

Impact of Norepinephrine and Selective β_1 -Adrenoceptor Blockers on the Growth of Retinal Tissue Explants

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We studied the effect of norepinephrine and selective β_1 -adrenoceptor blockers on the growth of retinal tissue explants from 10-12-day-old chicken embryos in organotypic culture. The test drugs produced a dose-dependent effect on cell proliferation. Norepinephrine (10^{-12} M) and atenolol (10^{-4} M) are shown to significantly stimulate explant growth. Combined treatment with norepinephrine (10^{-12} M) and atenolol (10^{-4} M) results in cumulation of their stimulatory effects. The results suggest that the retina-stimulating effect of norepinephrine is based on its interactions with other types of adrenoceptors.

Key Words: *retinal tissue explant; norepinephrine; atenolol; metoprolol*

Drugs selectively blocking β_1 -adrenoceptors, *e.g.* atenolol and metoprolol, are widely used in clinical practice. These drugs exhibit no intrinsic sympathomimetic activity [6,7]. Experiments on organotypic tissue culture showed that atenolol in a high concentration of 10^{-4} M stimulates the growth of explants from heart tissue and spinal ganglia of 10-12-day-old chicken embryos. This concentration of atenolol almost 3-fold surpassed norepinephrine concentration required for stimulation of trophic processes under similar experimental conditions [2,3]. Thus, atenolol mimics trophotropic properties of norepinephrine. Further studies showed that metoprolol inhibits proliferation of myocardial cells [5] and neurite growth in sensory neurons of 10-12-day-old chicken embryos [4]. It was proven that trophic properties of norepinephrine observed in organotypic tissue culture are mediated by its effect on β_1 -adrenoceptors [1,4,5].

Here we studied the impact of norepinephrine and selective β_1 -adrenoceptor blockers on the growth of retinal tissue explants from 10-12-day-old chicken embryos.

MATERIALS AND METHODS

The retina of chicken embryo is formed at the early stages of embryo development [8] and has a structure similar to human retina. Experiments were carried out on 850 retinal tissue explants from 10-12-day-old chicken embryos. The explants were cultured on collagen-coated Petri dishes at 36.5°C and 5% CO₂ for 3 days (Sanyo) [2-4]. The nutrient medium contained 40% Hank's solution, 40% Eagle's 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. Selective β_1 -adrenoceptor blockers atenolol (Sigma) and metoprolol were added to the nutrient medium in concentration ranges of 10^{-10} - 10^{-4} M and 10^{-8} - 10^{-4} M, respectively. Norepinephrine (Sigma) was added to the nutrient medium in a concentration range from 10^{-9} to 10^{-12} M. The explants cultured in standard nutrient medium served as the control. The effects of the test drugs were quantitatively evaluated by morphometric analysis using Axiostar Plus microscope (Carl Zeiss), Nikon 8M digital camera and software for image processing.

The explants were studied intravitaly and on fixed histological slides stained with hematoxylin and eosin. Pigment epithelium cells, ganglion cells, rods and cones were present in the growth zones of the control and

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experimental explants. To standardize the results of retinal explant growth, the area index (AI) was calculated as the ratio of the total explant area to the area of the central zone. An ocular grid square was considered the arbitrary unit area. AI values were expressed in arbitrary units. AI control value was taken as 100%.

Statistical analysis of the data was performed using Student's *t* test and Microsoft Excel software.

RESULTS

In the first experimental series we studied the effect of norepinephrine in concentration range of 10^{-9} – 10^{-12} M on the growth of retinal tissue explants from 10–12-day-old chicken embryos (Fig. 1). Norepinephrine in a concentration of 10^{-9} M completely blocked the growth of retinal tissue explants ($n=25$; $p<0.05$). It should be noted that this concentration of norepinephrine inhibited the growth of heart explants [2] and neurites from spinal ganglia [3]. In concentrations of 10^{-10} and 10^{-11} M norepinephrine had virtually no effect on the growth of retinal explants. Norepinephrine added to the nutrient medium in a concentration of 10^{-12} M significantly stimulated the growth of retinal explants. AI of explants surpassed the control level by $40\pm2\%$ ($n=25$). It should be noted that this concentration of norepinephrine under similar experimental conditions significantly stimulated the growth of heart explants [2], but its trophic effect was less pronounced.

The effect of atenolol, hydrophilic β_1 -adrenoceptor blocker, was studied in concentrations of 10^{-4} , 10^{-6} , and 10^{-8} M (Fig. 2). In concentration of 10^{-8} M, atenolol virtually did not affect explant growth. Atenolol added in a concentration of 10^{-6} M significantly increased explant growth. AI surpassed the control value by $34\pm3\%$ ($n=27$; $p<0.05$). Atenolol in a concentration of 10^{-4} M significantly stimulated the growth of explants by $43\pm2\%$ ($n=28$; $p<0.05$). It should be mentioned that under similar experimental conditions atenolol (10^{-4} M) promoted the growth of heart explants and neurites from spinal ganglia of 10–12-day-old chicken embryos [4,5].

Combined treatment with norepinephrine (10^{-12} M) and atenolol (10^{-4} M) led to cumulation of their stimulatory effects. AI of experimental explants exceeded the control by $70\pm5\%$ ($n=27$; $p<0.05$, Fig. 3). These findings suggest that nonspecific retinal stimulation produced by atenolol (10^{-4} M) is associated with activation of β_1 -adrenoceptors in the retina. Norepinephrine in a three orders of magnitude lower concentration (10^{-12} M) appears to act specifically via another type of adrenoceptors.

In the next experimental series, metoprolol, a lipophilic β_1 -adrenoceptor blocker, was added to the nutrient medium in concentrations of 10^{-4} , 10^{-6} , and

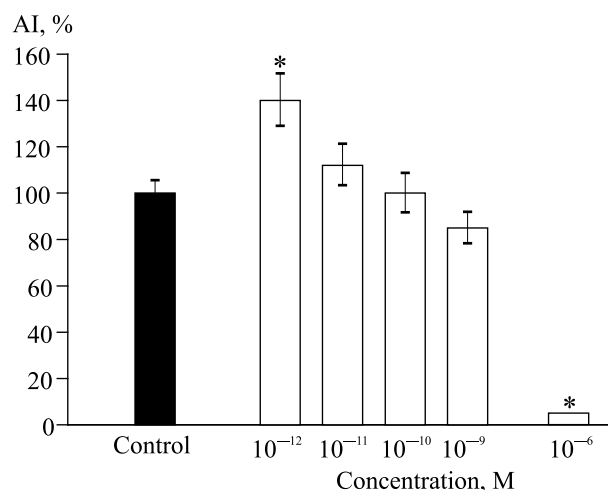


Fig. 1. Effect of norepinephrine on the growth of retinal tissue explants from 10–12-day-old chicken embryos (3 days of culturing). Here and in Figs. 2 and 3: * $p<0.05$ in comparison with the control.

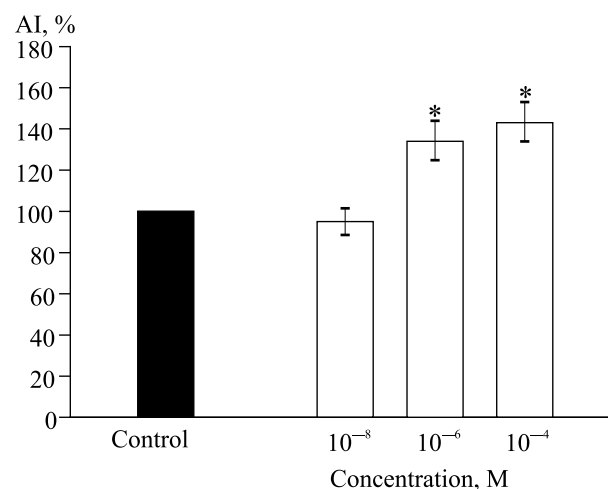


Fig. 2. Changes in AI of retinal tissue explants from 10–12-day-old chicken embryos after addition of atenolol to the nutrient medium.

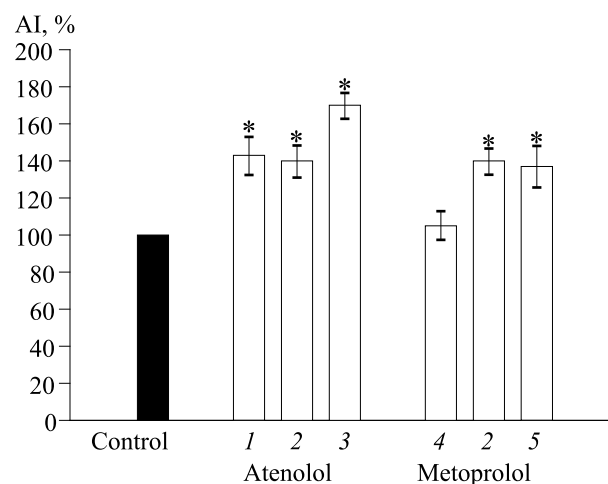


Fig. 3. Effect of norepinephrine plus β_1 -adrenoblockers atenolol or metoprolol on the growth of retinal tissue explants from 10–12-day-old chicken embryos. (3 days of culturing). 1) atenolol; 2) norepinephrine; 3) atenolol+norepinephrine; 4) metoprolol; 5) metoprolol+norepinephrine.

10^{-8} M. Metoprolol (10^{-4} M) significantly suppressed the growth of retinal explants from 10-12-day-old chicken embryos. AI of experimental explants was below the control value by $36.6 \pm 2\%$ ($n=25$; $p<0.05$). Metoprolol in concentrations of 10^{-6} and 10^{-8} M had practically no effect on the growth of retinal tissue explants; AI did not differ from control. Unlike atenolol, metoprolol produced no stimulating effect on the retina, which agrees with the neurotoxic side effects of the drug. The results are consistent with the data obtained by us on explants of myocardium and sensory ganglion neurites from 10-12-day-old chicken embryos [4,5].

Combined administration of metoprolol in a concentration of 10^{-8} M producing no effect on the growth of retinal explants and norepinephrine in a stimulating concentration of 10^{-12} M promoted the growth of retinal explants from 10-12-day-old chicken embryos (Fig. 3). AI of retinal explants ($n=30$; $p<0.05$) surpassed the control values by $37 \pm 2\%$ ($n=27$). This result suggests that the observed trophotropic effect of norepinephrine on retinal tissue of 10-12-day-old chicken embryos is not mediated entirely by activation of β_1 -adrenoceptors.

Experimental data on culturing of retinal tissue explants in a medium containing norepinephrine plus atenolol or metoprolol indicate that norepinephrine stimulate the growth of retinal explants via interactions with other types of adrenoceptors.

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