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Effect of NO Synthase Blockade on Myocardial Contractility of Hypokinetic Rats during Stimulation of β-Adrenoreceptors

R. I. Zaripova, N. I. Ziyatdinova, and T. L. Zefirov

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Stimulation of β-adrenoreceptors with low (10^{-8} and 10^{-7} M) or high (10^{-6} M) doses of isoproterenol in hypokinetic rats treated with L-NAME (a non-selective blocker of NO synthases) decreased or increased myocardial contractility, respectively. In control rats, all examined doses of isoproterenol used under blockade of NO synthases inhibited myocardial contractility.

Key Words: *nitric oxide; isoproterenol; rat; hypokinesia; myocardial contractility*

A long-term hypokinesia provokes significant alterations in the cardiovascular system. This state decreases myocardial contractility, energy resources for cardiac muscles, and cardiac output in company with unfavorable effects on the venous and arterial vessels.

NO plays an important role during adaptation of the organism to variable environment, the changes in motor activity included. NO synthase system is widely presented in various cardiac structures playing the key role in working cardiomyocytes, in the conduction system cardiomyocytes, and in the coronary blood vessels. NO is an effective vasodilator modulating the pacemaker and calcium currents in the myocardium. However, the role of NO in the regulation of myocardial contractility is not fully understood because of diversity of its intracellular targets (in some cases, with opposite effects on contractility) as well as uneven distribution of these targets in the heart [4-6]. NO down-regulates the responsiveness to stimulation of β-adrenoreceptors (β-AR) [7,9,11]. As for the effect of NO on myocardial contractility, the inotropic effect of β-AR stimulation can be modulated in two ways depending not only on NO, but also on catecholamines. Under constant NO concentration, the response to β-AR stimulation negatively correlates with concentration of catecholamines [10]. Nitric oxide can exert both negative and positive inotropic effects. On the one hand, its negative (antiadrenergic) effect results from β_3-AR-dependent activation of endothelial NO synthase (eNOS) via cytosolic modulation of the major targets in the classical signal pathway (β_3-AR)-cGMP-(cGMP-dependent protein kinase). Expression of inducible NO synthase (iNOS) and resulting increase of NO production down-regulate the release of calcium ions during stimulation of β-AR due to inhibition of ryanodine receptors. Stimulation of β-AR elevate the frequency of Ca^{2+} sparks [8,10]. On the other hand, eNOS-produced NO can exert the positive inotropic effect.

The decisive role in the development and regulation of myocardial contractility is played by sympathetic and parasympathetic branches of ANS [2]. The sympathetic cardiotropic effects are mediated via interaction of catecholamines with various subtypes of AR in cardiomyocytes. At present, the heart had been shown to possess the following palette of adrenergic receptors: α_1, α_2, β_1, β_2, and β_3. Of them, the major role in functional control of the heart in rats is given to β_1-ARs.

This work was designed to study the effect of non-selective blockade of NOS during activation of β-AR on contractility of myocardium in hypokinetic rats.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats. The control (normokinetic) rats (n=20) were

Department of Anatomy, Physiology, and Human Health Protection, Kazan Federal University, Kazan, Tatarstan, Russia. ***Address for correspondence:*** zefirovtl@mail.ru. T. L. Zefirov

maintained under vivarium conditions, while the experimental rats (n=20) were subjected to a 90-day hypokinesia (HK). The motor activity of growing rat pups was restricted by placing them into the Plexiglas restriction cages. The volume of these cases could be individually adjusted to the rat size by shifting a sliding partition panel. Daily HK session began at the age of 21 days. During the first 2 days, HK time was 1 h, thereupon it increased by 2 h in every 2 days. To HK day 25, the duration of the stay in the restriction cages amounted to 23 h; thereupon it remained constant. During the period with 22-23 h HK, the rats were released from the cages for 1-2 h [1]. The cages were placed on a wooden support for an optimal temperature conditions.

The contractile activity of myocardium was examined *in vitro* in a PowerLab setup equipped with a MLT 050/D Force Transducer (ADInstruments). The data were recorded in a PC with the help of Chart 5.5 software.

Narcotized rat was fixed onto a special platform, the thorax was opened, and the heart was isolated and placed into a Petri dish with oxygenated Krebs solution. The right ventricular strips with the length of 2-3 mm and the width of 1 mm were isolated and fixed in the vertical position. One of its ends was fixed to a support, while other end was attached to the force transducer. The strip was placed into a 10-ml tube filled with oxygenated Krebs solution. The strips were stimulated via platinum electrodes with 5-msec pulses (repetition rate 10 pulses per minute) employing an ESL-2 electric generator. After the strips had been placed into the reservoirs, there was a 40-50 min run-in period needed for their optimal loading. Then the initial (baseline) contractile parameters were recorded isometrically during 10 min. In the following 30-min period, the same parameters were recorded under the effect of pharmacological agents used at one of examined concentrations.

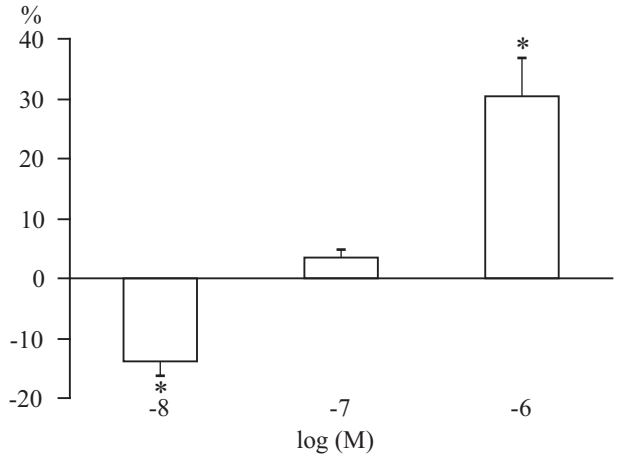

Fig. 1. Effect of ISO on contractility of right ventricular myocardium in HK rats. Here and in Fig. 2: *p<0.05 in comparison with initial values.

The changes in contractile force were assessed in percentage to the baseline values. The data were analyzed statistically using Student's t and Mann–Whitney U tests. The results are summarized as $m \pm SEM$.

RESULTS

The effect of isoproterenol (ISO), a long-acting agonist of β-AR, on myocardial contractile function, was examined at concentrations of 10^{-8} to 10^{-6} M. In control group, ISO increased the contractile force (CF) of ventricular myocardial strips at all examined concentrations. The maximum effect of ISO was observed at a concentration of 10^{-6} M that increased CF by 22%. In concentrations of 10^{-8} M and 10^{-7} M, ISO increased CF by 14.5 and 17.0%, respectively.

In experimental group, low ISO concentrations decreased CF by 13.9%. In contrast, 10^{-6} M ISO in-

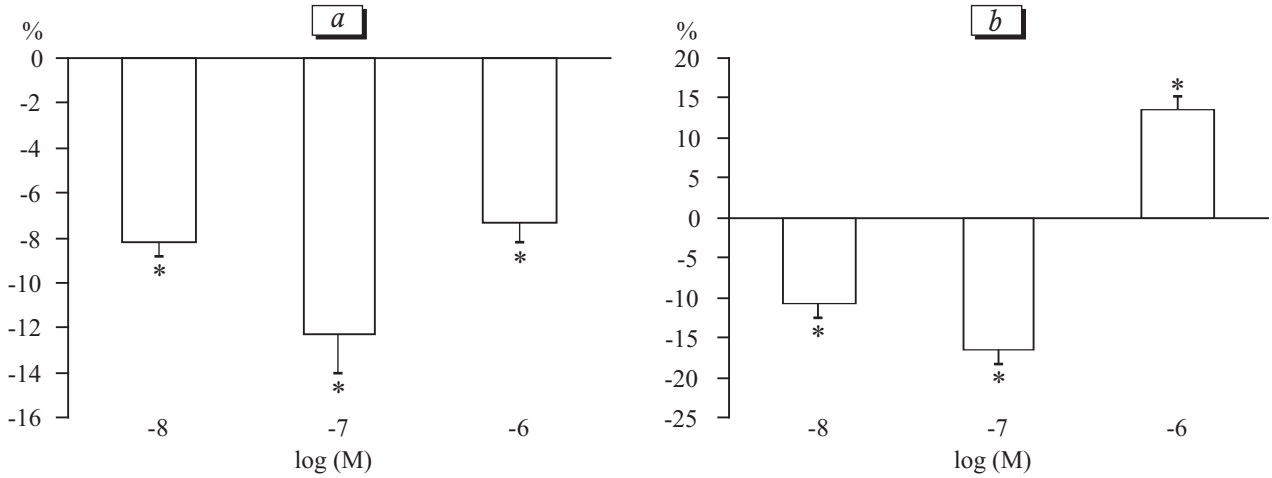

Fig. 2. Effect of nonselective NOS blockade on contractility of right ventricular myocardium in control (*a*) and HK (*b*) rats during stimulation of β-AR.

creased CF by 30.5% (Fig. 1), which was similar to the effect of high ISO concentration in the control group.

To reveal the effect of NO on ventricular myocardium strips of HK rats, the effect of ISO ion concentrations of 10^{-6}-10^{-8} M was examined during NOS blockade produced by 10 mg/kg L-NAME injected intraperitoneally 1 h prior to chest opening. In control group with inhibited NOS, ISO (10^{-6} and 10^{-8}M) decreased CF by 8.2 and 7.3%, respectively. In this group, the maximum inhibitory effect of ISO on myocardial contractile activity was observed at concentration of 10^{-7} M (Fig. 2, *a*). Thus, inhibition of NOS in the control group reversed the effect of NOS on CF. In HK rats with inhibited NOS, the low ISO concentrations (10^{-8} and 10^{-7} M) also diminished CF by 11 and 17%, respectively. In contrast, the highest ISO concentration (10^{-6} M) increased CF by 14% (Fig. 2, *b*).

Thus, stimulation of β-AR in the myocardium of HK rats against the background of NOS with low or high agonist concentrations decreased or increased myocardial contractility, respectively. Therefore, NO modulates the inotropic effect of ISO in relation to concentration of catecholamines.

It is an established fact that 30-day HK up-regulates synthesis and secretion of anti-inflammatory cytokines such as IL-1, IL-6, and TNF [3]. It is also known that IL-1 and TNF decrease the density of β_1-AR in parallel with up-regulation of β_2-AR expression. The available data showed that in right ventricular myocardium, the proportion of β_1- and β_2-AR varies from 87 and 13 to 62 and 38, respectively. Stimulation of various subtypes of β-AR results in various dynamics in myocardial contractility. Therefore, the present data on the opposite effects of ISO on myocardial contractility in normal and HK rats can be explained by simultaneous stimulation of different subtypes of β-AR, whose proportions can be disturbed by hypokinesia.

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