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 [Mg²⁺] >> [ATPfree]). 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-isofoms, 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 α₁ isoform has a much lower affinity for ouabain than the α₂ or α₃ isoform. It is believed that β-isomers 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 [Mg²⁺], 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, for kidney preparations: 141 mM NaCl, 15 mM KCl, 146 mM NaCl, 10 mM KCl, 50 mM Tris-HCl, pH 7.7; medium for brain preparations was as follows: 141 mM NaCl, 15 mM KCl, 57 mM Tris-HCl, pH 7.7.

If each isoform is dimeric, then we will 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:

\[ U = \frac{1}{V} = \frac{K_1K_2 + (K_1 + K_2)x + x^2}{(V_1 + V_2)K_1K_2 + (V_1K_2 + V_2K_1)x} \] (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 (Kᵢ). Taking into account that \( K_i \gg K_1 \) we have:

\[ K_i = \frac{V_1K_1^3}{(1 + \frac{V_2K_2}{V_1K_1})} = K_1 \] (3)

If each isoform is dimeric, then we will have the same situation. In all 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 ouabain concentration:

- curve 1, (open circle) brain;
- curve 2, (closed circle) kidney.

Figure 1.

References:
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ouabain concentration; the \( K_i \) are 0.110 ± 0.005 and 0.102 ± 0.006. Since \( K_i > K_i \) [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 \( \alpha \)-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) \([\text{MgATP}] = 2.53 \text{ mM}, [\text{Mg}^2+] = 0.46 \text{ mM} \) and \([\text{ATPf}] = 0.46 \text{ mM}; b) \[\text{MgATP}] = 2.53 \text{ mM}, [\text{Mg}^2+] = 2.16 \text{ mM} \) and \([\text{ATPf}] = 0.10 \text{ mM}; c) \[\text{MgATP}] = 2.53 \text{ mM}, [\text{Mg}^2+] = 0.46 \text{ mM}, [\text{ATPf}] = 0.46 \text{ mM} \) and \( \text{EGTA} = 0.4 \). 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 shows 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.

REFERENCES