BBRC

Bioscience Biotechnology Research Communications

SPECIAL ISSUE VOLUME 12 NUMBER- (5)
SEPTEMBER 2019
Print ISSN: 0974-6455
Online ISSN: 2321-4007
CODEN BBRCBA
www.bbrc.in
University Grants Commission (UGC)
New Delhi, India Approved Journal

Biosc Biotech Res Comm Special Issue Vol 12 (5)September 2019

An International Peer Reviewed Open Access Journal for Rapid Publication

Published By:
Society For Science and Nature
Bhopal, Post Box 78, GPO,
462001 India

Indexed by Thomson Reuters, Now Clarivate Analytics USA

ISI ESCI SJIF 2019=4.186
Online Content Available:
Every 3 Months at www.bbrc.in

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Medical Communication

OPEN ACCESS BBRC Bioscience Biotechnology Research Communications

Biosc.Biotech.Res.Comm Special Issue Vol 12 No (5) Sep 2019 Pages: 248-253

The Reaction of the 3-Week-Old Rats Heart to A2-Adrenoceptors Stimulation

Timur Lvovich Zefirov, Anna Mihailovna Kuptsova, Luiza Irekovna Khisamieva, Lenar Ilsurovich Faskhutdinov, Nafisa Ilgizovna Ziyatdinova

Kazan Federal University Kazan Russia

ABSTRACT

Heart development is an accurate and complex process controlled by the nervous and humoral regulation of the body. Modern literature presents a sufficient number of results on the formation, development, and growth of sympathetic and parasympathetic nerve fibers in the heart. Adrenergic regulation of cardiac activity is realized through many subtypes of adrenergic receptors (AR). In the heart of mammals and humans, α2-adrenergic receptors (α2-AR) of presynaptic membranes take part in the modulation of the release of norepinephrine, inhibiting the release of acetylcholine from cholinergic synapses, stimulating platelet aggregation, and narrowing the vessels of certain organs. In the central nervous system, activation of postsynaptic α2-adrenergic receptors causes a protective reflex, a sedative effect, and lowers blood pressure. Non-selective stimulation of α2-AR in isolated hearts of guinea pigs leads to a drop in pressure developed by the left ventricle during sympathetic stimulation. The objective of the research was to identify the effect of stimulation of α2-AR in the heart of rats during the onset of the formation of sympathetic innervation of the heart. Ex vivo experiments were performed on Langendorff-isolated hearts. In vivo experiments were performed on a holistic organism. Studies on the whole organism showed that intravenous administration of clonidine hydrochloride leads to bradycardia in 3-week-old rats. Application of the α2-adrenergic receptor antagonist - clonidine hydrochloride in experiments on a Langendorff-isolated heart of 3-week-old rats also causes a decrease in heart rate. The severity of this effect has a concentration dependence. Clonidine had a multidirectional effect on the blood supply to the heart. An agonist at a concentration of 10–9 M and 10–6 M reduces, and at a concentration of 10–8 M increases the coronary flow. Clonidine hydrochloride at a concentration of 10-7 M causes the multidirectional effects of heart circulation of 3-week-old rats. The multidirectional effect of stimulation of α2-adrenergic receptors on CF can be associated with different localization (pre- and postsynaptic) of different subtypes of α2-AR, as well as the ability of these adrenoreceptors to bind to different G-proteins.

KEY WORDS: Alpha2-Adrenergic Receptors, Isolated Heart, Heart Rate, Coronary Flow, Sympathetic Cardiac Innervation, Rat

ARTICLE INFORMATION:

Corresponding Author Email: zefirovtl@mail.ru

PRINT ISSN: 0974-6455, ONLINE ISSN: 2321-4007 CODEN: BBRCBA
Received: 14th July, 2019 Accepted: 16th Sep, 2019

Online content available: http//www.bbrc.in/

Thomson Reuters ISI ESC / Web of Science Clarivate Analytics USA

NAAS Journal Score 2019 (4.38)

Science Journal Impact Factor 2019 (4.196)

INTRODUCTION

Heart development is an accurate and complex process controlled by the nervous and humoral regulation of the body. At different stages of postnatal ontogenesis, the receptors and mediators of the autonomic nervous system involved in the regulation of the cardiovascular system vary (Zefirov, 2011; Zefirov, 2015). Modern literature presents a sufficient number of results on the formation, development, and growth of sympathetic and parasympathetic nerve fibers in the heart. Data on sympathetic nerves were obtained in experimental studies on the heart specimens of adult transgenic mice using noradrenergic marker immunohistochemistry (Ernsberger & Rohrer, 2009). However, histochemistry methods in the ventricles of newborn rats showed no signs of sympathetic innervation.

Early signs of the formation of adrenergic innervation in the heart were detected in experiments with radioactively labeled noradrenaline. Norepinephrine was detected in the form characteristic of an adult organism only 5 weeks after birth. The ability to absorb norepinephrine determines the functional maturity of the sympathetic nerves, which is observed 21 days after birth, and then reaches the parameters of mature animals by day 30 of life (Robinson et al., 1997). Fluorescence microscopy of tissues detected no sympathetic fibers up to 3-weeks of life of rats. Only 5 weeks after birth, the formation of sympathetic innervation, characteristic of adult animals, is observed, which continues to grow until the seventh week (Lipp & Rudolph, 1972).

Parasympathetic innervation of the heart and the main structures of the cholinergic innervation of the heart complete its development by the middle of the embryonic period (Kirby, 2007). Studies have shown that vagal efferent innervation is observed in the heart of mouse embryos and matures in the postnatal period of development (Hildreth et al., 2008; Fregoso & Hoover, 2012). The effects of stimulation of muscarinic cholinergic receptors with carbachol, causing bradycardia, are observed in the

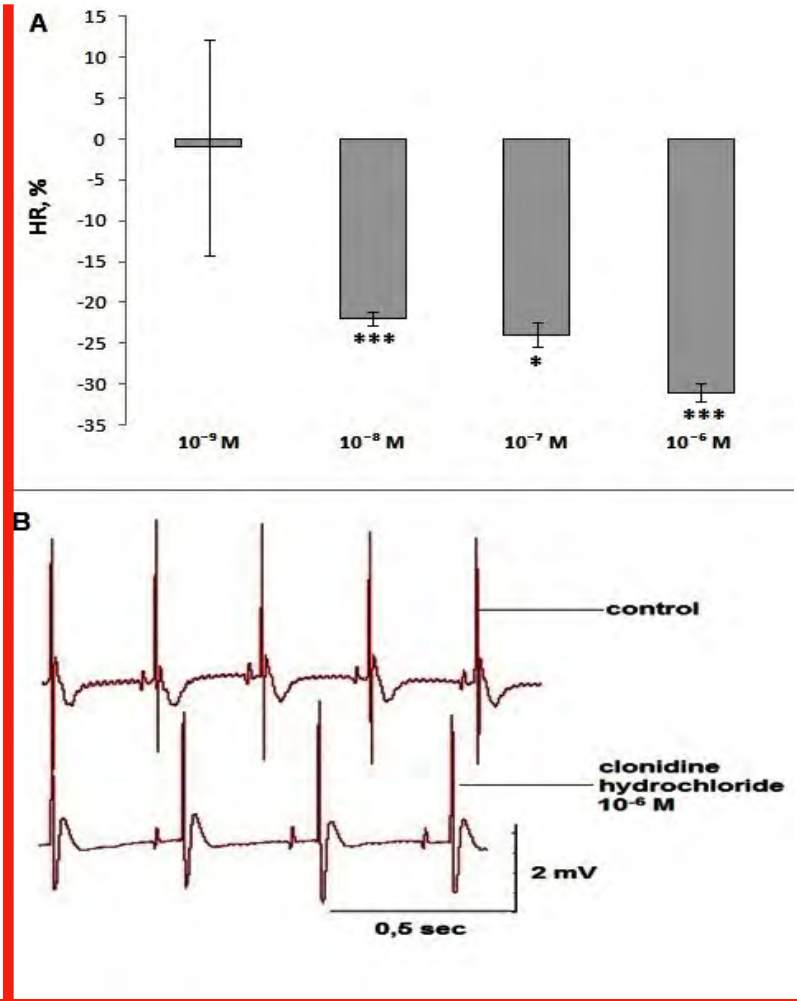

Fig. 1A: Dose-dependent effect of clonidine hydrochloride on heart rate in an isolated heart of 3-week-old rats. The ordinate axis is heart rate (HR, %), the abscissa axis is the concentration of clonidine hydrochloride (mol). Note: the reliability is shown in comparison with the initial values: * - p <0.05, * - p <0.05.Fig. 1B. The effect of stimulation of α2-adrenergic receptors at a concentration of 10-6 M on the heart rate of a Langendorff-isolated heart of 3-week-old rats (original entry).**

heart of the mouse on day 10.5 of embryonic development (Chen et al., 2006).

In 1995, a group of authors guided by Hansen C.A. revealed specific features of the expression of specific G-proteins in the heart at different stages of postnatal ontogenesis (Hansen et al., 1995). The second group of researchers believes that the regulation of the development in the postnatal ontogenesis of the relationship of GPCR with various G-proteins may not depend on the presence of certain protein structures (Robinson, 1996).

Adrenergic regulation of cardiac activity is realized through many subtypes of adrenergic receptors (AR) (Hongo et al., 2016). In the heart of mammals and humans, α2-adrenergic receptors (α2-AR) of presynaptic membranes modulate the release of norepinephrine (Rump et al., 1995), inhibiting the release of acetylcholine from cholinergic synapses, stimulating platelet aggregation, and narrowing the vessels of some organs (Dudek et al., 2015). In the central nervous system, activation of postsynaptic α2-adrenergic receptors causes a protective reflex, sedation, and lowers blood pressure (Knaus et al., 2007). Non-selective stimulation of α2-AR in isolated hearts of guinea pigs leads to a drop in pressure developed by the left ventricle caused by sympathetic stimulation (Hongo et al., 2016). Changes in the activity of the sympathoadrenal system are one of the main factors in the development of pathologies of the cardiovascular system. However, there are a few detailed studies of adrenergic interactions in the heart with different levels of adrenergic innervation. Subject to the above facts, the objective of the study was to identify the effect of stimulation of alpha2-AR in the heart of rats during the onset of the formation of sympathetic innervation of the heart.

METHODS

The experiments were carried out in compliance with the animal ethics standards on 21day-old rats at the early formation of sympathetic innervation of the heart (Robinson, 1996). The experiments were performed on urethane-anesthetized (800 mg/kg mass) white mongrel rats weighing 25-30 g. In ex vivo experiments, the isolated heart of rats was studied using a standard technique on a Langendorff apparatus (ADinstruments, Australia). Details of the method are described previously (Ziiatdinova, 2019). We used a non-selective α2-AR agonist - clonidine hydrochloride, manufactured by Sigma, in a concentration range of 10-9-10-6 mol.

In vivo experiment included ECG using an EC 1T-03M electrocardiograph. Steel needle electrodes were superimposed on the rat limbs. The second standard lead was used. Further, for the introduction of a pharmacological agent on the right thigh, the skin was cut out and the right femoral vein was exposed. After stabilization of the

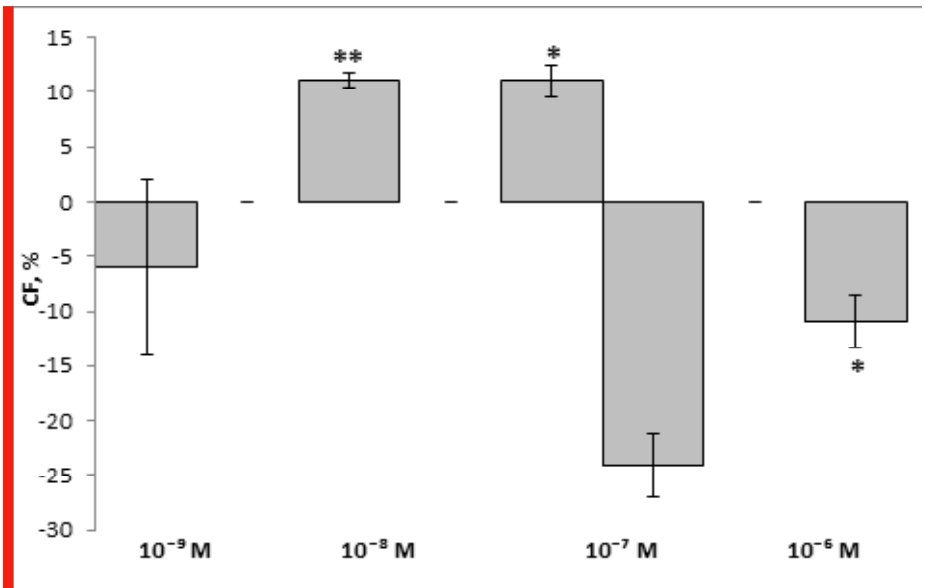

Fig. 2: Dose-dependent effect of clonidine hydrochloride on CF in an isolated heart of 3-week-old rats. The ordinate axis is CF (%), the abscissa axis is the concentration of clonidine hydrochloride (mol). Note: the reliability is shown in comparison with the initial values: * - p <0.05, ** - p <0.05.

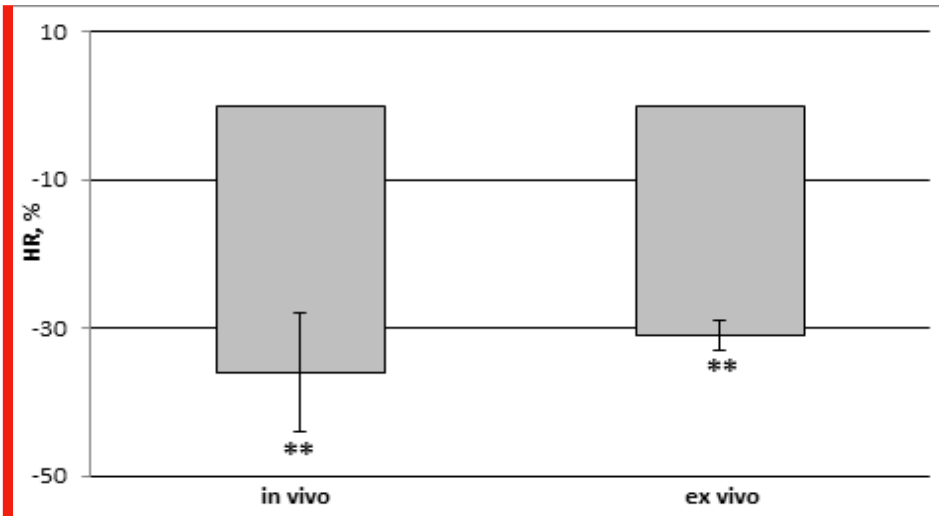

Fig. 3:Changes in the heart rate of 3-week-old rats with the application of an α2-adrenoreceptor agonist in vivo and ex vivo. Note: the reliability is shown in comparison with the initial values: ** - p <0.05.

heart rate, the non-selective α2-AR agonist - clonidine hydrochloride was administered at a dose of 0.01 mg/kg of animal weight. Mathematical analysis of the results of the study was carried out in Excel using the paired Student's t-test.

RESULTS AND DISCUSSION

A study of the effect of the α2-AR agonist on the Langendorff-isolated heart of 3-week-old rats gave the following results. An agonist at a concentration of 10–9 M increased the heart rate from 140.4±21 bpm to 163.8±28.7 bpm by the 6th minute of the experiment. Then, by the 15th minute of observation, heart rate was restored to its initial values and amounted to 141.3±19.4 bpm. An alpha2-adrenoreceptor agonist - clonidine hydrochloride 5 minutes after application led to a decrease in CF from 1.1±0.1 ml/min to 0.8±0.1 ml/min (p<0.05), i.e. the decrease was 21%. Then, the coronary flow tended to recover to 0.99±0.1 ml/min by the 15th minute of the experiment.

Administration of clonidine (10-8M) to 3-week-old rats reduced the heart rate from 230.1±19.9 bpm to 200.2±16.4 bpm (p <0.01) at the 10th minute of observation. The maximum decrease in 22% at the final minute of observation was 181.4±18.9 bpm (p<0.001) (Fig. 1A). Perfusion with an α2-AR agonist promoted increase (by 10%) in the coronary flow from 1.67±0.1 ml/min to 1.85±0.01 ml/min (p <0.01) by the 15th minute of observation (Fig. 2).

Application of an agonist at a concentration of 10-7 M reduced heart rate by 7 minutes from 228.9±18.7 bpm to 185.5±8.6 bpm (p <0.05). By the 15th minute of observation, the decrease was 34%, the heart rate decreased to 175±9.2 bpm (p <0.05) (Fig. 1A, Fig. 4). Perfusion of an isolated heart with an α2-AR agonist exerted multidirectional effects on the coronary flow. In one group of animals, agonist-induced perfusion increased (by 11%) the blood supply to the heart from 1.46±0.1 ml/min to 1.63±0.1 ml/min (p <0.05) by the 14th minute of the experiment (Fig. 2). In another group of animals, clonidine hydrochloride reduced the coronary flow from 1.63±0.1 ml/min to 1.24±0.1 ml/min. The reduction in coronary flow in this group of 3-week-old rats was 24%.

Application of an α2-AR agonist at a concentration of 10-6 M decreased the heart rate from 213.4±15.3 bpm to 172.5±9.6 bpm (p <0.01) by the 7th minute of the experiment. By the final minute of observation, the heart rate was 147.8±8.6 bpm (p <0.001) (Fig. 1A, 1B). Bradycardia was 31% of the baseline. The coronary flow of an isolated heart decreased by 11% when applied with clonidine hydrochloride from 1.37±0.1 ml/min to 1.22±0.1 ml/min (p <0.05) by the 14th minute of the experiment (Fig. 2).

The whole-body studies revealed that an alpha2-adrenergic receptor agonist - clonidine hydrochloride, causes bradycardia in 3-week-old rats. The maximum decrease was observed at the 40th minute of the experiment and amounted to 36% (p <0.01) of the initial value (Fig. 3).

SUMMARY

The whole-body studies revealed that intravenous

administration of clonidine hydrochloride causes bradycardia in 3-week-old rats. Application of an α2-adrenergic receptors agonist - clonidine hydrochloride in experiments on a Langendorff-isolated heart of 3-week-old-rats causes a decrease in heart rate. The severity of this effect has a concentration dependence. Clonidine had a multidirectional effect on the blood supply to the heart. An agonist at a concentration of 10–9 M and 10–6 M reduces, and a concentration of 10–8 M increases the coronary flow. Clonidine hydrochloride at a concentration of 10-7 M causes the multidirectional effects of heart circulation in 3-week-old-rats.

CONCLUSION

Ex vivo results of 3-week-old rats showed that application of an α2-adrenergic receptor agonist - clonidine hydrochloride in a concentration range in a perfused solution causes a decrease in heart rate. Comparison of the results of a decrease in heart rate on a whole organism and on an isolated heart showed that the effect of stimulation of α2-adrenergic receptors on heart rate is higher in a whole body. Perhaps this is due to the effect on the heart of central and peripheral regulatory mechanisms. The previously obtained results of the influence of an α2-adrenergic receptor agonist on the chronotropic function of the heart of adult animals showed that clonidine hydrochloride also causes negative chronotropic and hypotensive effects (Zefirov, 2014). In the isolated heart of adult animals, the α2-adrenergic receptor agonist had multidirectional effects on heart rate and CF (Ziiatdinova, 2018). Activation of α2-adrenergic receptors has a multidirectional effect on the coronary flow of an isolated heart of 3-week-old rats. The multidirectional effect of stimulation of α2-adrenergic receptors on CF can be associated with different localization (pre- and postsynaptic) of different subtypes of α2-AR, as well as the ability of these adrenoreceptors to bind to different G-proteins. On the one hand, the interaction of alpha2-adrenergic receptors with inhibitory GI and Go proteins, which reduce the activity of adenylate cyclase, is known. However, α2-AR can also bind to Gs proteins, which increase the activity of adenylate cyclase. Thus, our results suggest possible participation of α2-AR in the regulation of the cardiac function at the stage of formation of sympathetic innervation.

CONFLICT of INTERESTS

The author declares that the presented data contain no conflicts of interest.

ACKNOWLEDGEMENT

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and supported by the RFBR (№ 17-04-00071), RFBR and Government of the Republic of № 18-44-160022.

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