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Peculiar Aspects in Influence of α_1-Adrenoceptor Stimulation on Isolated Rat Heart

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The study examined the effect of α_1-adrenoceptor stimulation with methoxamine on chronotropic function of isolated heart perfused *ex vivo* according to Langendorff and cardiac chronotropy *in vivo*. Stimulation of α_1-adrenoceptors in isolated heart induced gradually developing bradycardia, which progressed during several minutes. Similar stimulation *in vivo* produced a short-term bradycardia probably terminated by the compensatory influences in the whole organism. Comparison of the data obtained in both experimental paradigms during α_1-adrenoceptor stimulation revealed unidirectional changes in cardiac chronotropy characterized with time-related peculiarities.

Key Words: *heart; chronotropy; α_1-adrenoceptor*; *methoxamine*; *rat*

The heart has a wide repertoire of receptors, which interact with transmitters and modulate intensity of intracellular signaling systems. It is an accepted view that the catecholamines activate predominantly β_1-, β_2-, and α_1-adrenoceptors (ARs), which interact with G proteins and modulate the second messenger systems. All ARs are metabotropic and coupled to G proteins. At this, β_1-ARs activate predominantly Gs proteins, while α_1-ARs and β_2-ARs stimulate Gq and Gi proteins, respectively [4-7].

Involvement of individual types of β-ARs in the control of cardiac rhythm and myocardial contractility, as well as their effects on pathological processes in the myocardium had been extensively studied [1,3,10-12]. However, the novel studies focused on the effects of combined blockade of several types of ARs on the cardiac activity harvested new data and showed that the combined blockade of β_1-, β_2-, and α_1-ARs was more effective than the selective block of β-ARs, which is widely used in the treatment of cardiac pathologies [4]. This finding prompted a view about significant effect of α-ARs on genesis of cardiac failure. Specifically, it had been shown that α-ARs are implicated in physiological hypertrophy in male mice [8]. In neonatal rats, stimulation of α-ARs with phenylephrine provokes hypertrophy of ventricular cardiomyocytes triggered via Gq protein- and protein kinase C-dependent pathways [4]. Stimulation of α_1-ARs elevates the concentration of myofibrillar proteins due to up-regulation of protein synthesis. In addition, α_1-ARs are involved in the development of myocardial hypertrophy via protein kinase C pathways [8]. The effects of blockade of various subtypes of α-ARs are age-dependent [2,13,14]. Probably, the cardiac α_1-ARs control numerous adaptive processes such as up-regulation of inotropy, gene transcription, protein synthesis, and glucose metabolism in parallel with inhibition of apoptosis [9]. These findings attest to the need of further studies to develop the novel approaches in the treatment of arrhythmias [8].

This work was designed to compare the effects of stimulation of α_1-ARs on cardiac chronotropic function *in vivo* and *ex vivo*.

MATERIALS AND METHODS

The experiments were carried out on random-bred albino rats weighing 200-250 g and aging 20 weeks (n=28). The rats were anesthetized intraperitoneally with 25% urethane (800 mg/kg body weight). In the

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in vivo series, methoxamine (MX, an agonist affecting all subtypes of α_1-ARs, Sigma) was injected into the right femoral vein in a dose of 0.1 mg/kg. During experiments, ECG was recorded and processed on-line with a PC.

In the *ex vivo* series, the heart was rapidly isolated and completely arrested by placing it into cold physiological saline (2-5°C). Aorta was cannulated with caution to prevent touching and injuring the aortic valve and to exclude penetration of the test solution into the lumen of left ventricle. The heart was perfused in a Langendorff System (ADInstruments) with carbogen-oxygenated Krebs–Henseleit solution containing (in mM): 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 5.5 glucose (pH 7.3-7.4) at 37°C. The retrograde perfusion was driven by constant hydrostatic pressure of 60-65 mm Hg. To stimulate α_1-ARs, MX had been used at the concentrations of 10^{-10}, 10^{-9}, and 10^{-8} M. To measure the intraventricular pressure, a latex water-filled balloon was placed into the lumen of the left ventricle via an orifice made posterior to the left atrial auricle. The end-systolic pressure was set at the level of 10-15 mm Hg. The left ventricular pressure was recorded with a MLT844 pressure transducer (ADInstruments) and used to calculate the HR. The signals were recorded in a PowerLab 8/35 system (ADInstruments) with the help of LabChart Pro 8.0 software.

The data were processed statistically using Microsoft Excel software and Student's *t* test.

RESULTS

In the *in vivo* series, MX (0.1 mg/kg) decreased HR from 347±21 to 321±18 bmp (n=5, p<0.05). On postinjection seconds 30 and 60, HR values were 327±21 and 334±21 bmp, respectively (Fig. 1). This short-term bradycardia was followed by recovery of initial cardiac activity. On postinjection minute 2, HR was 343±20 bmp, and it remained rather stable to the end of experiment (Fig. 1). On postinjection minutes 15 and 30, the HR values were 342±20 and 349±11 bmp, respectively.

In the *ex vivo* series, the chronotropic action of MX was examined at smaller concentrations of this agent (10^{-10}, 10^{-9}, and 10^{-8} M). Prior to MX, the control Krebs–Henseleit solution was applied which produced no effect on HR.

In contrast to *in vivo* experiments, the lowest tested concentration of MX (10^{-10} M) produced no significant effect on HR during the first 2 min postinjection (n=7, Fig. 1). Then, HR gradually decreased and attained 28% initial value to postinjection minute 5 (p<0.01, Fig. 1). On postinjection minutes 7 and 9, HR was below the initial level by 29% (p<0.001) and 25% (p<0.01), respectively. The maximum drop of HR by 35% (p<0.05) was observed on postinjection minute 15 (Figs. 1, 2).

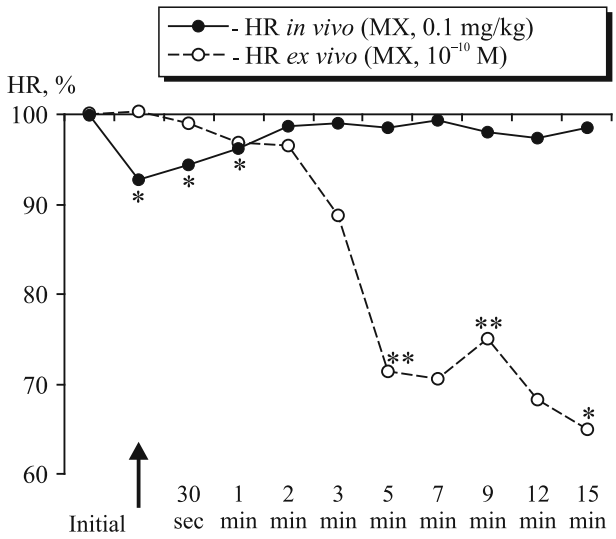

Fig. 1. Effect of MX on HR *in vivo* and *ex vivo*. The arrow indicates HR measured during 0-30 sec postinjection. HR shown at 30 sec was measured during 30-60 sec postinjection. *p<0.05, **p<0.01 in comparison with initial level.

Perfusion of isolated heart with MX (10^{-9} M, n=7) produced no significant chronotropic effect (Fig. 2). In 2 min after agonist application, HR slightly increased from 222±10 to 229±14 bmp. The maximum changes in HR were observed on minute 12: HR dropped to 190±20 bmp.

When tested at a concentration of 10^{-8} M (n=9), MX significantly decreased HR. However, it produced a transient and insignificant increase in HR in 30 sec after administration from 169±30 to 173±29 bmp**.** Then, HR gradually decreased: in 2 min this parameter was below the baseline by 17%. On minute 3 after MX administration, HR decreased to 137±26 bmp

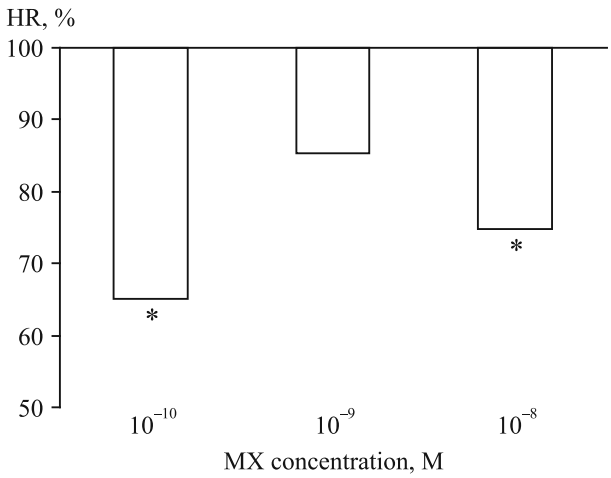

Fig. 2. Effect of MX on HR of the hearts isolated from 20-week-old rats. *p<0.05 in comparison with initial level.

(p<0.05). Bradycardia peaked on minute 7 after administration with HR of 126±22 bmp, *i.e.* 25% of the baseline (p<0.05, Fig. 2).

Although the density of α_1-ARs in the heart is lower than that of β-ARs, the former play a rather important role in the regulation of cardiac functions. According to common view, activation of α_1-ARs upregulates the myocardial contractility in the normal heart, although the opposite effect was also reported [6]. The present study showed that stimulation of α_1-ARs with MX induced bradycardia both in isolated heart and *in vivo*. However, the periods needed for the development of MX-induced bradycardia were significantly different in both experimental paradigms. In isolated heart, bradycardia developed gradually during several minutes. In contrast, the *in vivo* experiments demonstrated a short-term bradycardia, which can be explained by engaging the reflectory compensatory mechanisms of the whole organism. Paradoxically, the block of α_1-ARs with prazosin also induced bradycardia [2,14]. Similar cardiac effect induced by stimulation and block of α_1-ARs can result from a number of reasons. On the one hand, α_1-ARs can trigger various systems of second messengers exerting the opposite effects on cardiac rhythm. On the other hand, block of α_1-ARs can activate the intracellular mechanisms not directly implicated into performance of these receptors.

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