On the molecular mechanism of Na,K-ATPase isoforms

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There exist data according to which the molecular mechanism of the Na,K-ATPase of kidney differs from that of Na,K-ATPase of brain in nonexistence of the Mg-dependent mode (free $[Mg^{2+}] >> [ATP_{free}]$). On the other hand, there are several isoforms in brain, but only one in kidney. As a result of investigating the influence of ouabain on Na,K-ATPase, it turned out the OPM mode is not engendered by the variety of α-isoforms, but may be dependent on β-isomerization.

Keywords: ouabain, Na,K-ATPase

At present three isoforms of Na,K-ATPase are known, whose existence is determined by different α subunits. The isoforms differ in their amino acid composition and ouabain sensitivity. It is known that the α_1-isoform has a much lower affinity for ouabain than the α_2- or α_3-isoform. It is believed that β-isoforms exist, which have not yet been investigated [1].

As a rule the Na,K-ATPase reaction takes place with the stoichiometry $3Na^+/2K^+/1ATP$, but under certain circumstances it may change. For example, under excess $[Mg^{2+}]$, Na,K-ATPase works in the so-called OPM mode, during which a different stoichiometry occurs [2]. It should be noted that the OPM mode is characteristic for brain and is not observed in kidneys.

Taking into account the above-mentioned facts, it is interesting to find out how the existence of the OPM mode is connected with the Na,K-ATPase isoforms.

The subjects of our research were: kidney microsomes treated with dodecyl sodium sulphate (SDS) [3]; brain microsomes treated with SDS [4]; synaptosomal membranes treated with SDS [5]. Na,K-ATPase activity was measured as a part of ouabain-sensitive total ATPase, by the methods established earlier [6]. The reagent medium for brain preparations was as follows: 146 mM NaCl, 10 mM KCl, 50 mM Tris-HCl, pH 7.7; for kidney preparations: 141 mM NaCl, 15 mM KCl, 57 mM Tris-HCl, pH 7.7. The concentration of ouabain was variable. The unit of activity is μMP_i/h per one mg of protein. The number of identical tests was 3–6. Identical test series were united by the weighted mean method.

If there are two isoforms with different ouabain sensitivity in the preparation , then the total Na,K-ATPase activity is represented by the following formula:

$$v = v_1 + v_2 = \frac{V_1}{1+\frac{x}{K_1}} + \frac{V_2}{1+\frac{x}{K_2}} = \frac{\alpha_0 + \alpha_1 x}{\beta_0 + \beta_1 x + \beta_2 x^2} \qquad (1)$$

where x is the concentration of ouabain, K_1 and K_2 are the apparent inhibition constants, and V_1 and V_2 are the maximum activities of the α_1- and α_2-isoforms.

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We would have the same analytical form if we assume that Na,K-ATPase is a dimer, the connexion of one monomer of which with ouabain gives partial inhibition, and total inhibition is reached when ouabain is bound with both monomers:

$$U = \frac{1}{V} = \frac{K_1 K_2 + (K_1 + K_2)x + x^2}{(V_1 + V_2)K_1 K_2 + (V_1 K_1 + V_2 K_2)x} \qquad (2)$$

The analysis of the derivatives and limits of the general formula (2) unequivocally gives a convex curve with an asymptote, whose intercept with the abscissa gives an apparent inhibition coefficient (K_i). Taking into account that $K_1 >> K_2$ [1] we have:

$$K_i = \frac{V_1 K_1^2 \left(1 + \frac{V_2 K_2^2}{V_1 K_1^2}\right)}{V_1 K_1 \left(1 + \frac{V_2 K_2}{V_1 K_1}\right)} \approx K_1 \qquad (3)$$

If each isoform is dimeric, then we will have the same situation. In all three cases the number of complete inhibitors remains invariant and the analytical form of the curve will be uniformly convex.

Figure 1 shows the dependences of the Na,K-ATPases from SDS-treated preparations of kidney and brain on

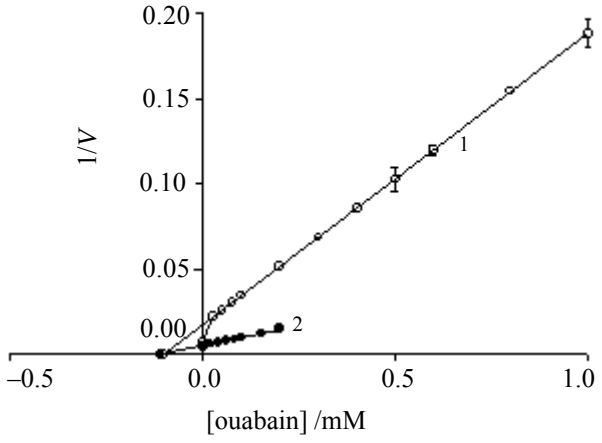

Figure 1. Dependences of the Na,K-ATPases from SDS-treated preparations of kidney and brain on ouabain concentration: curve 1, (open circle) brain; curve 2, (closed circle) kidney.

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ouabain concentration; the K_i are 0.110 ± 0.005 and 0.102 ± 0.006. Since $K_1 >> K_2$ [1], therefore the intersection of the two functions (curves) at a common point means that there is the same isoform in kidneys and brain according to sensitivity towards ouabain. If at the same concentrations we have for brain a convex curve and for kidney a linear one, then we can conclude that there is one isoform in kidney, and more than one in brain.

As was shown earlier [5], kidney has no OPM mode, but brain has. Therefore, it is interesting to investigate whether this deviation from linearity is connected with the OPM mode, i.e. whether the OPM mode is characteristic or not for a certain α-isoform.

For this purpose SDS-treated Synaptic Preparation was taken, in which the OPM mode is best apparent, and the inhibitory reactions of ouabain in the different modes of the molecular mechanism of Na,K-ATPase were compared. To this end, the following different reaction media were taken: a) [MgATP] = 2.53 mM, $[Mg^{2+}]$ = 0.46 mM and $[ATP_f]$ = 0.46 mM; b) [MgATP] = 2.53 mM, $[Mg^{2+}]$ = 2.16 mM and $[ATP_f]$ = 0.10 mM; c) [MgATP] = 2.53 mM, $[Mg^{2+}]$ = 0.46 mM, $[ATP_f]$ = 0.46 mM and EGTA = 0.4. The addition of EGTA was motivated by the fact that EGTA is known to directly activate Na,K-ATPase and at the same time it inhibits the OPM mode, transferring Na,K-ATPase into the normal OPS mode [7].

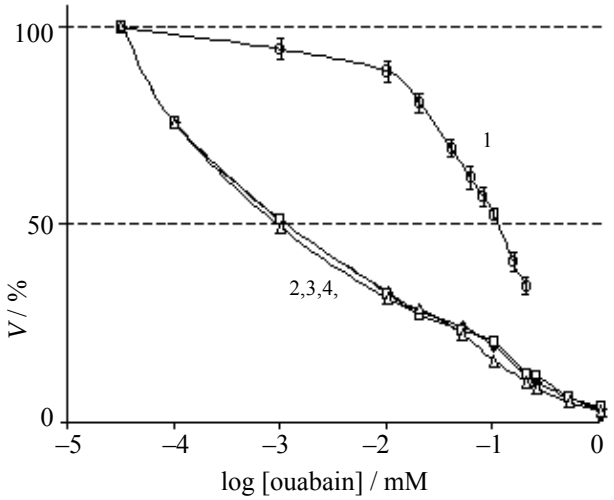

Figure 2. The influence of ouabain on the Na,K-ATPase activities of SDS-treated preparations of kidney (curve 1) and brain (curves 2,3 and 4) as a percentage of activity in the absence of ouabain.

Figure 2 shows the influence of ouabain on the preparation of brain under these conditions and on the preparation of kidney under the conditions mentioned earlier. 100% represents the activity of Na,K-ATPase without ouabain. The changes of degree of the reactions of ouabain on brain and kidneys are different. It is clear from the figure that in brain there is no statistically trustworthy difference between these three curves. This points to the fact that if the α-isoforms differ in sensitivity towards ouabain, then it is not they that cause the existence of the OPM mode in brain and its non-existence in kidney. Therefore we propose that this difference, from the view of the existence of OPM mode, is caused by the β-isoforms.

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