_{NKCC} + J_{KCC}\right],$$



Simulation of the RVD response. The dependence of the relative cell volume on time (bold line, model and thin line, experiment with indication of the standard error). The upper scale shows the changes in total extracellular ion concentration.

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$$\frac{dn_X}{dt} = AJ_X,$$

$$\frac{dV}{dt} = AV_W P_W \left[\frac{n_{Na} + n_K + n_{Cl} + n_X}{V} - \Pi_e\right],$$

$$E_m = F\frac{n_{Na} + n_K - n_{Cl} + zn_X}{AC_m}.$$

The Na^+, K^+, and Cl^- fluxes through the ion channels were simulated using the Fick and Nernst–Planck diffusion equations, with the Goldman–Hodgkin–Katz transformations taken into account [2, 5]:

$$J_{Na} = P_{Na}\varepsilon(u)\left[[Na^+]_{out}\exp\left(-\frac{u}{2}\right) - \frac{n_{Na}}{V}\exp\left(\frac{u}{2}\right)\right],$$

$$J_K = P_K\varepsilon(u)\left[[K^+]_{out}\exp\left(-\frac{u}{2}\right) - \frac{n_K}{V}\exp\left(\frac{u}{2}\right)\right],$$

$$J_{Cl} = P_{Cl}\varepsilon(u)\left[[Cl^-]_{out}\exp\left(\frac{u}{2}\right) - \frac{n_{Cl}}{V}\exp\left(-\frac{u}{2}\right)\right],$$

$$J_X = -P_X\varepsilon(u)\left[\frac{n_X}{V}\exp\left(\frac{zu}{2}\right)\right],$$

where $u = FE_m/RT$ and the function $\varepsilon(u) = \frac{u}{\left[\exp\left(\frac{u}{2}\right) - \exp\left(-\frac{u}{2}\right)\right]}$. The equation for the flux of organic anions from the cell, J_X takes into account that $[X]_{out} = 0$.

The flux J_P provided by the Na/K pump, was determined on the basis of a stationary state of the enzyme function [3, 4]. The fluxes via the KCC and NKCC cotransporters were determined by the transmembrane difference in ion concentrations and were proportional to the permeability parameters Q_{NKCC} and Q_{KCC} [6]:

$$J_{NKCC} = Q_{NKCC}([Na^+]_{out}[K^+]_{out}[Cl^-]^2_{out} - [Na^+]_{in}[K^+]_{in}[Cl^-]^2_{in});$$

$$J_{KCC} = Q_{KCC}([K^+]_{out}[Cl^-]_{out} - [K^+]_{in}[Cl^-]_{in}).$$

The parameters of permeability P_{Na}, P_K, and P_{Cl}; cotransport Q_{KCC} and Q_{NKCC}; and density of Na/K pump corresponding to a stationary state were chosen so that the intracellular concentrations $[Na^+]_{in}$, $[K^+]_{in}$, and $[Cl^-]_{in}$ and the resting membrane potential corresponded to the known experimental data for such cells [10, 14]. The extracellular osmolarity, Π_e, was controlled by the experimental protocol used while the intracellular osmolarity was determined by the total concentration of Na^+, K^+, and Cl^- ions and the organic ions X. The amount of the organic anions X was selected so that the initial total concentration of intracellular osmolytes was equal to the extracellular concentration (280 mM). The mean charge of the organic ions, z, was chosen to provide an approximate electric neutrality of the intracellular medium.

The equations were integrated using a fourth-order Runge–Kutta method with an integration step of 10^{-5} s, which is considerably shorter than the recording interval and the characteristic times of the simulated processes.

The RVD mechanism in the model is described as an increase in the permeabilities for the ions K^+, Cl^-, and organic anions X, which leads to a drastic increase in the fluxes of the corresponding ions from the cell. The required dependences were manually selected using minimization of the sum of the squared differences between relative cell volumes at each time moment as the criterion for similarity between the experimental and calculated data.

According to the experimental data, the cell volume increases at the beginning of swelling (for 0.9 s) as an ideal osmometer, which suggests the absence of a considerable outflux of the osmolytes during this period. In the model, this is reflected by a delay in switching on the RVD processes by 0.9 s after the change in ambient osmolarity. Then, it was assumed as a first approximation that the permeabilities are controlled by the current cell volume. The results of calculations allowed us to select the degree of increase in permeabilities and the type of their dependences on the volume. At $\frac{V}{V_0} > 1.3$, the permeabilities P_K, P_{Cl}, and Q_{KCC} were multiplied by the coefficient $G_{max} = 1100$; as for the organic ions (P_X), the increase was twofold smaller. In the interval $1 < \frac{V}{V_0} < 1.3$, the permeabilities gradually increased (the coefficient of increase G depended on the volume). An acceptable agreement with the experiment is achieved for $G \propto \left(\frac{V}{V_0} - 1\right)^2$, where $G = G_{max}$ at $\frac{V}{V_0} = 1.3$ (a continuous function). This smoothing has no effect on the interval of volume increase, since the value of $1.3V_0$ is reached before the RVD delay time of 0.9 s. The calculations demonstrated that the described model satisfactory described the cell behavior (figure).

The constructed model is preliminary, because it takes into account mainly the mechanical activation of RVD. However, even this level of modelling makes it possible to assess the physiological mechanisms of cell adaptation. In particular, the calculations suggest that the effective RVD requires at least three orders of magnitude increase of the ion permeabilities. In addition, this suggests a special importance of the fluxes via the KCl cotransporter (J_{KCC}) for the decrease in K^+ intracellular concentration and in the fluxes of organic anions X (J_X), which determine an equilibrium cell volume in a hypoosmotic medium. Moreover, the model provides for a preliminary quantitative estimate of the cell membrane water permeability during swelling caused by a hypotonic shock. This experimental–

theoretical approach can be useful for studying the effects of various agents on the cell transport mechanisms.

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