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GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

α_{2C}-Adrenergic Receptor Blockade Inhibits Langendorff-Isolated Rat Heart Work

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We studied the effect of selective α_{2C}-adrenergic receptor antagonist JP-1302 in concentrations of 10^{-9}-10^{-6} M on inotropy, chronotropy, and coronary flow in the Langendorff-isolated rat heart. JP-1302 in all studied concentrations decreased the left-ventricular myocardium force contraction, HR, and coronary flow. The maximum inotropic, chronotropic, and vascular effects were observed when the antagonist was applied to the perfused solution in a concentration of 10^{-7} M. The least pronounced decrease in the studied parameters was observed at JP-1302 concentrations of 10^{-8} and 10^{-9} M. The obtained data indicate the participation of this subtype of α_2-adrenergic receptors in the regulation of activity of isolated adult rats heart.

Key Words: *heart; α_{2C}-adrenergic receptor; myocardium; JP-1302; rat*

Currently, nine subtypes of adrenoreceptors (AR) are recognized: α_{1A}, α_{1B}, α_{1D}, $\alpha_{2A/D}$, α_{2B}, α_{2C}, β_1, β_2, and β_3 [1]. The participation of α_2-AR in various physiological functions, in particular in the regulation of the cardiovascular system and CNS has been shown [2].

All three α_2-AR subtypes were detected by immunoblotting in rat cardiac tissue, in the right atrium and left ventricle. The mRNA levels of the three α_2-AR subtypes in the right atrium and left ventricle do not differ significantly [3]. The expression of α_2-AR is maximal in rat fetal heart tissue, although it decreases with increasing the gestational age. Indirect immunofluorescence microscopy with subtype-specific antibodies and Western blotting showed the presence of $\alpha_{2A/D}$-AR and α_{2C}-AR in the population of fetal cardiomyocytes [4]. In the human heart, mRNAs of all three α_2-AR subtypes were detected by PCR [5].

It was previously thought that α_2-AR in the mammalian heart are located on the presynaptic membranes and only modulate the regulatory influences by affecting the release of norepinephrine, but latter α_2-AR were identified in vascular smooth muscle cells, on presynaptic membranes of adrenergic fibers, and on postsynaptic membranes of cardiomyocytes [6-8]. The question of the presence and functional significance of α2-AR in the heart of humans and animals remains the subject of active research [1,8,9]. Further studies of the role of different α_2-AR subtypes will help to clarify their functional significance in the heart regulation.

The aim of this work was to study the effect of selective blockade of α_{2C}-AR by JP-1302 on activity of the isolated rat heart.

MATERIALS AND METHODS

The study was performed in accordance with the principles of the European Convention for the Protection

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of Vertebrate Animals Used for Experimental or Other Scientific Purposes (Strasburg, 1986) and the recommendations of the Local Bioethical Committee of Kazan (Volga region) Federal University (Protocol No. 39, December 22, 2022). Experiments were performed on heart preparations of white outbred 20-week-old rats (n=32) of both sexes.

In a study on a Langendorff-isolated heart, Krebs–Henseleit solution for warm-blooded animals was used. The solutions were saturated with carbogen (95% O_2, 5% CO_2), the pH of the solutions was maintained at 7.3-7.4 at 37°C. The α_{2C}-AR blocker JP-1302 (Tocris) was used in concentrations of 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M. These concentrations of the blocker were selected based on the published reports and the results of our previous studies [10,11].

The rats were anesthetized with 25% urethane solution (800 mg/kg body weight) and the chest was opened. The heart was isolated and washed with ice-cold Krebs–Henseleit working solution (2°C). The isolated heart was cannulated through the aorta, and gravitational retrograde perfusion with oxygenated working solution was performed under a constant hydrostatic pressure of 60-65 mm Hg using a Langendorff unit (ADInstruments) at 37°C. To measure the pressure in the left ventricle (LV), a latex balloon filled with distilled water was inserted through a hole made behind the left ear. Changes in LV pressure were recorded using an ML T844 pressure transducer (ADInstruments). HR (bpm), left-ventricular developed pressure (LVDP, mm Hg), and coronary flow (CF, ml/min) were calculated from the curve recorded on a PowerLab 8/35 setup (ADInstruments) using the LabChart Pro program (ADInstruments). Perfusion with JP-1302 solution was carried out for 20 min.

Statistical analysis of the results obtained on the isolated heart was performed using the LabChart Pro 8 software. The data are presented as the $M\pm SEM$; n is the number of independent experiments. There were 8 animals in each series of experiments with a certain concentration of the blocker, the total number of experimental animals was 32. Significance of differences was assessed using one-way ANOVA followed by post hoc Tukey's test for related groups and adjustment for multiplicity of comparisons; paired and unpaired Student's t test was also applied. The differences were considered statistically significant at p<0.05.

RESULTS

Perfusion of the isolated heart of 20-week-old rats with an α_{2C}-AR blocker at a concentration of 10^{-9} M decreased LVDP from 88.63±5.1 to 81.33±3.9 mm Hg (p<0.05) at the 10th minute of the experiment. The maximum decrease in LVDP from 88.63±5.1 to 75.30±4.09 mm Hg was observed by the 19th minute (by 13.72±6.08% from the initial value; p<0.05; Fig. 1). Blockade of α_{2C}-AR also reduced HR from 247.06±8.98 to 237.86±7.94 bpm (p<0.01) by the 12th minute. The maximum decrease in HR by 6.61±2.34% (p<0.01; Fig. 2) was observed at the 16th minute (to 230.13±7.73 bpm; p<0.05). The CF of the isolated rat heart against the background of blockade decreased from 11.68±0.58 to 10.68±0.45 ml/min (p<0.05) by the 16th minute of the experiment (by 7.97±3.15% of the initial value; p<0.05) (Fig. 3). Then, the CF did not change.

When the blocker JP-1302 was added in a concentration of 10^{-8} M, the force of LV contraction decreased from 83.99±6.09 to 78.42±4.99 mm Hg (by 5.96±2.74%; p<0.05; Fig. 1). The blockade led to bradycardia in some animals (n=4) and to tachycardia in others (n=4). Bradycardia developed 10 min after administration of the blocker: HR decreased from 217.45±12.22 to 210.04±10.54 bpm (p<0.05); the maximum decrease in

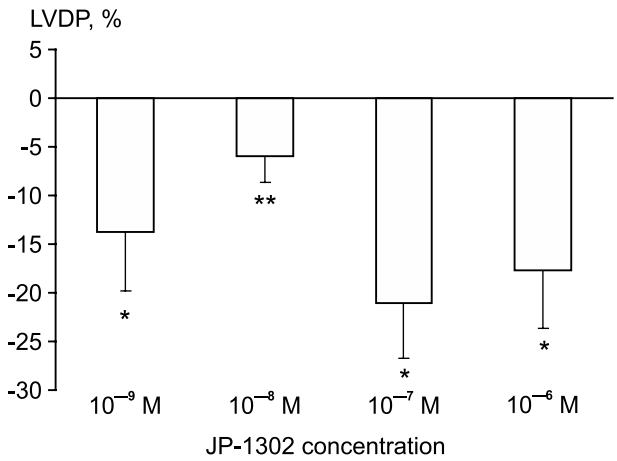

Fig. 1. Effect of α_{2C}-AR antagonist (JP-1302) on LVDP in the heart of 20-week-old rats. *p<0.05, **p<0.01 in comparison with baseline.

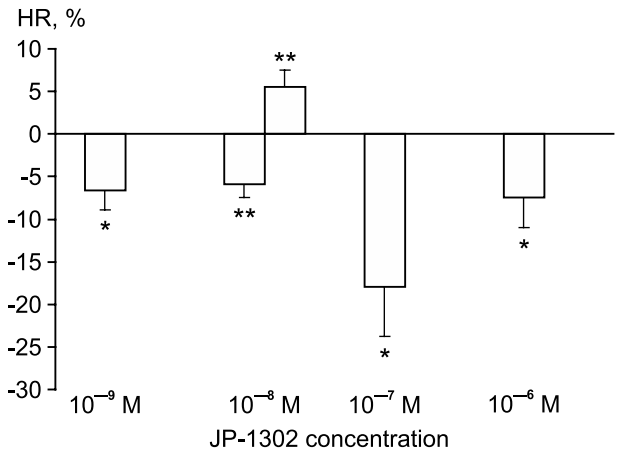

Fig. 2. Effect of α_{2C}-AR antagonist (JP-1302) on HR in 20-week-old rats. *p<0.05, **p<0.01 in comparison with baseline.

HR to 204.24±9.11 bpm (5.86±1.62%) was observed at the 19th minute of the experiment ($p<0.01$; Fig. 2). An increase in HR from 229.12±27.56 to 241.44±27.68 bpm (by 5.54±1.97%; $p<0.05$) (Fig. 2) was recorded at the 18th minute of observation. During perfusion with the blocker, CF decreased from 9.25±1.02 to 7.85±0.9 ml/min ($p<0.01$) by the 17th minute (by 14.05±4.81% of the initial value; $p<0.01$) (Fig. 3).

The addition of JP-1302 in a concentration of 10^{-7} M decreased LVDP from 71.42±10.55 to 55.67±6.76 mm Hg ($p<0.05$) by the 15th minute of the experiment. At the 20th minute, blockade of α_2-AR led to the maximum decrease in LVDP to 54.43±7.06 mm Hg ($p<0.05$), which corresponded to 21.07±5.68% of the initial value ($p<0.01$; Fig. 1). The maximum chronotropic effect during perfusion of JP-1302 was observed at the 20th minute. HR decreased from 227.98±7.58 to 185.47±9.64 bpm ($p<0.01$) by 17.91±5.85% ($p<0.01$; Fig. 2). By the 19th minute of the experiment, CF decreased from 7.93±1.17 to 6.25±1.04 ml/min ($p<0.05$) with the addition of the blocker, by 19.82±7.28% of the initial value ($p<0.05$; Fig. 3).

Perfusion of the isolated heart of mature rats with an α_{2C}-AR blocker at a concentration of 10^{-6} M decreased LVDP from 96.96±8.86 to 81.83±10.88 mm Hg ($p<0.01$) at the 15th minute of the experiment — by 17.71±5.96% ($p<0.01$; Fig. 1). By the 11th minute, the blockade reduced HR from 237.9±14.67 to 227.98±15.17 bpm ($p<0.05$). The maximum decrease in HR to 220.56±16.62 bpm was observed at the 18th minute (7.47±3.47%; $p<0.05$; Fig. 2). The blockade reduced CF of the isolated rat heart from 8.35±0.98 to 7.42±0.85 ml/min ($p<0.05$) by 10.16±3.93% ($p<0.05$; Fig. 3).

Thus, blockade of α_{2C}-AR at concentrations of 10^{-9}-10^{-6} M affects the inotropy, chronotropy, and CF in the isolated rat heart. The contraction force of the LV myocardium, HR, and CF decreased after application of all studied concentrations of α_{2C}-AR antagonist (JP-1302). However, the use of a blocker in a concentration of 10^{-8} M resulted in both bradycardia and tachycardia. Our previous *in vivo* studies have shown that intravenous administration of an α_{2C}-AR antagonist increased HR in 20-week-old animals [11]. In experiments on myocardial strips from the right atrium, a negative inotropic effect was observed, while in the ventricles it was positive in all studied concentrations of the JP-1302 antagonist [12]. The results confirm the significant role of α_{2C}-AR in the regulation of the isolated heart.

Experiments on *Adra2C*-knockout animals showed that the α_{2C} isoform predominantly controls the secretion of catecholamines from adrenal chromaffin cells by a Ca^{2+}-dependent feedback mechanism [13,14]. Hence, the blockade of α_{2C}-AR in the whole organism

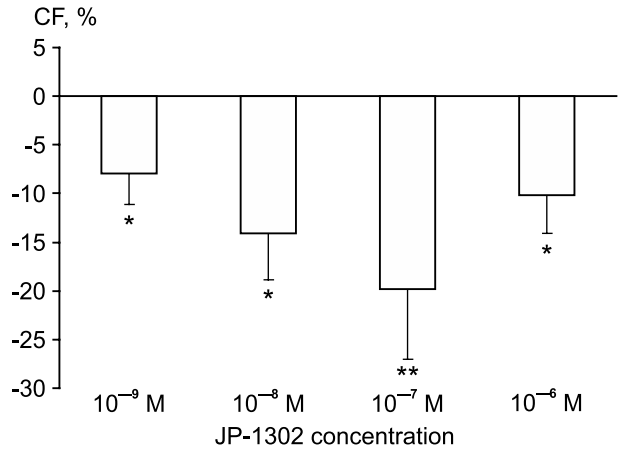

Fig. 3. Effect of α_{2C}-AR antagonist (JP-1302) on cardiac CF in 20-week-old rats. *$p<0.05$, **$p<0.01$ in comparison with baseline.

leads to tachycardia, which we have previously shown experimentally. However, the results obtained on Langendorff-isolated hearts and on myocardial strips indicate the possibility of a different mechanism for regulating cardiac activity. Experiments on isolated cardiomyocytes showed that NO and cyclic guanosine monophosphate (cGMP) are central intracellular messengers of α_2-AP signaling in ventricular myocytes. α2-AR agonist guanabenz stimulated NO production by activating the endothelial NO synthase (eNOS) isoform via the PI3K-Akt/PKB signaling pathway. It is known that activation of Akt/PKB and eNOS causes phosphorylation and S-nitrolysis of phospholamban and Ca^{2+}-ATPase of the sarcoplasmic reticulum (SERCA) by activating Ca^{2+} pumping into the reticulum [15]. It is possible that blockade of α_{2C}-AR suppresses eNOS, resulting in a decrease in LVDP. The results obtained indicate that the most pronounced effect is observed when using the blocker JP-1302 at a concentration of 10^{-7} M, and this concentration is the most adequate for experiments on studying the dynamics of LVDP. The results of our studies show that the effects of α_{2C}-AR blockade depend on the presence of different levels of cardiac activity regulation, what should be considered when applying medicines in the clinic.

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