Role of Na/K-ATPase in Regulation of Neurite Growth in Sensory Neurons

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We studied the effects of acetylcholine and norepinephrine on the growth of neurites in organotypic culture of 10-12-day-old chick embryo sensory neurons. Acetylcholine (10^{-8} M) and norepinephrine (10^{-13} M) stimulated the growth on sensory neuron neurites. Experiments with combined application of acetylcholine and norepinephrine against the background of ouabain showed that the nonspecific action of the test neurotransmitters is related to modulation of Na/K-ATPase activity.

Key Words: *Na/K-ATPase; signal transducer; organotypic culture; α-isoform*

Na/K-ATPase is an important regulator of cell functions, which underlies normal activity of all cells. Recent studies focus on the role of Na/K-ATPase in the plasmalemma of various cells, where it can be involved in signal transduction [2,3,13,14]. Activity of Na/K-ATPase is controlled by several transmitters [7,11]. Acetylcholine (Ach), catecholamines, and insulin activate this enzyme [1,9,11]. Endogenous ouabain, which is considered by some authors as a hormone, modulates activity of Na/K-ATPase in a dose-dependent manner [4,11].

Tissue culture technique made it possible to study bioactive substances under conditions, which exclude the influence of humoral, neural, hormonal, and other factors acting in the whole organism [6,8,10]. The organotypic culture of spinal ganglia of 10-12-day-old chick embryo is a classical test-system for the study of neurite growth [5,6,10]. In neural tissue culture, the intensity of neurite growth clearly reflects stimulatory or inhibitory effects of examined agents.

Our aim was to examine the involvement of Na/K-ATPase in the regulation of neurite growth in sensory neurons.

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MATERIALS AND METHODS

Experiments were carried out on explants (n=300) of sensory ganglia of chick embryos cultured for 3 days on a collagen matrix in Petri dishes at 36.5°C [6]. The culture medium contained 40% Hank's solution, 40% Eagle's medium, 5% chick embryonic extract, and 15% fetal calf serum supplemented with insulin (0.5 U/ml), glucose (0.6%), glutamine (2 mM), and gentamicin (100 U/ml). The control explants were cultured only in this nutrient medium. Ouabain (a selective inhibitor of Na/K-ATPase), Ach, and norepinephrine (NE) were added to the culture medium in concentrations of 10^{-8}, 10^{-7}-10^{-9}, and 10^{-13}-10^{-11} M, respectively. These reagents were from Sigma.

Visual control was performed using a microscope with a MTH-13 TV unit (series 10, Alpha-Telecom). The explants were qualitatively assessed using PhotoM 1.2 software. The area index (AI) was calculated in relative units as the ratio of explant area with the growth zone to the initial explant area. The data were processed statistically using Student's t test. The AI values were expressed in percents of the control taken as 100%.

RESULTS

After 3-day culturing, the control explants of spinal ganglia were presented by two clear-cut zones: the

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central zone consisting of non-migrating differentiating neuroblasts and the peripheral zone (the growth zone). The peripheral zone had a characteristic halo around the ganglion. This halo contained growing neurites, fibroblast-like cells, and glia. During culturing, the developmental differences were revealed between cultures grown in the presence and absence of the examined agents. Addition of stimulating agents to the culture medium induced the formation of intensive growth area around the ganglia.

In series I, we studied the effect of Ach (10^{-7}-10^{-9} M) on neurite growth in spinal ganglia of 10-12-day-old chick embryos (Fig. 1). When applied in concentration of 10^{-9} M, Ach produced no effect on neurite growth, so AI were similar in the control and experimental cultures. By contrast, 10^{-8} M Ach produced a significant neurite-stimulating effect: AI (n=27) increased by 27±2% compared to the control (n=25, p<0.05). Ach in a concentration of 10^{-7} M only insignificantly stimulated neurite growth by 20±1% (n=23) compared to the control (n=24).

Thus, we showed for the first time that Ach in low concentration produces a neurotrophic action and stimulates neurite growth in sensory ganglia of 10-12-day-old chick embryo.

In series II, the effect of NE (10^{-15}-10^{-11} M) on neurite growth was studied (Fig. 2). In a concentration of 10^{-15} M NE demonstrated a pronounced neurite-stimulating activity: AI of experimental ganglia (n=25) surpassed the control values (n=25) by 30±3% (p<0.05). By contrast, when applied in a concentration of 10^{-12} M, NE produced no effect on AI. Moreover, in a concentration of 10^{-9} M NE inhibited the growth of explants and decreased AI by 28±2% (n=27) in comparison with the control (n=23, p<0.05).

To examine whether the revealed neurite-stimulating effects of Ach and NE are related to regulation of Na/K-ATPase activity, these agents were introduced into the culture medium in combination with ouabain (Fig. 3, *1*, *2*). When this selective inhibitor of Na/K-ATPase was used individually in a concentration of 10^{-8} M, it completely inhibited neurite growth. Individual application of Ach (10^{-8} M) and NE (10^{-15} M) stimulated the growth of neurites by 27±2% (n=27, p<0.05) and 30±3% (n=25, p<0.05), respectively. Combined application of Ach (10^{-8} M) and ouabain (10^{-8} M) abolished the inhibitory effect of ouabain: AI did not differ from the control (Fig. 3, *1*). Partial elimination of inhibitory effect of ouabain was also observed during combined application of NE (10^{-15} M) and ouabain (10^{-8} M): AI was below the control value (n=25) by only 17±2% (n=25, Fig. 3, *2*).

Our findings suggest that the neurite-stimulating action of Ach and NE is associated with the effect of these hormones on Na/K-ATPase activity. Ach can

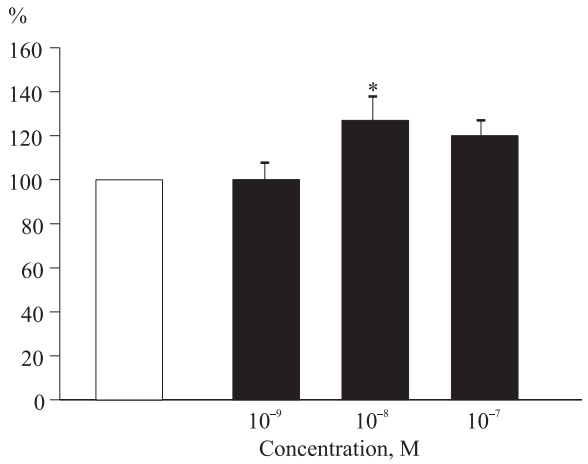

Fig. 1. Effect of acetylcholine on neurite growth in sensory neurons of spinal ganglia of 10-12-day-old chick embryo (3-day culturing). Light bar: control; solid bars: acetylcholine. Here and in Figs. 2, 3: ordinate: area index. *p<0.05 compared to the control.

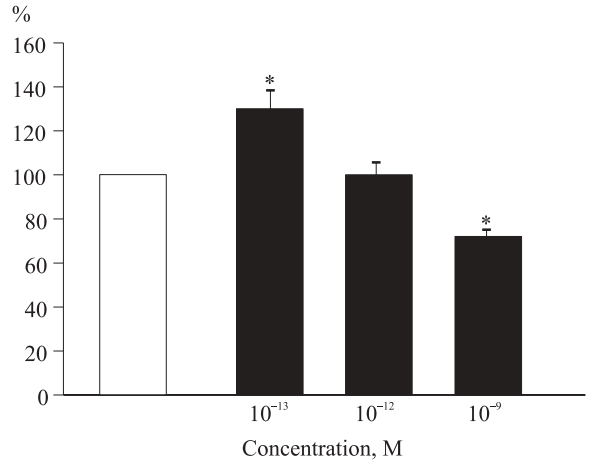

Fig. 2. Effect of norepinephrine on neurite growth in sensory neurons of spinal ganglia of 10-12-day-old chick embryo cultured for 3 days. Light bar: control; solid bars: norepinephrine.

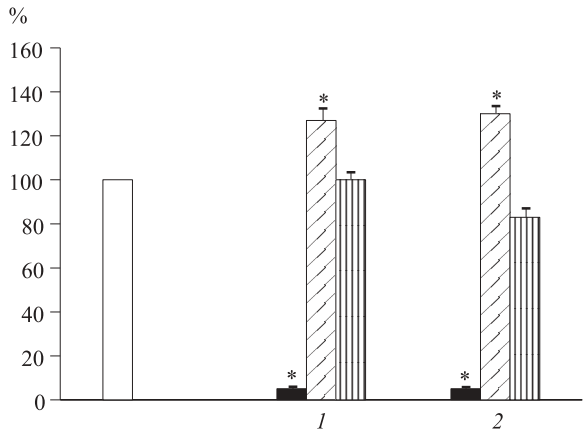

Fig. 3. Effects of combined application of ouabain and acetylcholine (norepinephrine) on neurite growth in sensory neurons of spinal ganglia of 10-12-day-old chick embryo (3-day culturing). Light bar: control; solid bars: ouabain; skew dashing: acetylcholine (*1*) or norepinephrine (*2*); vertical dashing: ouabain+acetylcholine (*1*) and ouabain+norepinephrine (*2*).

activate nicotinic or muscarinic cholinergic receptors or affect α-subunit of Na/K-ATPase, because the structure of the catalytic subunit is similar to that of nicotinic cholinergic receptors [1]. At the same time, the regulatory effect of NE on Na/K-ATPase is probably mediated via adrenoceptors. Thus, the regulation of enzyme activity is probably related to the level of phosphorylation of Na/K-ATPase α-subunit [12].

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