Effect of α_2-Adrenoceptor Stimulation on Cardiac Activity in Rats

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We studied the effect of α_2-adrenoceptor stimulation with clonidine on BP and cardiac activity in rats. Variations in BP and chronotropism of the heart were studied *in vivo* after bolus injection of clonidine. *In vitro* experiments were performed to evaluate a dose-dependent change in myocardial contractility of the atria and ventricles after treatment with clonidine in concentrations of 10^{-9}-10^{-5} M. α_2-Adrenoceptor stimulation with clonidine had a negative chronotropic and inotropic effect and induced the decrease in systolic BP of rats.

Key Words: *heart; chronotropism; inotropy; blood pressure; rat*

The sympathetic part of the autonomic nervous system has a wide range of cardiovascular effects, including the positive chronotropic, inotropic, lusitropic, and dromotropic effects [2,10]. Adrenergic receptors (AR) serve as an intermediary for the biological effects of the sympathetic nervous system. There are the following nine subtypes of AR: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 receptors [5]. Much attention was paid to studying the biological role of β_1, β_2, and β_3 receptors. Agonists and antagonists of β-AR are extensively used in practice [7,8,14]. The role of other AR in physiological activity of the heart is poorly understood. α_1-AR are involved in the regulation of blood flow and cause vasoconstriction of the basilar arteries [12]. Differences were found in the cardiac response to blockade of various subtypes of α_1-AR [3,15]. α_2-AR are the major regulators of sympathetic tone, neurotransmitter release, BP, and intraocular pressure. α_2-AR activation cause the sedative and analgesic effects [10]. α_2-AR are present in the vascular smooth muscles. α_2-AR suppress the sympathetic nervous system by the central mechanisms, which contributes to a decrease in systemic BP [11]. Norepinephrine release in the sympathetic nerve endings is regulated by presynaptic α_{2A}-AR and α_{2C}-AR [10], while genetic knockout of these subtypes of AR is followed by hypertrophy of the heart and cardiac insufficiency due to chronic increase in norepinephrine release in the heart and epinephrine hypersecretion in the adrenal medulla [4,9]. Nonselective activation of α_2-AR is usually accompanied by biphasic changes in BP. After a short phase of hypertension (more pronounced after intravenous injection), BP decreases below the baseline value. Oral administration of α_2-AR agonists has a hypotensive effect, which is used for the therapy of patients with hypertension. Biphasic variations in BP are probably mediated by 2 various subtypes of α_2-AR. α_{2B}-AR play a role in the initial phase of hypertension, while prolonged hypotension is realized via α_{2A}-AR [7,8,14]. α_2-AR blockade can produce a therapeutic effect in patients with coronary artery atherosclerosis [10]. α_{2B}-AR probably have a role in the development of acute heart attacks [13]. A possible role of α_2-AR in the regulation of various functions will be evaluated in further studies. This approach will allow us to develop new methods of treatment with medicinal agents that have an inhibitory or activating effect on various subtypes of α-AR.

Here we studied the effect of α_2-AR stimulation on chronotropic function, BP, and myocardial contractility of the atria and ventricles in rats.

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MATERIALS AND METHODS

Experiments were performed on 42 outbred albino rats aging 20 weeks. The animals were intraperitoneally narcotized with 25% urethane in a dose of 1000 mg/kg. In *in vivo* experiments, an α_2-AR agonist clonidine (Sigma) in a dose of 0.01 mg/kg was injected into the right femoral vein. ECG was recorded and analyzed in a computer system. BP in rats was measured using a SDK-1 device for noninvasive systolic pressure measurement. The pulse wave in the caudal artery was recorded using a clip pulse sensor. Air was pumped into the cuff, the artery was compressed, and the pulse wave disappeared. The pulse wave appeared in a certain moment of air discharge. This pressure in the cuff corresponded to systolic pressure in the caudal artery. The results were analyzed by LGraf software.

In *in vitro* experiments, the isolated heart was placed in a bath with the working solution. Two stimulating electrodes were connected to the solution. Myocardial strips (length 2-3 mm, diameter 0.8-1.0 mm) were excised from the right atrium and right ventricle according to the anatomical organization of the heart. The specimen was mounted vertically in a 20-ml reservoir, which contained the working solution oxygenated with carbogen (97% O_2 and 3% CO_2) at room temperature.

The specimen was subjected to electrical stimulation via 2 silver electrodes on an ESL-2 stimulator (frequency 6-10 impulses per min, signal amplitude 10 mV, duration 5 msec). The specimen was embedded into a reservoir. Optimal tension of the muscle fibers was progressively achieved during the treatment period (40-60 min). The optimal tension corresponds to a specific value of specimen extension, above which the strength of contraction decreases. The baseline parameters of contraction were recorded for 5 min after this period. Clonidine in concentrations of 10^{-5}-10^{-9} M was added. The strength of contraction (F) was expressed in grams. The results were analyzed by Chart 5 software on a Power Lab device (ADInstruments) using the Statgraphics software package. The significance of differences was evaluated by Student's and Wilcoxon tests (Microsoft Excel software).

RESULTS

In vivo stimulation of α_2-AR with clonidine in a dose of 0.01 mg/kg was followed by bradycardia. The mean *R-R* interval (Xm) increased from 174.00±9.69 to 239.0±19.46 msec ($p<0.05$) 1 min after administration of this agonist. Xm increased most significantly by the 15th minute after treatment (243.00±19.05 msec; $p<0.05$). Xm reached 232.00±16.87 msec by the 20th minute after clonidine injection ($p<0.05$). Xm was 217.00±20.26 msec after 30 min, which exceeded the baseline value by 25.00±5.67% ($p<0.01$). By the 60th minute, Xm was 111.00±1.14% of the baseline value ($p<0.01$; Fig. 1). Clonidine injection was followed by the decrease in systolic pressure from 80.00±3.74 to 51.0±3.2 mm Hg ($p<0.01$). BP remained low until the 20th minute of the study (62.00±3.74 mm Hg; $p<0.01$). Systolic pressure progressively increased and reached 81.0±18.7 mm Hg by the 60th minute (Fig. 1).

The influence of α_2-AR stimulation with clonidine on the strength of myocardial contractions in the atria and ventricles was studied *in vitro*. The agonist was used in doses of 10^{-9}-10^{-5} M to evaluate the sensitivity of myocardial α_2-AR. Experiments on isolated myocardial strips from the atria and ventricles showed that clonidine has a dose-dependent negative inotropic effect. Clonidine in a concentration of 10^{-9} M decreased the strength of myocardial contractions in the atria and ventricles by 5.00±1.93 ($p<0.05$; Fig. 2)

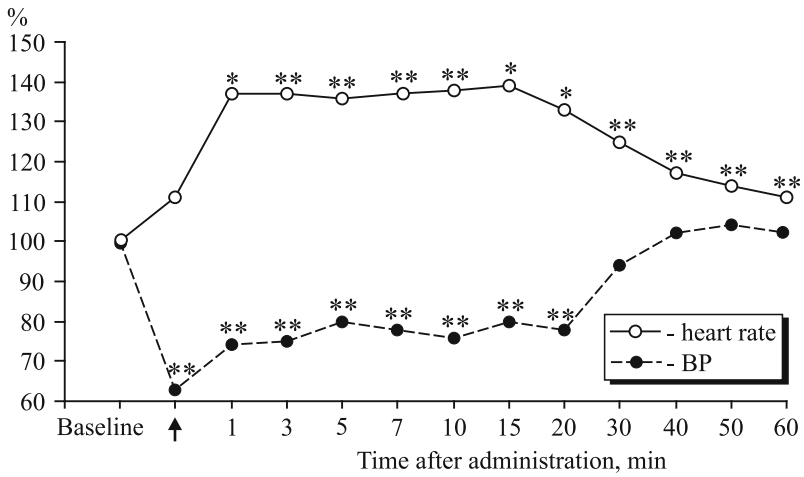

Fig. 1. Effect of α_2-AR stimulation with clonidine on the heart rate and BP. Arrow: administration. Here and in Figs. 2 and 3: *$p<0.05$ and **$p<0.01$ in comparison with the baseline.

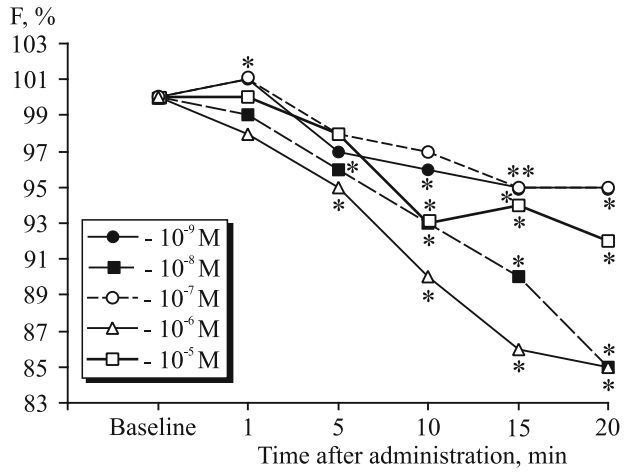

Fig. 2. Effect of α_2-AR stimulation with clonidine on contractility of the atrial myocardium.

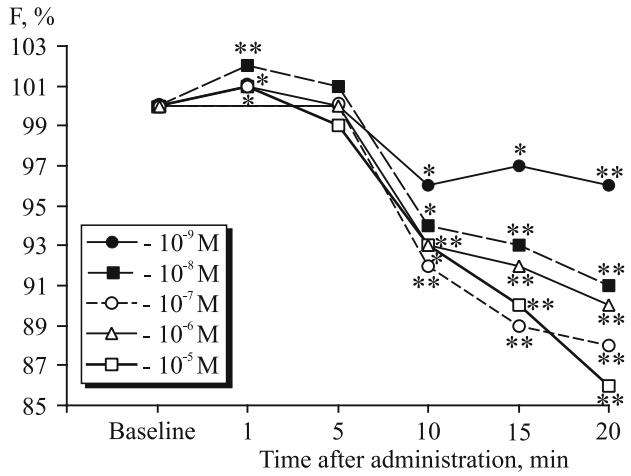

Fig. 3. Effect of α_2-AR stimulation with clonidine on contractility of the ventricular myocardium.

and 4.00±0.87% (p<0.01; Fig. 3), respectively. Addition of this agonist in a concentration of 10^{-8} M to the perfusion solution was followed by a decrease in the strength of contractions in the atria and ventricles by 15.00±5.40 (p<0.05; Fig. 2) and 9.00±2.26% (p<0.01; Fig. 3), respectively. After treatment with 10^{-7} M clonidine, the strength of contractions in the atria and ventricles decreased by 5.00±2.12 (p<0.05; Fig. 2) and 12.00±2.40% (p<0.01; Fig. 3), respectively. The agonist in a concentration of 10^{-6} M decreased the strength of myocardial contractions in the atria and ventricles by 15.00±3.99 (p<0.05; Fig. 2) and 10.00±2.74% (p<0.01; Fig. 3), respectively. This agent in a concentration of 10^{-5} M decreased the strength of myocardial contractions in the atria and ventricles by 8.00±3.01 (p<0.05; Fig. 2) and 14.00±2.58% (p<0.01; Fig. 3), respectively.

These data suggest that clonidine produced a modulatory effect on the analyzed reactions of the cardiovascular system. Our previous experiments have demonstrated age-related features of *in vivo* α_2-AR blockade on chronotropic function of the heart [1]. It should be emphasized that α_2-AR blockade with yohimbine did not produce significant changes of cardiac activity in adult rats. Clonidine *in vivo* had the negative chronotropic and hypotensive effects. These effects were revealed immediately after administration of clonidine. Bradycardia persisted for 1 h after clonidine injection. The hypotensive effect was observed for a shorter period of time (Fig. 1). *In vitro* experiments showed that clonidine in concentrations of 10^{-9}-10^{-5} M has a dose-dependent negative inotropic effect on isolated myocardial strips from the atria and ventricles. A negative inotropic effect developed by the 10th minute of the study. It should be emphasized that addition of clonidine was followed by an immediate slight increase in contractility of the atrial (10^{-9} M) and ventricular myocardium (10^{-9}, 10^{-8}, and 10^{-5} M; Figs. 2 and 3). It was shown that α_2-AR bind to inhibitory Gi and Go proteins and decrease the activity of adenylate cyclase. However, some authors reported that α_2-AR can bind to Gs proteins, which contributes to the increase in adenylate cyclase activity. It was hypothesized that α_2-AR decrease cAMP level under the influence of agonists in low concentrations. At the same time, stimulation of α_2-AR with agonists in high concentrations is followed by the increase in cAMP level [6]. The opposite effects of α_2-AR stimulation can be related to different localization of these receptors. Stimulation of postsynaptic and presynaptic receptors can produce various effects on the target. We revealed only the inhibitory effect of α_2-AR stimulation. The negative chronotropic and hypotensive effects are probably mediated by the central and peripheral mechanisms. *In vitro* experiments revealed a direct effect of clonidine on myocardial contractility in rats.

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REFERENCES

1. T. L. Zefirov, N. I. Ziatdinova, L. I. Khisamieva, and A. L. Zefirov, *Bull. Exp. Biol. Med.*, **151**, No. 6, 664-666 (2011).
2. N. I. Ziyatdinova, R. E. Dementeva, L. I. Khisamieva, and T. L. Zefirov, *Bull. Exp. Biol. Med.*, **156**, No. 1, 1-3 (2013).
3. N. I. Ziatdinova, A. L. Zefirov, and T. L. Zefirov, *Bull. Exp. Biol. Med.*, **152**, No. 1, 19-21 (2011).
4. M. Brede, G. Nagy, M. Philipp, *et al.*, *Mol. Endocrinol.*, **17**, No. 8, 1640-1646 (2003).
5. O. E. Brodde and K. Leineweber, *Eur. J. Pharmacol.*, **500**, Nos. 1-3, 167-176 (2004).
6. K. Gyires, Z. S. Zádori, T. Török, and P. Mátyus, *Neurochem. Int.*, **55**, No. 7, 447-453 (2009).
7. K. Leineweber, *Ann. Med.*, **36**, Suppl. 1, 64-69 (2004).

8. M. J. Lohse, *Trends Mol. Med*., **10**, No. 2, 55-58 (2004).
9. A. Lymperopoulos, G. Rengo, and W. J. Koch, *Trends Mol. Med*., **13**, No. 12, 503-511 (2007).
10. M. Philipp, M. Brede, and L. Hein, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **283**, No. 2, R287-R295 (2002).
11. M. Philipp and L. Hein, *Pharmacol. Ther*., **101**, No. 1, 65-74 (2004).
12. R. Shannon and M. Chaudhry, *Am. Heart J*., **152**, No. 5, 842-850 (2006).
13. A. Smapir, P. Heinonen, T. P. Tuomainen, *et al*., *J. Am. Coll. Cardiol*., **37**, No. 6, 1516-1522 (2001).
14. T. L. Zefirov, N. I. Ziyatdinova, and A. L. Zefirov, *Neurophysiology*, **35**, No. 6, 415-421 (2003).
15. N. I. Ziyatdinova, R. E. Dementieva, L. I. Fashutdinov, and T. L. Zefirov, *Bull. Exp. Biol. Med*., **154**, No. 2, 184-185 (2012).