Na+,K+-activation of the Mg^{2+}-dependent cycle of Na,K-ATPase

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When the Mg^{2+}/ATP_{free} ratio is high, in the molecular mechanism of the Na,K-ATPase system the Mg-dependent cycle is created, the most peculiar trait of which is dephosphorylation of the Mg-bound phosphoenzyme. Under these conditions, with the aid of a specially designed method (analysis of the geometrical forms of kinetic curves), the dependence of the number of essential Na^+-, and K^+-activators on the concentration of the Na^+, K^+ and Mg^{2+} ions, and the MgATP-complex, was investigated. At relatively low [Na^+], an increase of [K^+] elicits an increase of the number of essential Na^+-activators – from 3 to 4. At [K^+] = 0, the ouabain-sensitive ATPase system does not equal zero and K ions become the activators as modifiers with partial effect. The activation is a result of the binding of one or two K ions. In the regime of Mg-dependent dephosphorylation the apparent activation constant of Na ions does not depend on the MgATP and Mg^{2+} concentrations. On the basis of these experimental facts the principal scheme of the Mg-dependent Na,K-ATPase cycle is deduced, in which instead of the Na^+:K^+ transport stoichiometry being 3:2 we can have the ratios 3:0, 3:1 or 3:2. Therefore, certain cation-transporting sites become the non-transporting regulatory ones.

Key words: multisite kinetics, Na,K-ATPase

1. INTRODUCTION

The sodium-potassium-pump (Na,K-ATPase) has two specific rôles affecting neural functions: (1) creating the cation asymmetry that, together with the differential permeability, establishes the Nernstian resting membrane potential; and (2) contributing directly to the membrane potential by means of the electrogenic nature of the pump. It is accepted that Na,K-ATPase functions with a fixed stoichiometry in such a way that three Na^+ ions are ejected and two K^+ ions are taken up when each molecule of ATP is hydrolyzed.

The sharp and considerable changes of ionic gradients and, consequently, of Na,K-ATPase activity, are not to be expected when an instantaneous change of the membrane potential occurs. Therefore, Na,K-ATPase may significantly influence the value of the membrane potential only when the enzyme is entirely inactivated. However, if under certain conditions Na,K-ATPase can change the stoichiometry of ionic transport, then it acquires the rôle of a regulator of the membrane potential and, hence, of neuronal function.

Earlier it was shown that when the free Mg ions ([Mg^{2+}] >> [ATP_f], subscript f denoting free) are redundant, Na,K-ATPase has an alternative pathway of dephosphorylation and it is assumed that in this case the coefficient of electrogenicity is increased, i.e. the stoichiometry of the transport varies [1]. The present work is devoted to the investigation of this particular problem.

2. METHODS

Partially purified Na,K-ATPase was obtained from a fraction of the synaptic membranes of albino rat brains by means of treatment with sodium dodecylsulphate [2]. The Na,K-ATPase was measured as the ouabain-sensitive part of the total ATPase, while the ouabain-insensitive part was represented by Mg-ATPase. The reagent medium consisted of 50 mM Tris-HCl buffer, pH 7.8, and various concentrations of ATP, $MgCl_2$, NaCl, and KCl. Mg-ATPase activity was determined by adding 0.2 mM ouabain to a medium containing 145 mM KCl and the same concentrations of ATP and $MgCl_2$ as in the reagent medium. The concentrations of MgATP-complex, free Mg^{2+} and free ATP (ATP_f) were defined by considering that the dissociation constant for MgATP is 0.085. The temperature of the assay was 37 °C. The concentration of enzyme and the time of incubation were chosen in such a way that the concentration of the product did not exceed 5% of the initial concentration of MgATP, and that the linear dependence of the concentration of the protein be preserved. Inorganic phosphorus and protein were assessed by the classical Fiske-Subbarow and Lowry methods. Bovine serum albumin (BSA) was used as a standard. The Mg-ATPase activity was less than 5% of total ATPase activity. Na,K-ATPase activity data are presented in μM P_i per mg protein per hour, as means ± S.E.M; the minimum number of identical samples was four. In the case of indirect measurements, the laws of extension of an average error in the method of small selections were used (Student's distribution).

2.1 Evaluation method for the number of essential activators

The enzymatic velocity as a function of a single variable has the following analytical form:

$$\frac{V}{e_0} = \frac{x^n \sum_{i=0}^{p} \alpha_i x^i}{\sum_{i=0}^{s} \beta_i x^i}, \quad s = n + m + p \qquad (1)$$

where V is ATPase activity, the constants α_i and $\beta_i \geq 0$, n is the number of essential activators, m is the number of complete inhibitors, and p the number of modifiers with partial effect.

The principle of the method elaborated here to define the quantity of essential activators is based on the following property of the function $U(r, t)$:

$$U(r,t) = \left[\frac{t^n \sum_{i=0}^{s} \beta_i t^{-i}}{\sum_{i=0}^{p} \alpha_i t^{-i}} \right]^{\frac{1}{r}} \qquad (2)$$

For this function there exists an open interval (t_0, ∞), on which the function is concave for $r < n$ and is convex for $r > n$. For $r = n$ the function has an asymptote. This means that if the experimental points belong in the correct interval (t_0, ∞), then the regression line constructed using these points has the minimal weighted average quadratic error (MU) and linearity test quotient (F_{exp}) only when $r = n$. Following a power transformation, when $r = n$ the function $U(n,t)$ has an asymptote, the equation of which is:

$$U_n = A_n + B_n t = n\sqrt{\frac{\beta_0}{\alpha_0}} \cdot \left[\frac{1}{n}\left(\frac{\beta_1}{\beta_0} - \frac{\alpha_1}{\alpha_0} \right) - t \right] \qquad (3)$$

With the aim of demonstrating the method, we consider as an example the dependence of Na,K-ATPase activity (V) on the concentration (x) of Na^+ ions, when the reagent medium consists of 0.85 mM Mg-ATP, 0.024 mM ATP_f, 3 mM $MgCl_2$, and 141 mM KCl. The initial experimental data for this example are presented in Table 1.

Stage I of this method: assessment of the experimental points' trustworthiness and the correct choice of working interval. We must ensure that the relative error of the points $\varepsilon_i \leq 0.15$; that the curve $\ln (1/V) = f(t)$ is convex, i.e. $(\ln 1/V)''_{tt} < 0$ (Fig. 1A), and that the curve $\ln (1/V) = f(\ln t)$ has no inflexion point, i.e. $(\ln 1/V)''_{tt} \neq 0$ (Fig. 1B).

Stage II. For each r parameter $U(r,t) = 1/\sqrt[r]{V}$ is calculated, its error being $\sigma(r,t) = \frac{\sigma(V_i)}{rV_i^r\sqrt{V}}$. The minimum value of $r = 1$, the maximum value $r = 8$, and the step $\Delta r = 0.1$. For every r the regression line $U_r = A_r + B_r t$ is constructed (Fig. 1C), the sequence of signs of the expression $U_r - u(r,t)$ is defined, and MU and F_{exp} are calculated:

$$MU = \left[\sum_{i=1}^{k}\left(\frac{U_r - U(r,t)}{\sigma(r,t)} \right)^2 \right]^{\frac{1}{2}}; F_{exp} = \frac{k\sum_{i=1}^{k}(U_r - U(r,t))^2}{(k-2)\sum_{i=1}^{k}(\sigma(r,t))^2} \qquad (4)$$

where k is the number of points (the number of identical points is constant).

Stage III. The value $r = \overline{R}$ is defined as that for which we have the minimum values of MU and F_{exp} (Fig. 1D), and $r = R_1$ and $r = R_2$ when the distribution of the signs of $U_r - u(r,t)$ begins (R_1) and ends (R_2) to change, thus defining the interval $[R_1 R_2]$, within which there exists the possibility that for certain t, $[U_r - u(r,t)] = 0$. In the first and the second approximations we define the distance of $\overline{R}$ from the real value of n (the number of essential activators) as:

$$\left(\overline{R}-n\right)_I = \frac{(R_2-R_1)\cdot t_1 t_2}{(t_1+t_2)}; \left(\overline{R}-n\right)_{II} = \frac{(\overline{R}-R_2)t_2^2 + (\overline{R}-R_1)t_1^2}{(t_2-t_1)^2} \qquad (5)$$

where t_1 and t_2 are the first and the last points of the working interval. In our specific example we have $\overline{R} = 3.8$, $\left(\overline{R}-n\right)_I = -0.045$ and $\left(\overline{R}-n\right)_{II} = -0.131$

Stage IV. On the basis of the above procedures we have finally:
1. The number of essential activators $N = 4.001 \pm 0.036$;
2. $\overline{R} = 3.800 \pm 0.034$; $(R-n)_1 = -0.045$;
3. The coefficients of the equations of the asymptote $r = 4$: $A_4 = 0.3095 \pm 0.0008$, and $B_4 = 1.9289 \pm 0.0062$.

The experimental results will be presented mainly as charts of the function $U(r,t)$ with indicated values of $r = n$. The criterion of trustworthiness, i.e. the correctness of the chosen working interval, is indicated by the fulfilment of the conditions: relative errors $\varepsilon_i \leq 0.15$, $(-\ln V)'_t < 0$, $(-\ln V)''_{tnt} \neq 0$, the minimum value of MU, and satisfactory values of $\left(\overline{R}-n\right)_I$ and $\left(\overline{R}-n\right)_{II}$.

Table 1. Initial experimental data.

x/mM	3.75	5.75	10.75	15.75	17.75	20.75	25.75
V_i	2.18	5.75	17.17	28.84	35.4	37.46	45.27
σ_i	±0.26	±0.30	±0.83	±0.40	±0.89	±1.19	±0.87
$\varepsilon_i = \sigma_i / V_i$	0.12	0.05	0.05	0.01	0.03	0.03	0.02