Drug Modulation of Transductor Function of Na^+,K^+-ATPase

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Experimental proof of the hypothesis on modulation of the transductor function of Na^+,K^+-ATPase by the ouabain—Ca^{2+} chelate complex was obtained by the method organotypic tissue culture. Quantum chemical estimations detected two principally different modes of Ca^{2+} ion chelation by ouabain molecule. It is hypothesized that the ouabain—Ca^{2+}—Na^+,K^+-ATPase ligand-receptor complex is formed due to ion-ion bonds. The formation of the complex serves as the signal triggering the enzyme transductor function. It is experimentally proven that ouabain is incapable of inhibiting neurite growth in sensory neuron and heart tissue explants after removal of free calcium from the nutrient medium with EGTA.

Key Words: *organotypical tissue culture; ouabain; EGTA; Na^+,K^+-ATPase; signal transductor; quantum-chemical estimations*

Analysis of experimental data obtained on different objects has put forward the characteristics of Na^+, K^+-ATPase not linked with the pumping function. This enzyme is regarded as a transductor of intracellular signal routes [1,5,7,8]. This function of Na^+,K^+-ATPase manifests in modulation of proliferation and growth of cells of different tissues [2-4,7,8].

We studied the mechanism of drug modulation of the transductor function of Na^+,K^+-ATPase by ouabain molecule.

MATERIALS AND METHODS

The study was carried out on 300 explants of sensory ganglia and 350 heart tissue explants from 10-12-day chicken embryos cultured over 3 days on collagen-coated Petri dishes at 36.5°C and 5% CO_2 [2-4]. The nutrient medium contained 40% Hanks' solution, 40% Eagles' medium, 5% chicken embryo extract, and 15% FCS with insulin (0.5 U/ml), glucose (0.6%), glutamine (2 mM), and gentamicin (100 U/ml). Explants cultured in nutrient medium alone served as the control. Ouabain (selective inhibitor of Na^+,K^+-ATPase; Sigma) was added to the medium in a concentration of 10^{-8} M; EGTA (Sigma) was added to the culture medium in concentrations of 10^{-2}-10^{-5} M.

The growth of explants in tissue culture was controlled on vital (by phase contrast microscopy) and fixed preparations. The preparations were controlled visually using a microtelevision microscope attachment (Series 10, MTH-13, Alfa-Telecom). The quantity of explants was evaluated using PhotoM 1.2 software, the explant growth intensity was evaluated by the area index (AI), which was calculated as the proportion of the total area of the explant including peripheral growth zone to the initial area of the tissue fragment (area of the central zone).

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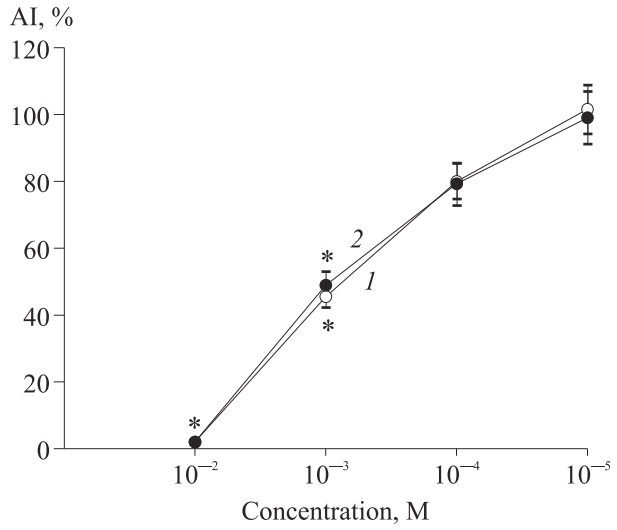

Fig. 1. Effect of EGTA on the growth of SG neurites (*1*) and heart tissue explants (*2*) from 10-12-day chicken embryos. *$p<0.05$ compared to control explants.

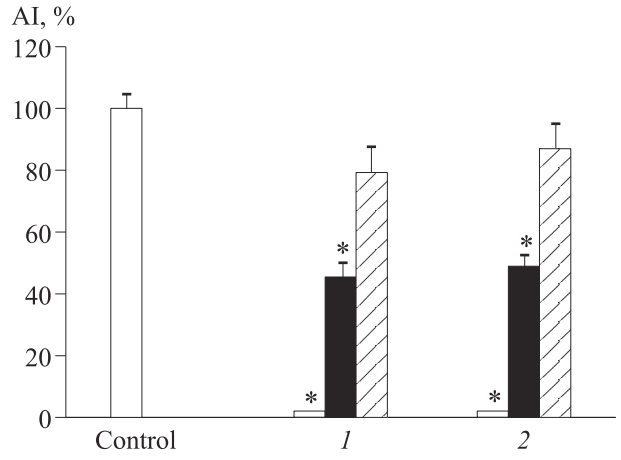

Fig. 2. Effect of EGTA in the presence of ouabain on SG neurite growth (*1*) and heart tissue explants (*2*) from 10-12-day chicken embryos. Light bars: ouabain; dark bars: EGTA; cross-hatched bars: ouabain+EGTA. *$p<0.05$ compared to the control.

The significance of differences in AI of control and experimental explants was evaluated using Student's *t* test. AI was expressed in percent; AI in the control was taken as 100%.

Complete optimization of geometrical parameters of possible conformations of the ouabain—Ca^{2+} complex was carried out by the *ab initio* method using GAMESS program with 6-31G* basis [6]. Calculations were carried out in the gas phase approximation.

RESULTS

Two zones formed in the control and experimental explants after 3 days in culture. The central zone consisted of non-migrating differentiating cells, while the peripheral zone of sensory ganglia (SG) consisted of growing ganglia, fibroblast-like cells, and glia. The growth zone in the heart tissue explants contained cardiomyocytes and some fibroblasts.

The growth of SG neurites and heart tissue explants from 10-12-day chick embryos was completely blocked with EGTA in a concentration of 10^{-2} M (Fig. 1). Addition of EGTA in a concentration of 10^{-3} M to the nutrient medium significantly inhibited the growth of SG neurites and heart tissue explants; AI of these explants was below the control by 45.5%. Addition of EGTA in a concentration of 10^{-4} M also inhibited significantly the growth of the studied explants; AI was below the control by 20% in both cases. Addition of EGTA in a concentration of 10^{-5} M did not modify the growth of SG neurites and heart tissue explants from 10-12-day chicken embryos. We previously showed that ouabain (selective inhibitor of Na^+,K^+-ATPase) in a concentration of 10^{-8} M virtually completely suppressed the growth of SG neurites and heart tissue explants from 10-12-day chicken embryos [2-4]. The inhibitory effect of ouabain was

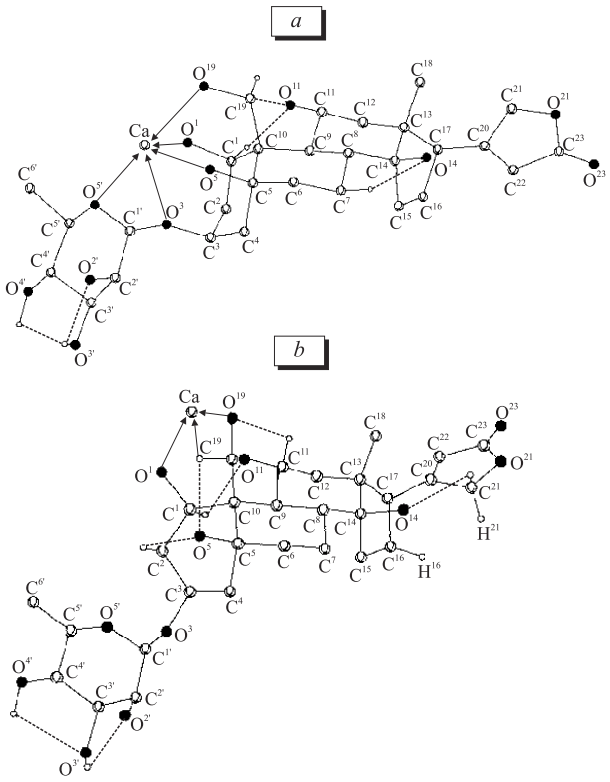

Fig. 3. Spatial structure of possible conformations of ouabain with Ca^{2+}. Hydrogen atoms are presented selectively. Intramolecular hydrogen bonds are shown by dotted lines, coordination Ca—O bonds by lines with arrows. *a*) Ca^{2+} forms coordination bonds with 5 oxygen atoms, A ring acquires armchair conformation; *b*) Ca^{2+} forms coordination bonds with 3 oxygen atoms, A ring acquires bathtub conformation.

almost completely abolished by culturing SG and heart tissue explants in a medium with EGTA (10^{-3} M) and ouabain (10^{-8} M) (Fig. 2), AI of SG and heart tissue explants just slightly differed from the control.

Since removal of free Ca^{2+} ions from nutrient medium with EGTA abolished the effect of ouabain on the growth of the studied tissue explants, we suggested that ouabain reacted with the transductor site for Na^+,K^+-ATPase binding not in the form of a free molecule, but as a chelate complex with Ca^{2+}.

Systematic analysis of the potential energy surface for the ouabain—Ca^{2+} system revealed possible conformations of chelate complexes. Quantum chemical estimations showed two principally different modes of Ca^{2+} ion chelation by the ouabain molecule (Fig. 3). In one case, Ca^{2+} forms coordination bonds with 5 atoms of oxygen O^1, O^3, O^5, O^{19}, and $O^{5'}$, the lengths of Ca—O bonds being 2.34-2.49 Å, charge on Ca atom q(Ca)=1.56 a.e., and the A ring ($C^1C^2C^3C^4C^5C^{10}$) acquiring the armchair conformation (Fig. 3, *a*). In the other case Ca^{2+} is bound by 3 oxygen atoms (O^1, O^{11}, O^{19}), with the Ca—O distances between 2.28 and 2.48 Å and q(Ca)=1.67-1.69 a.e., the A ring acquiring the armchair or the bathtub conformation (Fig. 3, *b*). The entalpic effects of chelation are about -170 kcal/mol for the former case and about -130 and -140 kcal/mol for the two conformations of A ring in the latter case.

The Na^+,K^+-ATPase molecule binding site responsible for realization of the enzyme transductor function differs from the highly conservative site of the pump regulating the pumping function [5]. Ouabain molecule realizes drug modulation of Na^+,K^+-ATPase transductor function by forming a chelate complex with Ca ion. Presumably, the ligand-receptor ouabain—Ca^{2+}—Na^+,K^+-ATPase complex forms due to ion-ion bonds.

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