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Research Article

Dose-Dependent Effect of Dopamine on Rats Myocardium Contractility in Postnatal Ontogenesis

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ABSTRACT:
A catecholamine-activated sympathetic-adrenal system plays special role in neuro-humoral regulation of body functions. It is known that the effect of dopamine on the function of the cardiovascular system differs from the effects of epinephrine and norepinephrine. Dopamine functions are realized through activation of D_1 and D_2 of dopamine receptors, which are found in human and rat heart. But dopamine also interacts with α- and β-adrenergic receptors. Regulatory effect of dopamine on myocardial contractility is least studied, especially during ontogeny. Objective of this study was to determine a dose-dependent effect of dopamine on the strength of myocardial contraction and the effect of different concentrations of dopamine upon dopamine receptor blockade on myocardial atria and ventricles of rats of different ages. The experiments were performed on white laboratory rats, aged 21 and 100 days, in compliance with all bioethical rules. Isometric contraction of atrial and ventricular myocardial strips were recorded on “Power Lab” (ADInstrumets). Reaction of myocardial contraction force to the increasing concentrations of dopamine (Sigma) was determined within the range of 10^{-9}-10^{-5} M. Dopamine receptors were blocked with the use of Droperidol (10^{-6}M) (“Sigma”). The reaction of contraction force in response to dopamine was calculated as a percentage of the original, which was taken as 100%. The differences significance was assessed by Student t-test. All age groups of animals have shown positive inotropic influence of dopamine at a dose of 10^{-9} M in interacting with dopamine receptors. At the same time, the reaction of the atrial myocardium to dopamine was higher in 21-day-old rats, and ventricles – in 100-day-old animals. Higher doses of dopamine (10^{-7} M, 10^{-6} M, 10^{-5} M) induce negative inotropic effects.After blocking the dopamine receptors with Droperidol, dopamine has a positive effect on the contractile force of the atrial myocardium of 21-day-old rats at a concentration of 10^{-5} M. 100-day-old rats, on the background of the blockade of dopamine receptors, showed a dose-dependent increase in the strength of atrial and ventricular myocardial contraction at all used concentrations of dopamine (10^{-9}-10^{-5}M). Therefore, high concentrations of dopamine (10^{-5}M) are involved in the regulation of myocardial adrenergic receptors via activation of adrenergic receptors after forming the adrenergic cardiac regulation.

Keywords: dopamine, myocardium, rat, contractility, ontogenesis

INTRODUCTION
The first reports on the synthesis of a precursor of norepinephrine - dopamine - were made more than a hundred years ago (G. Barger, P.C. Ewins, 1910; E. Mannich, W. Jacobsohn, 1910). It was shown that dopamine has weak peripheral sympathomimetic activity. Then, it was established that dopamine alters the cardiovascular system functions different from those that cause rush of epinephrine and norepinephrine [1]. Development of methods of fluorescence histochemistry made it possible to detect the distribution of dopamine both in the

brain structures and in peripheral tissues, which served as the basis for the assumption of an independent functional role of dopamine as a neurotransmitter [1].

Currently, there has been shown the presence of five different subtypes of dopamine receptors [2,3,4,5,6]. They are classified as D1-like receptors, including subtypes D1 and D5 - stimulating an adenylate cyclase, and D2-like receptors that include D2-, D3- and D4-receptor. They inhibit an adenylate cyclase and Ca-channels and activate K-channels, and these effects are carried out with involvement of G-proteins [7]. Dopamine in low doses (> 10^{-5}M) acts through the dopamine receptors, and the increase in its doses activates β and α-adrenergic receptors [8,9]. Pharmacological, biochemical and molecular techniques allowed revealing the presence of different subtypes of dopamine receptors in the heart [10,11,12,13,14,15,16]. D1-receptors are localized in human and rat heart [10,11,12], D2-receptors have also been found in rat heart [15,17], and D3 and D4-receptors – in the heart of guinea pig [16]. The positive inotropic effect of dopamine on the ventricles of the chick embryo heart is mediated through the stimulation of postsynaptic β-adrenergic receptors and dopamine receptors [13]. Dopamine as a brain neurotransmitter is of great interest, since the degeneration and deficiency of dopamine neurons in one of the main mechanisms of Alzheimer's and Parkinson's diseases. This impairs a vegetative balance of the sympathetic-parasympathetic interactions in heart towards the predominance of sympathetic influences during pre-symptomatic stage of the disease [18]. Therefore, the study of the reaction of the myocardium to different concentrations of dopamine in animals of different ages is very important. It is known that the sensitivity during ontogenesis to mediators and hormones is different, and dopamine may be a test object for the pre-symptomatic stage of development of neurodegenerative diseases.It should be noted that the effect of dopamine on myocardial contractility in a postnatal ontogenesis remains poorly studied. Objective of this study was to determine a dose-dependent effect of dopamine on the strength of myocardial contraction and the effect of different concentrations of dopamine upon dopamine receptor blockade on myocardial atria and ventricles of rats aged 21 and 100 days.

METHODS

Experiments were conducted on the isolated atrial and ventricular myocardia of 21- and 100-day-old rats, in compliance with all bioethical rules. Isometric contraction of myocardial strips of atria and ventricles were recorded on “Power Lab” (ADInstrumets) with a force sensor MLT 050/D (ADInstrumets). The rats were anesthetized with urethane (1200 mg/kg) and their heart was quickly taken out and placed in a petri dish with oxygenated process solution, connected to a stimulator “ЭСЛ-2”. Next, the muscle strips of atrial and ventricular mocardia of 2-3 mm and a diameter of 0.8- 1.0 mm were dissected. The upper end of the strip was attached by a thread to a force transducer, and a lower end was attached to the block, which was placed in a reservoir with a process solution (g/l: NaCl - 8g; KCl - 0.3g; $CaCl_2$- - 3ml; $MgSO_4$ - 0.5 ml ; NaH_2PO_4 - 0.04g; glucose - 2g; Trizma HCl - 2.4-3.9g/l). The solution was continuously aerated with carbogen 95% O_2 and 5% CO_2 , pH=7.4. The myocardium strips were stimulated during 5ms through platinum electrodes at a frequency of 6 stimuli for 21-day-old rats, and 10 stimuli for 100-day-old rats.The curve was recorded on the personal computer with the use of "Chart 5.0” software. During 30-40 minutes after dipping into reservoirs, the stabilization of myocardial strips contractions was achieved (running-in). At the end of this procedure, the initial contractile parameters were recorded, and then for 20 minutes with adding dopamine (10^{-9}-10^{-5}M) («Sigma») to the process solution. After the end of stimulation with dopamine the specimens were washed three times with process solution for 5 minutes and then the initial indicators were recorded for each subsequent dose. Contractile force was expressed in grams, the response to dopamine was calculated as a percentage of the original, which was taken as 100%. D-receptor blockade was achieved with Droperidol at 10^{-6} M concentration. Statistical

processing was carried out by determining M, m and σ, the significance of differences was calculated using the Student's t-test (* - $p<0.05$).

RESULTS

In the first series of the experiments the dose-dependent effect of dopamine (10^{-9}-10^{-5}M) on the contractile activity of the myocardium of 21- and 100-day-old rats was determined. In 21-day-old rats, 10^{-9} M dopamine concentration causes a positive inotropic effect on the atrial and ventricular myocardium. The contractile force in atria increased by 13.14% ($p<0.05$); in the ventricles - by 7.43% ($p<0.05$) (**Fig.1**). Adding 10^{-8}M dopamine to the process solution causes a decrease in contractile force of atrial myocardium by 9.18% ($p<0.05$), and increase in ventricular contractility force by 6.93%. Further experiments on 21-day-old rats showed that dopamine at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M had only a negative impact on myocardial inotropy. The contractile force in the atria decreased by 5.63% ($p<0.05$), 8.63% ($p<0.05$) and 10.72% ($p<0.05$), and in the ventricles by 10.68 %, 5.53% ($p<0.05$) and 13.02% ($p<0.05$), respectively (**Fig.1**).

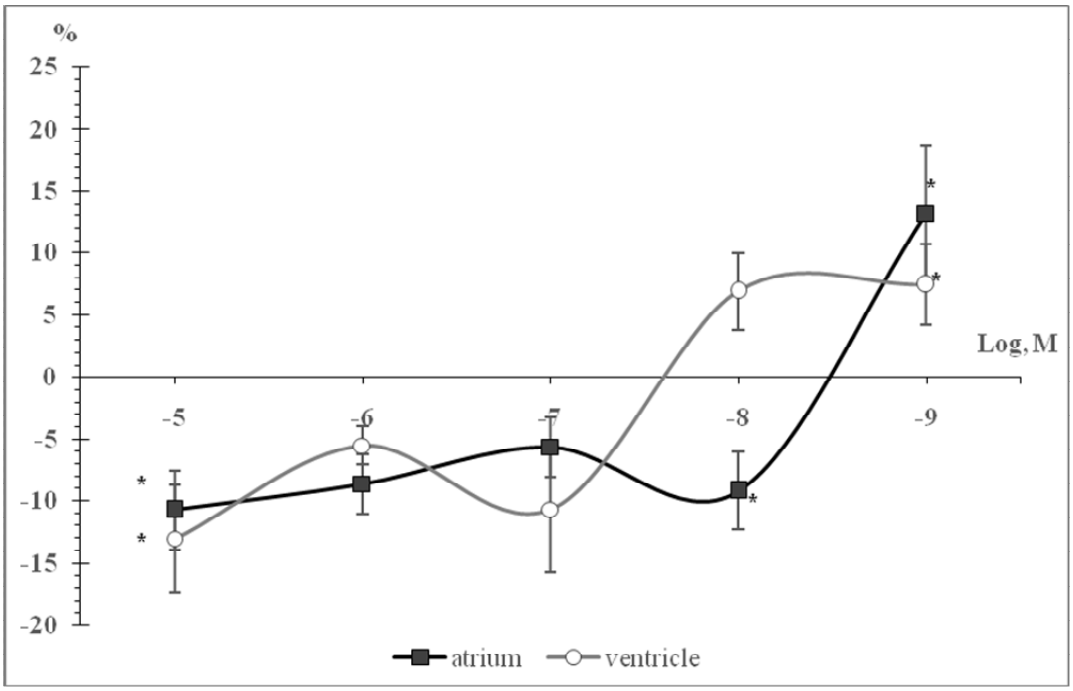

Fig. (1). Reaction of force of myocardium contraction of the atria and ventricles to dopamine in various doses in rats aged 21 days.

Note: reliability in comparison with the initial value: «*»- $p<0.05$.

In 100-day-old rats, the maximal effect on myocardial contractility was observed under the action of dopamine at a concentration of 10^{-9} M. The contractile force of myocardial strips increased by 7.83% ($p<0.05$), and the ventricles by 12.23% ($p<0.05$) (**Fig.2**).

Dopamine at a concentration of 10^{-8}M had no significant effect on the contractile force of the myocardium strips. Adding 10^{-7} M dopamine to the carbogen-perfusing process solution caused a decrease in contractile force of myocardial strips of the atria and ventricles by 4.34% and 4.30%, respectively.

The contractile force of the atrial myocardium in 100-day-old rats after addition of 10^{-6} M dopamine decreased by 5.41% ($p<0.05$), and the ventricles – by 10.67% ($p<0.05$). Dopamine at a concentration of 10^{-5} M reduces the contractile force of atrial myocardium by 5.24% ($p<0.05$), and the ventricles – by 7.33% ($p<0.05$) (**Fig.2**). Thus, in 21- and 100-day-old animals, dopamine at a concentration of 10^{-9} M enhances myocardial inotropy.

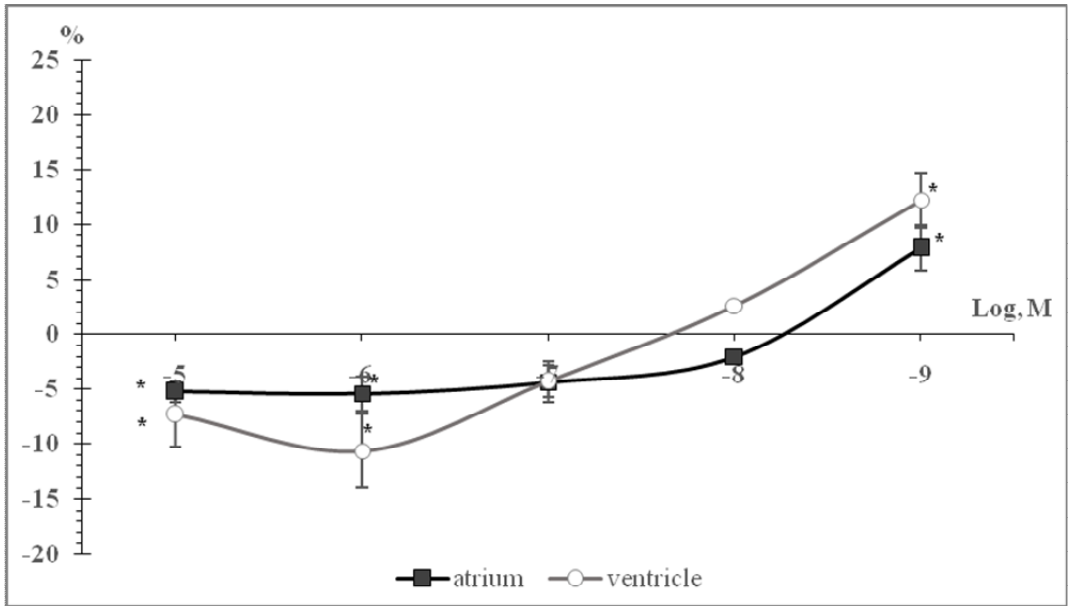

Fig. (2). Reaction of force of myocardium contraction of the atria and ventricles to dopamine in various doses in rats aged 100 days.

Note: reliability in comparison with the initial value: «*»- $p<0.05$.

Upon Droperidol-induced deprivation of D-receptors in 21-day-old rats, 10^{-9}M dopamine causes reduction in the contractile force of atrial myocardium by 12.51%, and in ventricles - by 16.09% (**Fig.3**). Against the background of Droperidol-induced blockade, 10^{-8}M dopamine causes a decrease in contractile force of atrial and ventricular myocardia by 10.43% and 11.31%, respectively. The contractile force of the atrial and ventricular myocardium in 21-day-old rats decreased also after administering dopamine at a concentration of 10^{-7} M. When adding 10^{-6} M dopamine, the contractile force of atrial myocardium increased by 15.05% ($p<0.05$). Against the background of Droperidol action, dopamine at a concentration of 10^{-5} M caused a positive inotropic effect in 21-day-old rats (**Fig.3**).

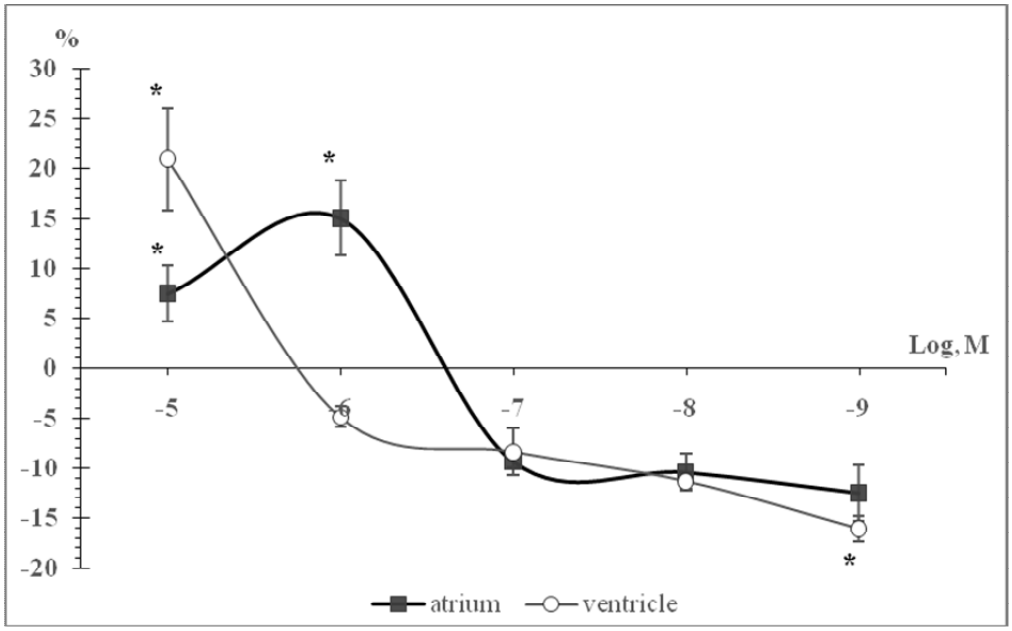

Fig. (3). Reaction of force of myocardium contraction of the atria and ventricles to dopamine at different doses 21 days of age at D-receptor blockade in the rat.

Note: reliability in comparison with the initial value: «*»- $p<0.05$.

In 100-day-old animals, on the background of the blocked D-receptors, the positive inotropic effect of the atria and ventricles of the myocardium persists at all concentrations tested (**Fig.4**). Thus, dopamine

at concentrations of 10^{-9}-10^{-6}M causes an increase in myocardial contractility by 5-9%. In 100-day-old rats, on the background of the blocked D-receptors, dopamine at a concentration of 10^{-5} M causes an increase in contractile force of atrial and ventricular myocardium by 18.09% ($p<0.05$) and 12.14% ($p<0.05$), respectively (**Fig.4**). Consequently, 21- and 100-day-old rats with blocked D-receptors show positive inotropic effect of atrial and ventricular myocardium under the influence of dopamine at a concentration of 10^{-5}M.

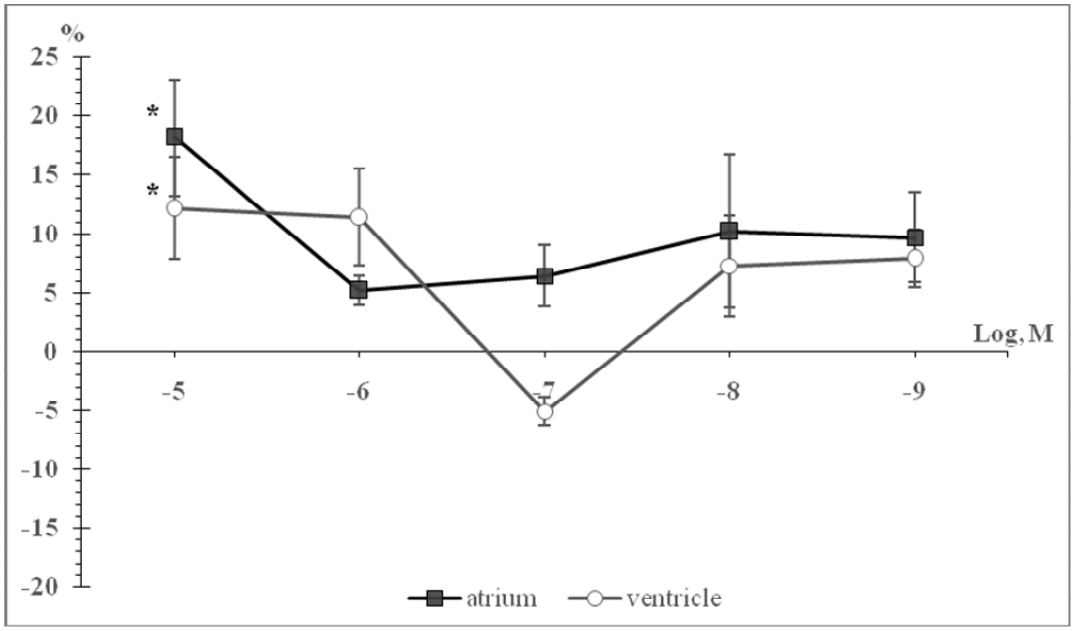

Fig. (4). Reaction of force of myocardium contraction of the atria and ventricles to dopamine at different doses 100 days of age at D-receptor blockade in the rat.
Note: reliability in comparison with the initial value: «*»- $p<0.05$.

SUMMARY:

The conducted studies allow us to make the following conclusion:

1. Dopamine at a concentration of 10^{-9} M has a positive inotropic effect on 21- and 100-day-old rats.
2. Increase in the dopamine dose up to 10^{-7}-10^{-5}M causes only negative inotropic effects on the heart.
3. In 100-day-old animals, on the background of the blocked D-receptors, the positive inotropic effect of dopamine is reached at a concentration of 10^{-5}M.

CONCLUSION:

Thus, it was found that low concentrations of dopamine (10^{-9} M) cause an increase in contractile force of myocardial strips. Possibly, this effect is associated with the binding of dopamine directly to dopamine receptors [8, 9, 19]. Following the blockade of D-receptors with Droperidol, the contractile force of ventricular and atrial myocardia in 21-day old rats was observed only under the action of high concentrations of dopamine (10^{-5} M). In 100-day-old rats, on the background of the blocked dopamine receptors, the increase in the contractile force of the atria and ventricles of the myocardium was observed at all concentrations of dopamine (10^{-9}-10^{-5}M). Perhaps, dopamine at high concentrations (10^{-5} M) is involved in the regulation of myocardial contractility by activation of adrenergic receptors after forming the sympathetic regulation of the heart. It should be noted that the formation of the adrenergic innervation in the rat heart occurs during week 3-6 of postnatal development. These findings suggest that the role of dopamine receptors in the regulation of cardiac activity is dose-dependent and depends on the age of the animals.

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