# PHARMACOLOGY AND TOXICOLOGY

# Role of Cardiac Glycosides in Regulation of the Growth of Retinal Tissue Explants

**E.V. Lopatina, A.V. Karetsky*, V.A. Penniyaynen**, and T.V. Vinogradova****

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We studied the role of cardiac glycosides in the regulation of the growth of retinal tissue explants from 10-12-day-old chicken embryos in organotypic culture. The studied compounds produced a dose-dependent effect on cell proliferation in retinal tissue explants. Ouabain ($10^{-13}$ M), strophanthin K ($10^{-13}$ M) and digoxin ($10^{-11}$ M) significantly stimulated explant growth. It was hypothesized that the physiological role of endogenous oubain is associated with regulation of tissue modeling.

**Key Words:** *$Na^+,K^+$-ATPase; cardiac glycosides; organotypic culture; retina tissue explants; chicken embryo*

Discovery of endogenous digitalis-like factors structurally similar to cardiac glycosides provided new insight into the process of tissue modeling. It was experimentally proved that ouabain in low concentrations specifically regulates signal transduction function of $\alpha_3$-isoform of $Na^+,K^+$-ATPase characterized by high affinity to this cardiac glycoside [1-5,11-14]. Signal transduction function of $Na^+,K^+$-ATPase is manifested in modulation of cell proliferation and growth in various tissues [2-4,7,11,12, 14]. We previously showed that cardiac glycosides regulate the growth of heart tissue explants and neuritis of sensory neurons of 10-12-day chicken embryos [2-4].

The aim of the study was to assess the role of cardiac glycosides in the regulation of the growth of retina tissue explants from 10-12-day chicken embryos in an organotypic culture.

## MATERIALS AND METHODS

Chicken embryo retina is formed at the early stages of embryonic development [9] and has a structure similar to human retina. Experiments were performed on 600 retina tissue explants from 10-12-day chicken embryos. They were cultured on collagen-coated Petri dishes at 36.5°C and 5% $CO_2$ for 3 days [2-4]. The nutrient medium contained 40% Hanks solution, 40% Eagle medium, 5% chicken embryonic extract, and 15% fetal bovine serum and was supplemented with 0.5 U/ml insulin, 0.6% glucose, 2 mM glutamine, and 100 U/ml gentamicin. Ouabain (Sigma) was added in concentration $10^{-8}$-$10^{-13}$ M. Strophanthin K and digoxin were added to the nutrient medium in concentration $10^{-6}$-$10^{-11}$ M. The explants cultured in the nutrient medium served as the control. Effects of the test compounds were quantitatively evaluated using morphometric analysis. Visual examination was performed using

Department of Experimental Pharmacology of V. A. Almazov Federal Heart, Blood and Endocrinology Center; *Phtysioophtalmology group, Saint-Petersburg Institute of Phtysiopulmonology; **Laboratory for physiology of excitable membranes of I. P. Pavlov Institute of Physiology, Saint-Petersburg, Russia. ***Address for correspondence:*** evl1112@infran.ru. E.V. Lopatina

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a microtelevision attachment to microscope (MTN-13 Alfa-Telecom series 10) and Photo M software. The explants were studied intravitally and on fixed preparations stained with hematoxylin and eosin. Pigment epithelium cells, ganglion cells, rods and cones were present in the growth zones of the control and experimental explants. A relative criterion area index (AI) was used for unification of the final results of the retina explant growth. AI was calculated as a ratio of the total area of the explant to the initial area of the central zone. The relative area unit was ocular grid quadrate of the microscope. AI values were expressed in relative units. AI value in the control was taken as 100%. Statistical analysis of the data was performed using Student's *t* test and Microsoft Excel software.

## RESULTS

After addition of ouabain to the incubation medium in concentration $10^{-8}$ M, no growth of retina tissue explants was observed (Fig. 1). In this concentration ouabain completely inhibited the growth of neuritis of sensory neurons [4] and heart tissue explants of 10-12-day chicken embryos [2,3]. A slight inhibition of retina tissue explant growth was observed in the presence of ouabain in a concentration $10^{-9}$ M (Fig. 1). AI did not virtually differ from the control (Fig. 1). In concentration $10^{-10}$ M, ouabain increased explant growth by 5%. We previously demonstrated that this concentration of ouabain significantly increased the growth of heart explants by 30% and inhibited the growth of neuritis of sensory neurons by 50% [4]. In concentration $10^{-11}$-$10^{-12}$ M ouabain insignificantly stimulated explant growth, while in concentration $10^{-13}$ M it significantly stimulated growth of retina tissue explants of 10-12-day chicken embryos. AI of explants surpassed the control level by 34% ($n$=25; $p$=0.05).

In the next set of experiments we studied the effect of strophanthin K ($10^{-6}$-$10^{-13}$ M; Fig. 2). Strophanthin K in concentration $10^{-6}$ M virtually completely blocked explant growth. Addition of the compound to the incubation medium in concentration $10^{-7}$ M inhibited the growth of retina tissue explants. AI was below the control level ($n$=25) by 34±6% ($n$=27; $p$<0.05). Explant growth was slightly suppressed in the presence of $10^{-8}$ M glycoside and insignificantly stimulated in the presence of this agent in concentrations of $10^{-9}$ and $10^{-11}$ M. Strophanthin K in concentration $10^{-13}$ M significantly stimulated the growth of retina tissue explants of 10-12-day chicken embryos. AI surpassed the control ($n$=27) by 30±6% ($n$=25; $p$<0.05). In this concentration strophanthin K stimulated growth of heart explants of 10-12-day chicken embryos by only 3.5% [3]. The compound produced a significant stimulating effect on heart tissue explants in concentration $10^{-14}$ M and lower [3]. Thus, the previously observed trophic effects of strophanthin K [3] were confirmed in experiments with retina explants. The effects of the compound on the growth of heart and retina tissue explants were dose-dependent and tissue-specific.

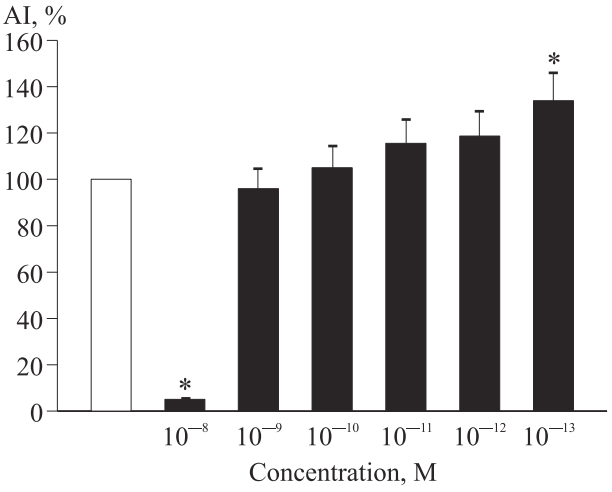


**Fig. 1.** Effect of specific $Na^+$, $K^+$-ATPase inhibitor ouabaine on the growth of retinal tissue explants of 10-12-day chicken embryos. Light bar: control, dark bars: ouabain. Here and on Figs. 2 and 3: *$p$<0.05 compared to the control.

Then we studied the effect of digoxin ($10^{-6}$-$10^{-11}$ M) on the growth of retina tissue explants of 10-12-day embryos (Fig. 3). Digoxin in concentration $10^{-6}$ M almost completely inhibited retina explants growth ($n$=29; $p$<0.05). After admini-

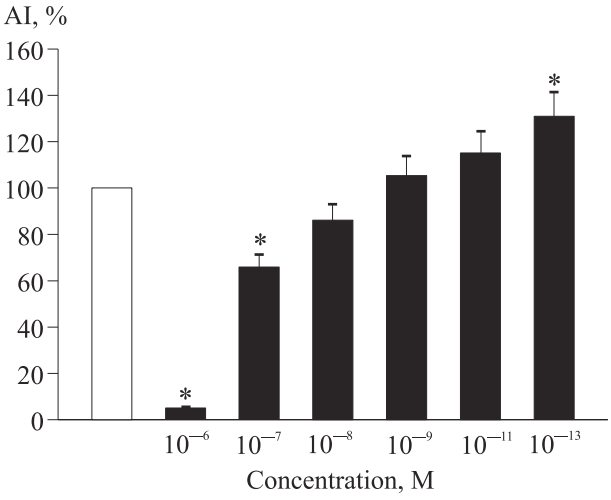


**Fig. 2.** Changes in AI of retina tissue explants of 10-12-day chicken embryos after addition of strophasntine K to the nutrient medium. Light bar: control, dark bars: strophanthin K.

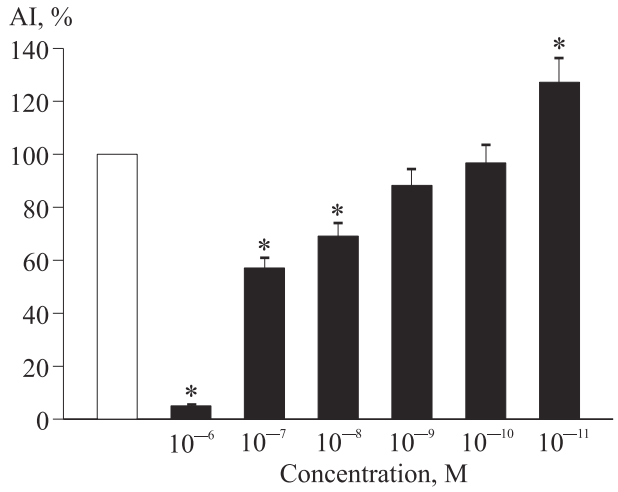


**Fig. 3.** Effect of digoxin on the growth of retinal tissue explants of 10-12-day-old chicken embryos. Light bar: control, dark bars: digoxin.

stration of the test cardiac glycoside to the nutrient medium in concentrations of $10^{-7}$-$10^{-8}$ M, a significant inhibitory effect was observed (Fig. 3). AI was below the control ($n$=25) by 43±6% ($n$=30; $p$<0.05) and 31±5% ($n$=25; $p$<0.05), respectively. Digoxin in concentration of $10^{-9}$ M slightly inhibited retina explant growth. Addition of $10^{-10}$ M digoxin to the nutrient medium did not affect explants growth (Fig. 3). Digoxin in concentration $10^{-11}$ M significantly stimulated the growth of retina tissue explants of 10-12-day chicken embryos. AI surpassed the control level by 27±5% ($n$=29; $p$<0.05; Fig. 3). Digoxin produced a pronounced inhibitory effect on the heart tissue [3].

Thus, it was found out that cardiac glycosides play a role in the regulation of the growth of retina explants. The effect of the compounds was tissue-specific [2-4]. We hypothesize that the physiological role of endogenous ouabain is related to the regulation of the tissue modeling process.

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