Effect of β-Adrenoceptor Blockers on Growth of Neurites in Spinal Ganglia in Organotypic Tissue Culture

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We studied the effects of norepinephrine and β_1-adrenoblockers atenolol and metoprolol on the growth of neuritis in organotypic tissue culture of spinal ganglia from 10-12-day chicken embryos. Atenolol (10^{-4} M) accelerated the growth of neurites in spinal ganglia by 45%, while metoprolol inhibited the growth of the explants almost in all examined concentrations (10^{-10}-10^{-4} M). Thus, β_1-adrenoblockers can produce opposite effects on neurite growth. Both atenolol and metoprolol abolished the neurite-stimulating effect of norepinephrine, probably, because this effect is mediated via β_1-adrenoreceptors of spinal ganglia.

Key Words: *organotypic tissue culture; atenolol; metoprolol; β_1-adrenoblockers; norepinephrine*

Antagonists of β-adrenoreceptors (*e.g.* atenolol and metoprolol) are now widely used in the therapy of cardiovascular diseases [6,8]. These drugs are characterized by cardioselectivity, absence of intrinsic sympathomimetic or membrane-stabilizing activities, and long-lasting effects.

Pharmacological preparations differ by solubility in water and lipids. Lipophilicity determines penetration of β-adrenoblockers across the blood-brain barrier [1], which can explain adverse side effects of these drugs on CNS.

Our aim was to examine the effects of selective β_1-adrenoblockers atenolol and metoprolol on the growth of neurites of spinal ganglia (NSG).

MATERIALS AND METHODS

The study was carried out on organotypic culture. We examined 1000 explants of spinal ganglia isolated from 10-12-day chicken embryos and cultured for 3 days in Petri dishes on a collagen matrix in a Sanyo incubator at 36.5°C and 5% CO_2 [2]. The culture medium contained 45% Hanks solution, 40% Eagle medium with insulin (0.5 U/ml), glucose (0.6%), glutamine (2 mM), gentamicin, 5% chicken embryonic extract, and 10% FCS.

Selective β_1-adrenoblockers atenolol (Sigma) and metoprolol (Leiras Hassle) were added to the culture medium in concentrations from 10^{-4} to 10^{-10} M. Norepinephrine (NE, Sigma) was added to the culture medium in a concentration of 10^{-13} M. The explants grown in drug-free medium served as the control.

Growth of the explants in tissue culture was monitored with a phase-contrast microscope on vital and fixed preparations. A microscope with MTH-13 attachment (series 10, Alfa-Telecom) was used for visual control of neurite growth.

Quantitative assessment of explant growth was performed using a PhotoM 1.2 software by the area index (AI) calculated as the ratio of total explant area including peripheral growth region to the initial area of tissue fragment (*i.e.* central area).

The data were processed statistically using Student's *t* test.

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RESULTS

After 3 days in culture, the control and experimental explants formed two sones. The central zone consisted of non-migrating differentiating cells, while the peripheral zone included growing neurites, fibroblast-like cells, and glia. The control and experimental cultures developed differently.

Atenolol in a concentration of 10^{-10} M decreased AI by 13% below the control value (Fig. 1), but had practically no effect on neurite growth in concentration of 10^{-8} M. Atenolol in a concentration of 10^{-6} M slightly stimulated NSG growth (AI surpassed the control by 24%). Addition of 10^{-4} M atenolol to the culture medium significantly stimulated NSG growth (AI surpassed the control level by 45%).

Metoprolol in a concentration of 10^{-4} M almost completely blocked NSG growth (Fig. 1). Metoprolol in a concentration of 10^{-6} M inhibited NSG growth: AI decreased by 55% below the control level. In a concentration 10^{-8} M metoprolol insignificantly inhibited NSG growth. The smallest concentration of this drug (10^{-10} M) produced no effect on NSG growth: AI did not differ from the control level (Fig. 1).

We previously showed that NE exhibits a pronounced neurite-stimulating activity [2]. NE in a concentration of 10^{-13} M significantly stimulated the growth of NSG: AI surpassed the control value by 30%. It was hypothesized that the neurite-stimulating effect of NE is mediated though adrenoreceptors on NSG. Therefore, in the next experimental series we examined the combined effect of NE (10^{-13} M) and atenolol (10^{-4} M). The stimulating effect in this case was absent; AI of experimental and control explants were similar (Fig. 2).

After combined application of metoprolol (10^{-10} M) and NE (10^{-13} M) no stimulating effect of NE was observed: AI of experimental explants did not differ from the control (Fig. 2).

These results attest to neurite-stimulating activity of atenolol (10^{-4} M). High effective concentration attests to non-specific action of the drug on the nervous tissue. Atenolol in the same concentration stimulated the growth of cardiac tissue explants from 10-12-day chicken embryos [3], which suggests that trophic effects of atenolol are tissue-unspecific. Probably, the neurite-stimulating activity of atenolol is realized via interaction with β_1-adrenoreceptors followed by activation of adenylate cyclase.

The study of the effects of metoprolol was of particular interest, because adverse side effects of this drug on the central and autonomic nervous system were reported [1,5,8]. The neurotoxic effects of this drug manifest in fatigue, insomnia,

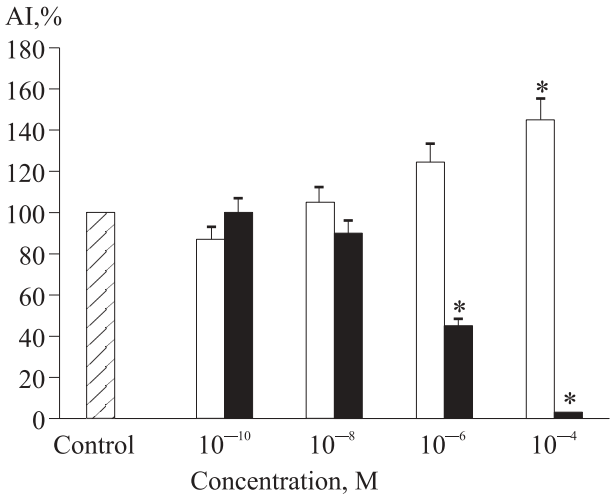

Fig. 1. Effect of atenolol (open bars) and metoprolol (dark bars) on the growth of NSG from 10-12-day chicken embryos. Here and in Fig. 2: *p<0.05 compared to the control.

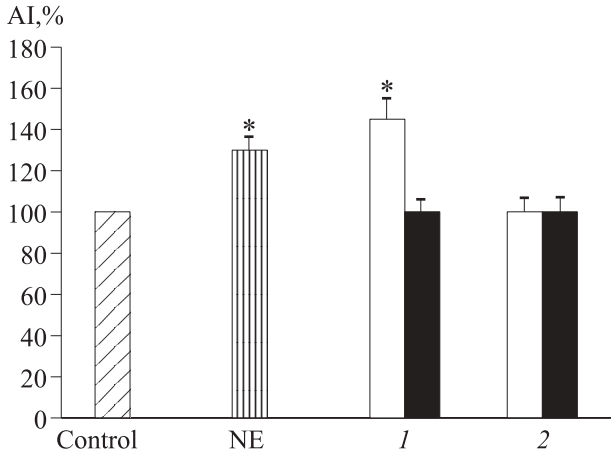

Fig. 2. Effect of NE on the growth of NSG from 10-12-day chicken embryos in the presence of β_1-adrenoceptor blockers. Open bars: 10^{-4} M atenolol (*1*) or 10^{-4} M metoprolol (*2*); dark bars the same drugs in combination with NE.

and partial loss of short-term memory. In contrast to atenolol, metoprolol inhibited the growth of NSG in almost all concentrations (10^{-8}-10^{-4} M). This property can underlie neurotoxicity of metoprolol.

The data on the effects of NE observed against the background of atenolol and metoprolol application showed that the neurostimulating action of NE was mediated via β_1-adrenoreceptors in NSG from 10-12-day chicken embryos.

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