DOI 10.1007/s10517-020-04943-1
Bulletin of Experimental Biology and Medicine, Vol. 169, No. 5, September, 2020

BIOPHYSICS AND BIOCHEMISTRY

Effect of α2-Adrenergic Receptor Stimulation on the Isolated Rat Heart against the Background of I_f Blockade

N. I. Ziyatdinova, A. M. Kuptsova, M. I. Sungatullina, and T. L. Zefirov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 169, No. 5, pp. 564-567, May, 2020
Original article submitted February 4, 2020

The study examined the effect of α_2-adrenoreceptor (α_2-AR) activation against the background of preliminary blockage of I_f on the performance of Langerndorff-isolated rat heart. Stimulation of α_2-AR in isolated rat hearts against the background of ZD7288 in concentrations of 10^{-9} M and 3×10^{-5} M changed the negative dynamics of myocardial inotropy to positive (by 25 and 38%; $p<0.05$). Activation of α_2-AR produced opposite effects on HR. I_f blockade abolished tachycardia caused by activation of α_2-AR; HR deceleration in response to α_2-AR agonist against the background of I_f blocker in a concentration 10^{-9} M was 41% ($p<0.05$). We observed negative dynamics of coronary flow (by 38%; $p<0.05$) in isolated adult rat hearts after application of α_2-AR agonist against the background of I_f blockade (10^{-9} M).

Key Words: *α2-adrenergic receptors; I_f; isolated heart; rat*

α_2-Adrenergic receptors (α_2-AR) are associated with heterotrimeric pertussis toxin-sensitive G_i/G_o and G_s proteins and affect the cascade of cell biochemical reactions [2]. In the heart of mammals and humans, α_2-AR activation inhibits the release of acetylcholine from cholinergic synapses [6], participates in modulating norepinephrine release [10], in the protective reflex, sedative effect, reduces BP [3], and mediates vasoconstriction. α_2-AR are located on the membranes of cardiomyocytes, vascular smooth muscles, cells of peripheral and central nervous system, intestinal and renal epithelium [8].

In the heart of mammals, α_2-AR performs the function of modulation of regulatory influences. Activation of α_2-AR inhibits cAMP synthesis by adenylate cyclase. Presumably, activation of α_2-AR with a low concentration agonist leads to a decrease in intracellular cAMP content, while higher concentrations of the agonist increase cAMP concentration [5]. I_f of HCN channels are modulated by cAMP level and the sympathetic and parasympathetic departments of the autonomic nervous system [9]. Modulation of I_f through cAMP is an essential element in HR regulation by the autonomic nervous system. It was shown that stimulation of adrenergic receptors activates I_f thereby increasing the chronotropic function of the heart through β-AR-mediated increase in cAMP level [9]. Available data indicate a significant effect of α_2-AR on cardiac performance: activation of α_2-AR induces bradycardia and reduces systolic BP in rats, produces different effects on contractility of rat atrial and ventricular strips [12], reduces inotropy and produces opposite effects on HR and coronary flow in Langendorff-isolated rat heart [14]. Non-selective stimulation of α_2-AR against the background of preliminary I_f blockade reduces the severity of tachycardia caused by norepinephrine [13]; phenylephrine against the background of I_f blocker ZD-7288 induces a significant two-phase HR deceleration [7].

Heart automatism and regulation of neuronal excitability are controlled by current through hyperpo-

Department of Human Health Protection, Kazan (Volga Region) Federal University, Kazan, Republic of Tatarstan, Russia. ***Address for correspondence:*** zefirovtl@mail.ru. T. L. Zefirov

0007-4888/20/1695-0635

larization-activated cyclic nucleotide-gated channels (HCN channels). This current in the nervous system is referred to as I_h (hyperpolarization-activated current). Recent ample molecular and pharmacological data confirmed the role of HCN channels and α_2-AR in the regulation of nervous system functions [4,11]. For instance, α_2-AR agonist dexmedetomidine has been shown to inhibit I_h [11]. Dexmedetomidine binds to α_2-AR on the neuronal membrane, which leads to activation of G-protein-coupled K^+ channels and inhibition of I_h leading to membrane hyperpolarization.

As HCN channels and α_2-AR are present in cardiomyocytes, I_f can also serve as an effector of adrenergic regulation of the heart through this receptor. Here we studied the effect of α_2-AR activation against the background of preliminary I_f blockade on the performance of Langendorff-isolated rat heart.

MATERIALS AND METHODS

Ex vivo experiments were performed on 20-week-old white outbred rats (n=21) on the Langendorff heart perfusion system (ADInstruments) in compliance with the principles of Good Laboratory Practice and ethical standards for the treatment of animals. The effects of α_2-AR agonist clonidine hydrochloride alone (control) and against the background of preliminary I_f blockage with ZD7288 (experiment) were compared.

The rats were intraperitoneally anesthetized with 25% urethane (800 mg/kg). The hearts were isolated, washed, and placed in cold Krebs—Henseleit solution (2-5°C). The isolated heart was mounted on a cannula through the aorta and retrogradely perfused under a constant hydrostatic pressure of 60-65 mm Hg at 37°C with oxygenated (95% O_2, 5% CO_2) solution. For α_2-AR stimulation clonidine hydrochloride (Sigma) was added in a concentration of 10^{-6} M; I_f blocker ZD7288 (Tocris) was added in concentrations of 10^{-9} and 3×10^{-5} M. Contractile activity of the myocardium was studied in the isovolumic mode using MLT844 pressure sensor (ADInstruments) with a latex balloon filled with water and inserted into the left ventricle. HR (bpm), left ventricular pressure (LVDP, mm Hg), and coronary flow (ml/min) were calculated from the curve. Registration was performed on a Power Lab 8/35 installation (ADInstruments) using the LabChart Pro program.

Statistical processing of the results was carried out using one-way ANOVA (Statistica 8.0) and paired and unpaired Student's t tests. The differences were significant at $p<0.05$.

RESULTS

To identify the relationship between I_f and α_2-AR, experiments were carried out with the introduction of α_2-AR agonist clonidine hydrochloride (10^{-6} M) against the background of preliminary blockade I_f ZD7288. I_f blocker was administered in high (3×10^{-5} M) or low (10^{-9} M) concentrations. Previous studies showed that I_f blocker ZD7288 in these concentrations caused significant changes in the functioning of the isolated heart [1].

Application of α_2-AR agonist (10^{-6} M) against the background of the preliminary I_f blockade with ZD7288 (10^{-9} M) induced a decrease in LVDP from 18.7±3.6 to 16.4±2.4 mm Hg by the 4th min followed by an increase in this parameter to 21.8±5.3 mm Hg (by the 15th min) and 23.4±5.9 mm Hg (by the 20th min; Fig. 1, *a*). The increase in LVDP was 25% (Fig. 2, *a*). Non-selective stimulation with clonidine hydrochloride against the background of ZD7288 (3×10^{-5} M) increased LVDP from 18.1±3.5 to 22.4±3.9 mm Hg ($p<0.01$) by the 5th min of the experiment. By the 20th min, LVDP increased to 25±4.4 mm Hg ($p<0.05$), *i.e.* by 38%. In the control group, application of α_2-AR agonist (10^{-6} M) to the perfused solution reduced LVDP by 26% ($p<0.001$) (Fig. 2, *a*).

Clonidine hydrochloride stimulation against the background of ZD7288 blockade (10^{-9} M) reduced HR from 139.9±15.7 to 83.1±14.5 bpm ($p<0.05$) by the 10th min of the experiment and to 82.1±15.2 bpm (by 41%; $p<0.05$) by the 20th min. Application of α_2-AP agonist against the background of I_f blockade (3×10^{-5} M ZD7288) reduced HR from 164.6±17.6 to 148.3±20.4 bpm ($p<0.05$) by the 5th min of the

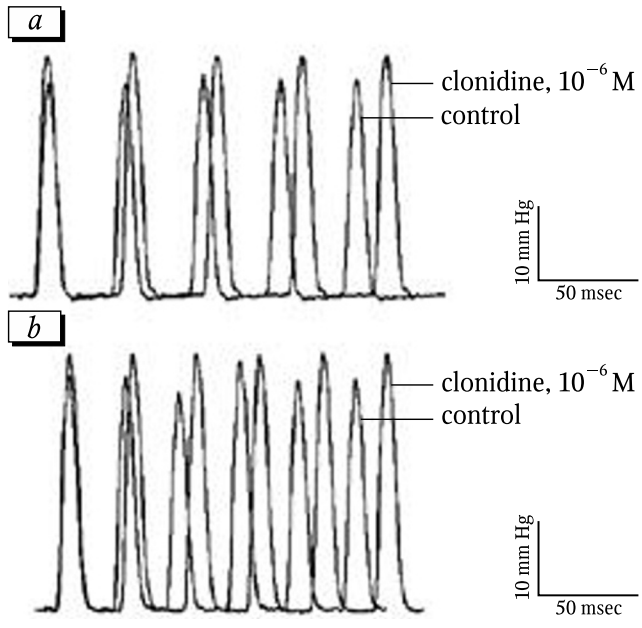

Fig. 1. Effect of α_2-AR agonist clonidine hydrochloride (10^{-6} M) against the background of I_f blockade with ZD7288 in concentrations of 10^{-9} M (*a*) and 3×10^{-5} M (*b*) on LVDP and HR of Langendorff-isolated rat heart (original record). Control: record prior to administration of α_2-AP agonist.

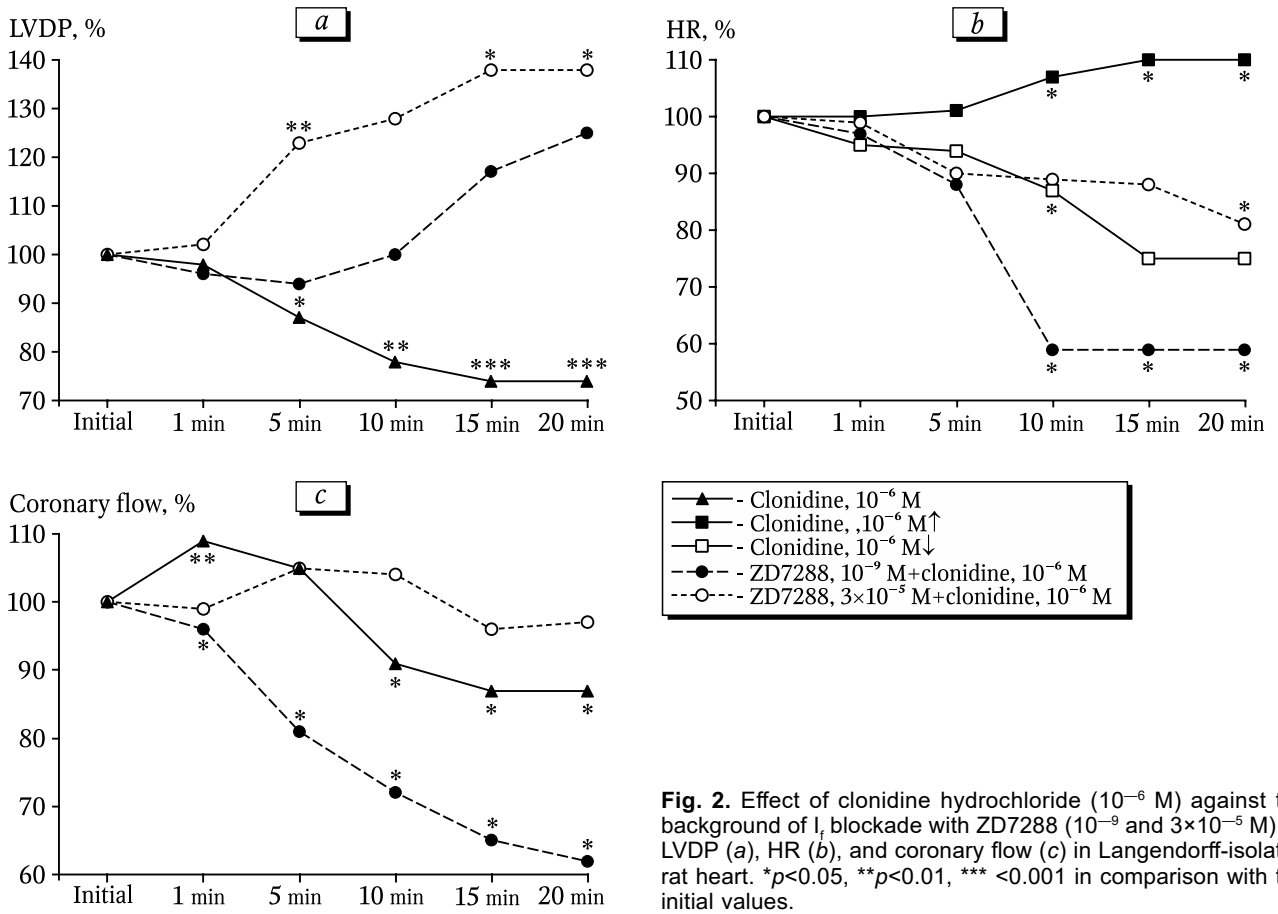

Fig. 2. Effect of clonidine hydrochloride (10^{-6} M) against the background of I_f blockade with ZD7288 (10^{-9} and 3×10^{-5} M) on LVDP (*a*), HR (*b*), and coronary flow (*c*) in Langendorff-isolated rat heart. *p<0.05, **p<0.01, *** <0.001 in comparison with the initial values.

experiment, to 140.0±21.4 bmp (p<0.05) by the 16th min, and to 133.5±21.4 bpm (*i.e.* by 19%) (p<0.05) by the 20th min. In the control group, application of α_2-AR agonist to the working solution induced opposite changes in HR (Fig. 2, *b*): in some hearts, HR increased from 148.0±16.6 to 162.0±12.9 bpm ($p\leq0.05$) by the 15th min (by 9%), while in others, this parameter decreased from 167.7±26.3 to 145.9±24.4 bpm ($p\leq0.05$) by the 10th min (by 25%).

Activation of α_2-AR against the background of preliminary I_f blockade (10^{-9} M) reduced coronary flow from 9.3±2.1 to 7.5±1.8 ml/min ($p\leq0.05$) by the 5th min, to 6.2±1.7 ml/min ($p\leq0.05$) by the 15th min, and to 5.8±1.9 ml/min ($p\leq0.05$) by the 20th min of the experiment (Fig. 2, *c*). Thus, the decrease in coronary flow was 38%. Addition of clonidine hydrochloride against the background of I_f blockade (3×10^{-5} M) increased coronary flow from 8.7±2.2 to 9.5±2.6 ml/min by the 5th min of observation. Then, this parameter decreased to 8.3±2.0 ml/min by the 15 min and to 8.5±2.2 ml/min by the 20 min (Fig. 2, *c*). The coronary flow changed by 3%. In the control group, application of α_2-AR agonist increased coronary flow from 3.80±0.09 to 4.10±0.09 ml/min (by 9%; $p\leq0.01$) (Fig. 2, *c*). By the 20th min of the experiment, we observed a gradual decrease in the coronary flow to 3.3±0.04 ml/min ($p\leq0.01$), *i.e.* by 13% of the initial value.

Comparative analysis of the effect of α_2-AR activation with clonidine hydrochloride without (control group) and against the background of I_f blocker ZD7288 (experiment) showed that stimulation of α_2-AR against the background of ZD7288 administration in high and low concentrations changed the dynamics of myocardial inotropy of the isolated heart of adult rats from negative to positive. Thus, stimulation of α_2-AR reduced LVDP by 26%, while application of α_2-AR agonist after I_f blocker in concentrations of 10^{-9} and 3×10^{-5} M increased LVDP by 25 and 38%, respectively. In the control group, activation of α_2-AR induced opposite changes in HR (decrease or increase). I_f blockade abolished the effect of tachycardia: only a decrease in HR was observed upon α_2-AR stimulation. Bradycardia in response to application of α_2-AR agonist against the background of the I_f blocker (10^{-9} M) was more pronounced (41%) than the isolated effect of the agonist (25%). The appearance of negative dynamics of the coronary flow was also observed in the isolated hearts of adult rats after addition of α_2-AR agonist against the background of I_f blockade (10^{-9} M).

Thus, our experiments on evaluation of the role of I_f and α_2-AR in adrenergic mechanisms of regulation of the function of adult isolated rat heart showed that preliminary blockade of I_f changed the dynamics of contractile activity of the heart, strengthened the bradycardic effect, and reduced blood supply in the isolated heart. Based on the literature data, several possible mechanisms of α_2-AR and I_f interaction in the regulation of isolated heart work can be proposed. Some researchers suggest that α_2-AR can be associated with not only cAMP, but also inositol phosphate regulation [11]. Hence, stimulation of α_2-AR can activate protein kinase C leading to Ca^{2+} release, which can partially coincide with the data on the dependence of I_f on Ca^{2+} level [11]. In addition, activation of α_2-AR with clonidine hydrochloride can directly inhibit I_f [6].

This study was performed within the framework of the State Support Program of Competitive Recovery of Kazan Federal University and supported by the Russian Foundation for Basic Research and Government of the Tatarstan Republic (grant No. 18-44-160022).

REFERENCES

1. Kuptsova AM, Ziyatdinova NI, Faskhutdinov LI, Biktemirova RG, Zefirov TL. Influence of HCN Channels on Isolated Heart Functions in Adult Rats. Uchen. zap. Kazan. Univer. Ser. Estestv. Nauki. 2018;160(4):568-578. Russian.
2. Gyires K, Zádori Z.S, Török T, Mátyus P. alpha(2)-Adrenoceptor subtypes-mediated physiological, pharmacological actions. Neurochem. Int. 2009;55(7):447-453.
3. Hongo M, Fujisawa S, Adachi T, Shimbo T, Shibata S, Ohba T, Ono K. Age-related effects of dexmedetomidine on myocardial contraction and coronary circulation in isolated guinea pig hearts. J. Pharmacol. Sci. 2016;131(2):118-125.
4. Inyushin MU, Arencibia-Albite F, Vázquez-Torres R, Vélez-Hernández ME, Jiménez-Rivera CA. Alpha-2 noradrenergic receptor activation inhibits the hyperpolarization-activated cation current (Ih) in neurons of the ventral tegmental area. Neuroscience. 2010;167(2):287-297.
5. Jasper JR, Lesnick JD, Chang LK, Yamanishi SS, Chang TK, Hsu SA, Daunt DA, Bonhaus DW, Eglen RM. Ligand efficacy and potency at recombinant α2-adrenergic receptors: Agonist-mediated (35S) GTPγS binding. Biochem. Pharmacol. 1998;55(7):1035-1043.
6. Knaus AE, Muthig V, Schickinger S, Moura E, Beetz N, Gilsbach R, Hein L. Alpha2-adrenoceptor subtypes-unexpected functions for receptors and ligands derived from gene-targeted mouse models. Neurochem. Int. 2007;51(5):277-281.
7. Kuptsova AM, Ziyatdinova NI, Zefirov TL. The role of If and ICa,L in α-adrenergic regulation of rats cardiac activity. J. Pharm. Res.. 2017;11(10):1270-1273.
8. Maltsev AV, Kokoz YM, Evdokimovskii EV, Pimenov OY, Reyes S, Alekseev AE. Alpha-2 adrenoceptors and imidazoline receptors in cardiomyocytes mediate counterbalancing effect of agmatine on NO synthesis and intracellular calcium handling. J. Mol. Cell. Cardiol. 2014;68:66-74.
9. Mengesha HG, Tafesse TB, Bule MH. If channel as an emerging therapeutic target for cardiovascular diseases: a review of current evidence and controversies. J. Pharmacol. 2017;8:874. doi: 10.3389/fphar.2017.00874
10. Rump LC, Riera-Knorrenschild G, Schwertfeger E, Bohmann C, Spillner G, Schollmeyer P. Dopaminergic and α-adrenergic control of neurotransmission in human right atrium. J. Cardiovasc. Pharmacol. 1995;26(3):462-470.
11. Yang YC, Meng QT, Pan X, Xia ZY, Chen XD. Dexmedetomidine produced analgesic effect via inhibition of HCN currents. Eur. J. Pharmacol. 2014;740:560-564.
12. Zefirov TL, Ziyatdinova NI, Khisamieva LI, Zefirov AL. Effect of α2-adrenoceptor stimulation on cardiac activity in rats. Bull. Exp. Biol. Med. 2014;157(2):154-157.
13. Ziyatdinova NI, Dement'eva RE, Khisamieva LI, Zefirov TL. Age-related peculiarities of adrenergic regulation of cardiac chronotropic action after If blockage. Bull. Exp. Biol. Med. 2013;156(1):1-3.
14. Ziyatdinova NI, Kuptsova AM, Faskhutdinov LI, Zefirov AL, Zefirov TL. Effect of α2-adrenoceptor stimulation on functional parameters of Langendorff-isolated rat heart. Bull. Exp. Biol. Med. 2018;165(5):593-596.