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Regulatory Role of Na,K-ATPase in the Growth of Heart Tissue Explants in Organotypic Culture

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We studied the effects of ouabain, norepinephrine, and acetylcholine on the growth of heart tissue explants from 10-12-old chicken embryos in an organotypic culture. Ouabain, norepinephrine, and acetylcholine stimulated the growth of explants. Our results suggest that trophic function of acetylcholine and norepinephrine is associated with regulation of Na,K-ATPase activity as a signal transducer.

Key Words: *Na,K-ATPase; signal transducer; organotypic tissue culture; α_3 isoform*

Adaptation of cardiomyocytes at various stages of ontogeny is associated with changes in gene expression, including total activation that can result in myocardial hypertrophy [5,8,10]. Published data show that α_3 isoform of Na,K-ATPase with high affinity for ouabain plays a role in the regulation of cardiomyocyte proliferation [7,8]. Activity of this enzyme as a transducer of intracellular regulation is most pronounced in the presence of cardiac glycoside ouabain [11].

Here we studied the regulatory role of Na,K-ATPase in the growth of heart tissue explants in organotypic culture.

MATERIALS AND METHODS

Experiments were performed with organotypic tissue culture. The study involved 300 heart tissue explants from 10-12-old chicken embryos. They were cultured in Petri dishes with collagen sublayer. Culturing was performed in a CO_2 incubator at 36.5°C for 3 days [4]. The nutrient medium included 40% Hanks solution, 40% Eagle medium, 5% chicken embryonic extract, and 15% fetal bovine serum containing 0.5 U/ml insulin, 0.6% glucose, 2 mM glutamine, and 100 U/ml gentamicin. Control explants were cultured in the nutrient medium. Selective Na,K-ATPase inhibitor ouabain in concentrations of 10^{-13}-10^{-18} M was added to the nutrient medium. Acetylcholine (ACh) and norepinephrine (NE) in concentrations of 10^{-8} and 10^{-15}-10^{-9} M, respectively, were placed in the medium. Ouabain, ACh, and NE were obtained from Sigma.

Visual examination was performed using an MTN-13 television attachment to microscope (Alfa-Telecom series 10). Explant growth was quantitatively studied by means of Photo M 1.2 software. It was evaluated by changes in the area index (AI). AI was calculated as the ratio of the total area of the explant and growth zone to the initial area of the explant (relative units). Growth of cells and tissues was studied morphometrically [3,4]. The significance of differences between the mean AI for the control and experimental explants was estimated by Student's *t* test. AI was expressed in percents. AI for the control explant was taken as 100%.

RESULTS

Explants flattened over the collagen substratum on day 1 of culturing. Proliferation and migration of cells was observed in the marginal zone of the explant. The

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growth zone contained proliferating cardiomyocytes (89% area) and few fibroblasts. The growth zone (*i.e.*, change in AI relative to the control) was repeatedly examined after 3 days.

In series I we studied the effect of selective Na,K-ATPase inhibitor ouabain in concentrations of 10^{-13}-10^{-18} M on the growth of heart tissue explants from 10-12-old chicken embryos (Fig. 1). Addition of this inhibitor to the incubation medium in concentrations of 10^{-13} and 10^{-12} M reduced AI by 20% (n=23) compared to the control (n=21). Ouabain in a concentration of 10^{-10} M stimulated explant growth. Under these conditions AI (n=25) exceeded the control by 33±2% (n=27, p<0.05). Administration of 10^{-8} M ouabain to the nutrient medium completely inhibited the growth of the explant. The study of organotypic culture demonstrated high sensitivity of cardiomyocytes from 10-12-old chicken embryos to ouabain. Embryogenesis of cardiomyocytes is characterized by expression of α_3 isoform of Na,K-ATPase with high affinity for selective inhibitor ouabain [9]. Fibroblast-like and endothelial cells do not contain this isoform of the enzyme [6,8]. Probably, ouabain in very low concentration regulates activity of the Na,K-ATPase α_3 isoform in cardiomyocytes. Published data [1,7, 8,11] and results of our experiments suggest that the Na,K-ATPase α_3 isoform transduces signals to membrane ion channels and cell nucleus, which results in modulation of myocardial growth and development.

In series II we studied the effect of NE in concentrations of 10^{-15}-10^{-9} M on the growth of heart tissue explants (Fig. 2). Administration of NE to the nutrient medium was followed by a decrease in the explant growth zone. Under these conditions AI (n=27) decreased by 31±2% compared to the control (n=23, p<0.05). After treatment of the nutrient medium with NE in a concentration of 10^{-12} M, AI (n=23) was above the control by 18.0±2.5% (n=25, Fig. 2). Published data show that NE can increase Na,K-ATPase activity under certain conditions [8]. NE (10^{-12} M) and ouabain were simultaneously added to the nutrient medium to evaluate whether the trophic effect of NE is associated with the regulation of Na,K-ATPase activity (Fig. 3, *c*, *d*). After combined administration of NE and 10^{-8} M ouabain, AI (n=25) was below the control by 26.6± 2.0% (n=25, p<0.05, Fig. 3, *d*). NA (10^{-12} M) and ouabain (10^{-10} M) potentiated the stimulatory effects of each other (Fig. 3, *d*). Explant AI exceeded the control by 43±2% (n=25, p<0.05). It can be hypothesized that the tropic effect of NE is not mediated by Na,K-ATPase.

In series III we studied the effects of ACh. Administration of ACh in a concentration of 10^{-8} M to the culture medium stimulated the growth of heart tissue explants. It manifested in an increase in AI

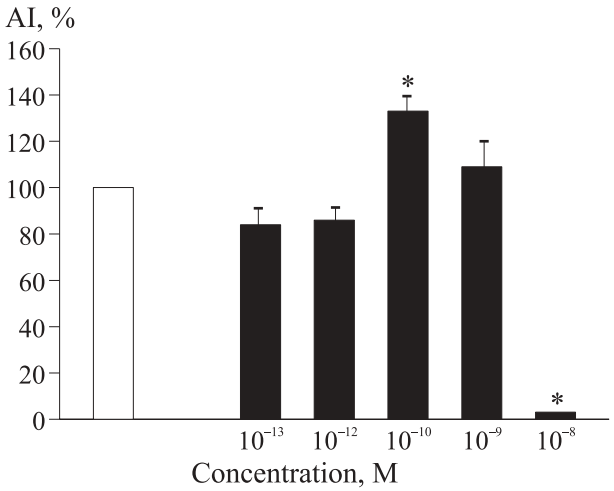

Fig. 1. Effect of specific Na,K-ATPase inhibitor ouabain on the growth of heart tissue explants (day 3 of culturing). Light bar: control. Dark bars: ouabain. AI, area index. Here and in Figs. 2 and 3: *p<0.05 compared to the control.

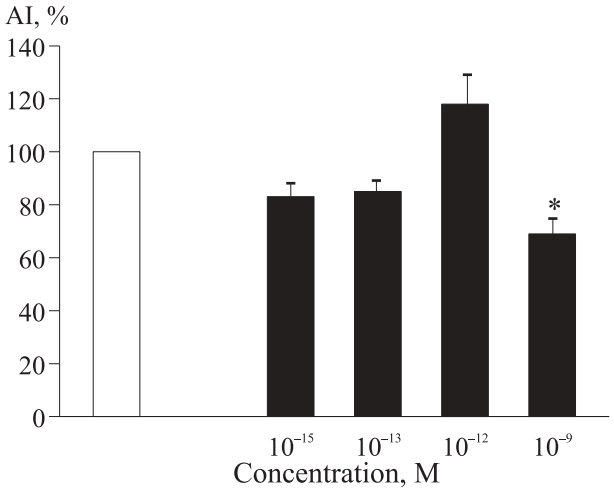

Fig. 2. AI of heart tissue explants from 10-12-day-old chicken embryos after addition of norepinephrine to the nutrient medium. Light bar: control. Dark bars: ouabain.

(n=25) by 27±2% compared to the control (n=20, Fig. 3, *a*, *b*).

ACh regulates Na,K-ATPase activity in the plasma membrane of various cells [2,7,9,10]. Therefore, we studied the effect of combined treatment with ACh and ouabain (Fig. 3, *a*, *b*). The inhibitory effect of ouabain in a concentration of 10^{-8} M was partially blocked under these conditions. AI was below the control by 20±2% (n=25). After combined administration to the nutrient medium, ACh and 10^{-10} M ouabain did not potentiate the effects of each other. AI exceeded the control by 28% (n=20, Fig. 3, *a*, *b*). The absence of potentiation of the effects under these conditions indicates that the test substances have the same target (α_3 isoform of Na,K-ATPase).

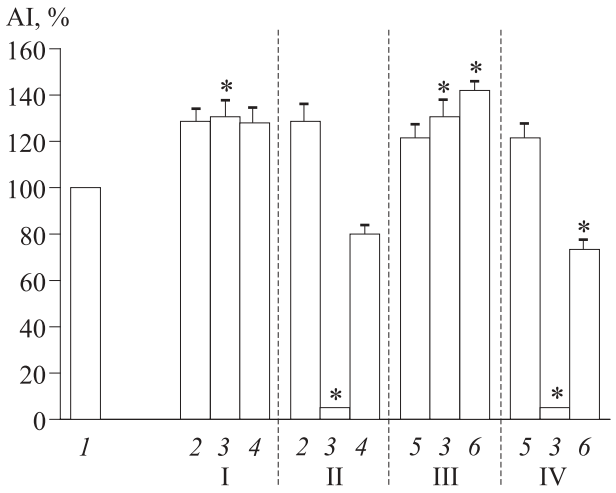

Fig. 3. Effects of acetylcholine and norepinephrine on the growth of heart tissue explants from 10-12-day-old chicken embryos in the presence of ouabain. Treatment of the nutrient medium with ouabain in concentrations of 10^{-10} M (I, III) and 10^{-8} M (II, IV). Control (*1*); acetylcholine (*2*); ouabain (*3*); acetylcholine and ouabain (*4*); norepinephrine (*5*); and norepinephrine and ouabain (*6*).

Our results and published data [1-3,11] suggest that under certain conditions α_3 isoform of Na,K-ATPase operates as a signal transducer and modulates cell growth and development.

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REFERENCES

1. B. V. Krylov, A. V. Derbenev, S. A. Podzorova, *et al*., *Ros. Fiziol. Zh*., **85**, 225-236 (1999).
2. E. V. Lopatina and I. I. Krivoi, *Vestn. St. Petersburg Gos. Univ*., Ser. 3, No. 4, 72-79 (1997).
3. V. A. Penniyainen, B. V. Krylov, N. I. Chalisova, *et al*., *Tsitologiya*, **45**, No. 4, 377-379 (2003).
4. N. I. Chalisova, S. G. Zhuravskii, V. A. Penniyainen, *et al*., *Ibid*., **41**, 48-52 (1999).
5. E. V. Shlyakhto and A. O. Konradi, *Arterial. Gyperten*., **4**, No. 3, 23-28 (2002).
6. G. Blanco and R. W. Mercer, *Am. J. Physiol*., **275**, 633-655 (1998).
7. T. Clausen and O. B. Nielsen, *Acta Physiol. Scand*., **152**, 365-373 (1994).
8. A. Mobasheri, J. Avila, I. Cozar-Castellano, *et al*., *Biosci. Rep*., **20**, No. 2, 51-90 (2000).
9. K. J. Sweadner, *Neuroglia*, **1**, 259-272 (1995).
10. B. Swynghedaw, *Physiol. Rev*., **79**, No. 1, 216-248 (1999).
11. Z. Xie, *Cell Mol. Biol*., **47**, 383-390 (2001).