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NOREPINEPHRINE EFFECT ON MYOCARDIAL CONTRACTILITY IN RATS AT HYPOKINESIA

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Abstract:

Hypokinesia for warm-blooded animals and humans is a stressor agent, and the activation of the NO-system is one of the body mechanisms of reduction of stress influences. A prolonged hypokinesia causes significant changes in myocardial contractility and cardiac phases as a consequence of the formation of the phase syndrome of "myocardial hypodynamia". Objective of the research was to study the effect of hypokinesia on myocardial contractility in rats at administration of various doses of norepinephrine under blocking NO-synthases (NOS) and β-adrenergic receptors (β-AR). The effect of a non-selective agonist of adrenergic receptors - norepinephrine - was studied in the concentration range of 10^{-5} - 10^{-7} on atrial and ventricular myocardial contractility in mature rats of control (unlimited motor activity) and experimental (90-day hypokinesia) groups on the background of a NOS inhibitor - L-NAME at a dose of 10 mg/kg (intraperitoneally, 1 hour before autopsy) and β-AR blockade with Obsidan (0.1% solution at a concentration of 0.8 mg/kg), with the use of "PowerLab" ("ADInstruments") device, with the force sensor "MLT 050/D" ("ADInstruments"). The contractile force reaction in response to pharmacological agents was calculated in percentage of the original (100%). On the background of the β-AR and NOS blockade, the administration of norepinephrine at all studied concentrations causes a decrease in contractility of the strips of ventricular myocardium in both experimental groups. In the atria, the studied substance at a concentration of 10^{-5}M causes a slight increase in the contractility of myocardial strips.

Keywords: heart, inotropy, hypokinesia, postnatal ontogenesis.

1. Introduction

The study of the effect of limiting the motor activity (hypokinesia) on the body is a relevant problem of physiology. Hypokinesia is one of the most pressing medical and social problems caused by lifestyle, occupational activity, prolonged bed rest, a significant decrease in physical activity, especially among children and persons not engaged in

physical labor. A prolonged hypokinesia causes significant changes in myocardial contractility and cardiac phases as a consequence of the formation of the phase syndrome of "myocardial hypodynamia", as well as the changes in adrenergic and cholinergic regulation of cardiac activity. In addition, this state contributes to the weakening of the heart muscle, reduction of the cardiac energy potential and its cardiac output, as well as the tone of venous and arterial vessels. X-ray kymography shows a decrease in heart size by 10 - 20%. ECG detects signs of impared myocardial trophism with signs of slowing conduction and shifts in the phase structure of the cardiac cycle.

The NO-synthase system is widely represented in various cardiac structures. Nitric oxide (NO) controls vascular tone, blood pressure, proliferation of endothelial and vascular smooth muscle cells, participates in causing atherosclerosis and hypertension, and regulates the myocardial contractility. NOS inhibition can induce negative inotropic effect, which suggests that the endogenous formation of NO supports the myocardial contractility [1, 2, 3].

There are NO-dependent mechanisms of the body's response to hypokinesia and immobilization stress [4]. It is known that hypokinesia is accompanied by the development of hypoxia, which, in turn, causes an increased synthesis of nitric oxide and NO^{3-} and NO^{2-} ions [5]. We have found data about increasing amount of NO at chronic immobilization. Experimental EPR spectroscopy shows that after the transition of rats to hypokinesia mode, starting with the age of 21 days, there is an increase in nitric oxide production in the heart, liver and spinal cord of mature rats. The results suggest that the prolonged hypokinesia causes strengthening of NO synthesis processes in the body. The highest increase in NO in the atrial and ventricular tissue was observed after 30-day hypokinesia. Most likely, this is due to the age peculiarities – the beginning of puberty, which may be a stress – a limiting reaction. It continues during 60-day hypokinesia and is leveled off after 90-day immobilization [4, 6].

Hypokinesia for warm-blooded animals and humans is a stress factor, and the activation of the NO-system is one of the body mechanisms of prevention of stress damages. Excessive formation of NO can significantly reduce the tone of the smooth muscle cells, impair endothelial function and inhibit myocardial contractile function, which is observed at septic and hemorrhagic shock, and acute myocardial infarction.

The sympathetic and parasympathetic divisions of the autonomic nervous system play a decisive role in the regulation of cardiac rhythm [7, 8]. The sympathetic effects on the heart is implemented through the influence of catecholamines on various adrenergic receptors of the heart cells. Currently, the presence of α_1,α_2 and β_1,β_2,β_3 -AR in the heart is proven. It is known that the main AR involved in the regulation of heart function in rats are β_1-AR and α_1-AR. Non-selective agonists of β-AR are isoproterenol, epinephrine, and norepinephrine. Norepinephrine is also a non-selective

agonist of α-adrenergic receptors. Therefore, objective of the research was to study the effect of hypokinesia on myocardial contractility in rats at administration of various doses of norepinephrine under blocking NOS and βAR.

2. Methods

Experiments were conducted on male and female herding laboratory outbred white rats. The animals were divided into two groups (each of 20 animals): 1) control group – 110-day-old animals, kept under standard vivarium conditions, at unrestricted motion activity; 2) experimental group - animals, kept at restricted physical activity for 90 days. Restriction of motor activity of growing rats was achieved by placing them in the pen-case cages. The cage volume was changed by moving a partition wall, in accordance with the size of the animal. Hypokinesia started from age of 21 days: the first two days hypokinesia time was 1 hour, and further increased by 2 hours every 2 days [9]. By day 25, the time of stay in the cages reached 23 hours, and thereafter remained constant until the end of the experiment. At 22-23-hour hypokinesia, animals were released from the cages for 1-2 hours. If necessary, the rats were washed with warm water, the cages were washed too and dried. The pen-case cages were placed in special boxes for optimum temperature conditions.

Myocardial contractile activity in the *in vitro* experiment was studied on strips of the atria and ventricles. Reaction of myocardial contractility to the action of agonists and blockers was determined on "PowerLab" ("ADInstruments") device, with a force sensor "MLT 050/D" ("ADInstruments"). The curve was recorded on the personal computer with the use of "Chart 5.5" software.

An anesthetized rat was fixed on an operating table, then the thorax was opened, the heart was removed and placed in a petri dish with the oxygenated Krebs solution, with the connected "ECL-2" stimulator. Then the strips of 1.5-2 mm long and >1mm width were cut out from the right ventricle and atrium. Myocardial strips were fixed vertically with one end to a force sensor, and the other - to the point of support, then each strip was immersed in a separate 10 ml reservoir with the Krebs solution.

Composition of Krebs solution (g/l): NaCl- 8 g; KCl- 0,3 g; CaCl2- 3 ml; MgSO4 – 0.5 ml; NaH2PO4- 0.04 g; Glucose – 2 g; Trizma HCl- 2.4-3.9 g/l; Trizma base- 0.25 g/l (Sigma). The solution was continuously aerated with carbogen 95% O_2 and 5% CO_2, pH was maintained in the range of 7.3-7.4.

The process solution was prepared on the day of the experiment. pH level was determined using a pH-meter ionomer EXPERT 001, with ESK 10601/7 electrode. To maintain the pH level in the range of 7.3-7.4, the solution was added with Trizma basic and acidic buffers ("Sigma"). Myocardial strips were stimulated through platinum electrodes with a

frequency of 10 stimuli per min., each of 5 msec. After dipping into the reservoir, the slice was left for 40-50 minutes, during which an optimal stretch was gradually applied to the muscle fibers. At the end of this procedure, the initial contractile parameters were recorded, and then for 30 minutes with adding pharmacological agents to the process solution.

We used L-NAME at a dose of 10 mg/kg as a non-selective NOS blocker. L-NAME was administered intraperitoneally 60 min prior to autopsy.

We used the following substances in our experiment, added with a micropipette directly to the bath:

- Obsidan – nonspecific blocker of β-AR (0.1% solution at a concentration of 0.8 mg/kg);
- norepinephrine (NE) in the concentration range of 10^{-5}M – 10^{-7}M.

We evaluated the percentage of change in force of isometric contraction of the atrial and ventricular myocardial strips caused by the effect of pharmacological agents as compared with the initial parameters. The initial contractile parameters were taken as 100%.

The average of the measured value and standard error of mean M ± SEM were obtained upon statistical processing. The significance of differences was determined using the Student t-test. Differences were considered significant at $p<0.05$.

3. Results

The exact role of NO as a modulator of myocardial contractility and force-length relation remains unclear. The reason for this is the diversity of its intracellular targets, which sometimes can have the opposite effect on contractility. Based on the experimental conditions and tissues, NO usually has no effect on the basic function, but causes a decrease in the reactivity to stimulation of β-AR [10,11,12].

We carried out the determination of the reaction of contractile function of the atrial and ventricuar myocardium of mature rats to the action of an agonist of adrenergic receptors - norepinephrine - in the concentration range of 10^{-5} – 10^{-7} on the background of a NOS inhibitor – L-NAME, and the blockade of β-AR with Obsidan.

The NOS blockade in the control group of rats caused reduction in contractility of atrial myocardium strips at administration of NE at a concentration of 10^{-7}M on the background of β-AR blockade up to 49.3±5.6% ($p<0.05$). A further increase in the NE concentration caused an increase in myocardial strips by 8.1±1.9%. The studied substance at a concentration of 10^{-5}M also caused increase in contractility of myocardial strips by 8.2±2.1% (Figure 1).

The NOS blockade caused reduction in contractility of ventricular myocardium strips at administration of NE at a concentration of 10^{-7}M on the background of β-AR blockade up to 37.4±6.2% ($p<0.05$). A further increase in the NE concentration also caused reduction in myocardial strips by 15.8±1.1% ($p<0.05$). Administration of norepinephrine at a concentration of 10^{-5}M caused reduction in myocardial contractility by 10.5±3.1%.

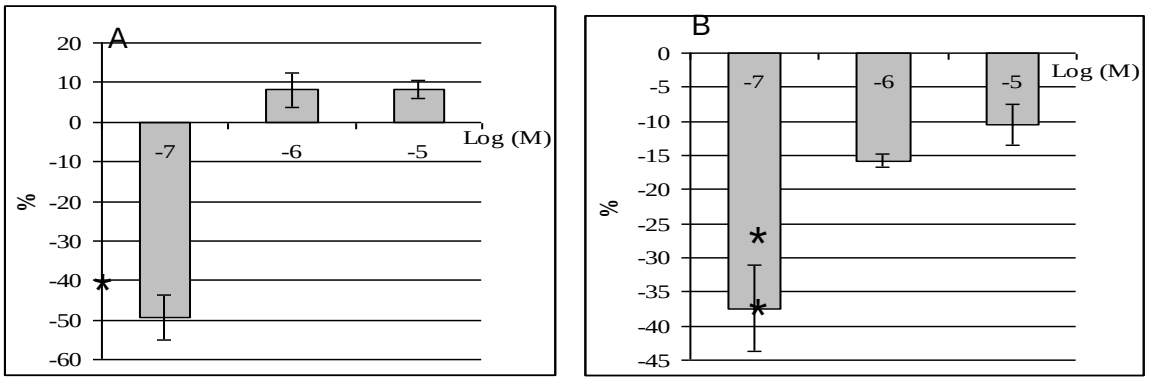

Fig. (1). Effect of norepinephrine on the contractility of atrial (A) and ventricular (B) myocardium in the control group of rats at NOS and β-AP blockade. Concentrations, M.

Note: * - significance as compared to initial value: $p<0.05$.

The NOS blockade in the experimental group of rats caused reduction in contractility of atrial myocardium strips at administration of NE at a concentration of 10^{-7}M on the background of β-AR blockade up to 32.9±4.9% ($p<0.05$). A further increase in the NE concentration caused reduction in myocardial strips by 27.9±6.5% ($p<0.05$). The studied substance at a concentration of 10^{-5}M caused increase in contractility of myocardial strips by 6.1±0.8%. The contractility of ventricular myocardium strips at administration of NE at a concentration of 10^{-7}M on the background of β-AR blockade reduced up to 20.5±6.2% ($p<0.05$). Norepinephrine concentrations of 10^{-6}M and 10^{-5}M also reduce the contractility of myocardial strips by 14.2±5.1% and 34.9±5.1%, respectively ($p<0.05$) (Figure 2).

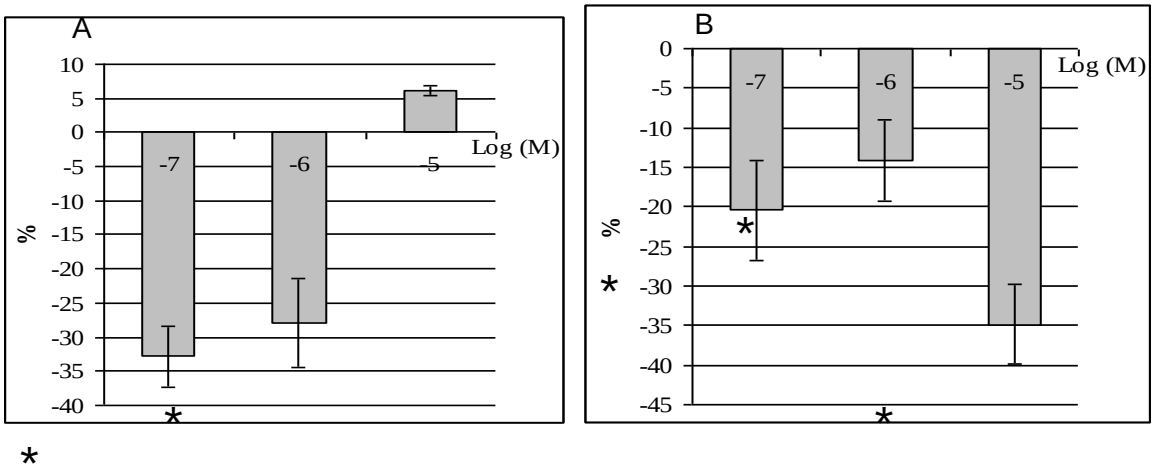

Fig. (2). Effect of norepinephrine on the contractility of atrial (A) and ventricular (B) myocardium in the experimental group of rats at NOS and β-AP blockade. Concentrations, M.

Note: * - significance as compared to initial value: $p<0.05$.

4. Summary

Both experimental groups have shown that, on the background of the β-AR and NOS blockade, the administration of norepinephrine causes reduction in contractility of the myocardial strips at all studied concentrations, and decrease in contractility of the strips of atrial myocardium at a concentration of 10^{-7}M.

5. Conclusion

Thus, on the background of the β-AR blockade with propanol and NOS-blockade with L-NAME, the administration of norepinephrine at all studied concentrations causes a decrease in contractility of the strips of ventricular myocardium in both experimental groups. In the atria, norepinephrine at a concentration of 10^{-5}M causes a slight increase in the contractile force of myocardial strips.

The results of our experiments revealed no significant influence of 90-day hypokinesia on the myocardial contractility in rats in response to the administration of norepinephrine on the background of NOS and β-AR blockade.

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