DOI 10.1007/s10517-018-4220-9
Bulletin of Experimental Biology and Medicine, Vol. 165, No. 5, September, 2018

PHYSIOLOGY

Effect of α_2-Adrenoceptor Stimulation on Functional Parameters of Langendorff-Isolated Rat Heart

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 165, No. 5, pp. 532-535, May, 2018
Original article submitted December 18, 2017

We studied the effect of α_2-adrenoreceptor agonist clonidine hydrochloride in concentrations of 10^{-9}-10^{-6} M on inotropy, chronotropy, and coronary flow in Langendorff-isolated heart of adult rats. It was found that α_2-adrenoreceptor agonist changed all studied parameters. Left ventricular myocardium contraction force decreased after application of all tested concentrations, the maximum effect was observed at a concentration of 10^{-6} M. Stimulation of α_2-adrenergic receptors in concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M produced a two-phase effect (initial increase and a subsequent decrease) on the coronary flow. Clonidine hydrochloride in the maximum concentration (10^{-6} M) caused a decrease in HR in one group and an increase in the other.

Key Words: *α_2-adrenergic receptors; chronotropy; inotropy; coronary flow; isolated heart*

Adrenergic receptors (AR) are important targets in the treatment of various human diseases such as hypertension and heart failure, mental and neurological pathologies, asthma and glaucoma [13]. α_2-AR were described as presynaptic receptors that inhibit the release of various transmitters from neurons in the central and peripheral nervous systems [2] and called autoreceptors. There is evidence that α_2-AR activation with norepinephrine or epinephrine can modulate transmitter release from non-adrenergic neurons, including acetylcholine, dopamine, serotonin, and probably other transmitters [9] and therefore, α_2-AR were called heteroreceptors [2]. α_2-AR have been identified in many organs and tissues: on presynaptic membranes of adrenergic fibers, postsynaptic membranes of cardiomyocytes, in vascular smooth muscles, central and peripheral nervous system, intestinal and renal epithelium [3,7,10]. α_2-AR were revealed in rat heart by immunoblotting [5], mRNA of all α_2-AR subtypes were found in human heart by PCR [8].

α_2-Autoreceptors regulate cardiac activity, participate in the regulation of night spontaneous locomotor activity, prolong slow sleep phase, and shorten the paradoxical sleep [2]. Activation of α_2-heteroreceptors by selective agonists produces bradycardia, hypotension, sedative, antinociceptive, and hypnotic effects, increased baroreceptor reflex sensitivity, improves the effectiveness of inhalation anesthesia and hypothermia, and reduces intraocular pressure [2,4].

It has been demonstrated *in vitro* that α_2-AR activation with clonidine can produce a variety of effects on contractility of atrial and ventricular myocardium strips [15]. α_2-AR blockade in rats without sympathetic heart innervation led to a short-term increase in contraction force of atrial myocardium with its subsequent decrease [6]. Activation of presynaptic

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α_2-AR are thought to reduce norepinephrine release from synapses and thereby reduces heart contraction force [11]. *Ex vivo* studies on isolated rat fetal heart showed that α_2-AR agonist dexmedetomidine prevented the positive inotropic effect that occurred upon α_1- and α_2-AR activation with isoproterenol [12]. In humans, non-selective α_2-AR agonist clonidine significantly reduced BP [1]. The nature of HR shifts upon α_2-AR stimulation is ambiguous. Some authors believe that α_2-AR stimulation causes a negative chronotropic effect, which is most likely produced by inhibition of norepinephrine release from sympathetic nerve endings [5,13]. According to other data, α_2-AR activation with clonidine can produce positive chronotropic effect [14] or no effect on HR [8], and α_2-AR blockade with yohimbine reduced HR in 1- and 3-week-old rats, and in 6- and 20-week rats it had no influence on this parameter [14].

We studied the dose-dependent effect of α_2-AR agonist clonidine hydrochloride on the coronary flow, inotropy, and chronotropy of the Langendorff-isolated heart of adult rats.

MATERIALS AND METHODS

Experiments were carried out *ex vivo* on isolated hearts of 20-week-old white outbred rats (n=16) in compliance with ethical requirements for animal care. The rats were intraperitoneally anesthetized with 25% urethane (800 mg/kg).

The hearts were quickly removed and placed in a cold Krebs—Henseleit solution (2-5°C). A cannula was inserted into the aorta, and oxygenated (95% O_2, 5% CO_2) solution was delivered so that the cannula tip did not touch or damage the aortic valve in order to prevent solution entry into the left ventricle. The isolated hearts were perfused with Krebs—Henseleit solution using a Langendorff chamber (ADInstruments) at 37°C and constant carbogen oxygenation. Retrograde gravity perfusion was performed at a constant hydrostatic pressure of 60-65 mm Hg. 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, left ventricular developed pressure (LVDP), and coronary flow (CF) were calculated from the curve. The recording was carried out on the Power Lab 8/35 apparatus (ADInstruments) using the LabChart Pro software. For α_2-AR stimulation clonidine hydrochloride (Sigma) was used in concentrations of 10^{-9}-10^{-6} M.

The data were statistically processed using Student's t test.

RESULTS

Addition of 10^{-9} M of clonidine hydrochloride to the perfused solution reduced LVDP from 40.9±2.2 to 32.3±2.1 mm Hg ($p \leq 0.05$), that is by 21% (Fig. 1). After agonist administration, HR decreased from 210±5 to 193.0±5.9 bpm ($p \leq 0.05$) at minute 15 (Fig. 2), the reduction was 8%. CF in case of perfusion with clonidine decreased by 14%: from 4.9±0.1 to 4.2±0.2 ml/min ($p \leq 0.01$) by minute 15 (Fig. 3).

By minute 5 of isolated heart perfusion with 10^{-8} M clonidine hydrochloride, LVDP decreased from 34.2±3.6 to 24.6±2.9 mm Hg ($p \leq 0.05$) and by minute 15, it decreased to 28.0±3.7 mm Hg ($p \leq 0.05$,

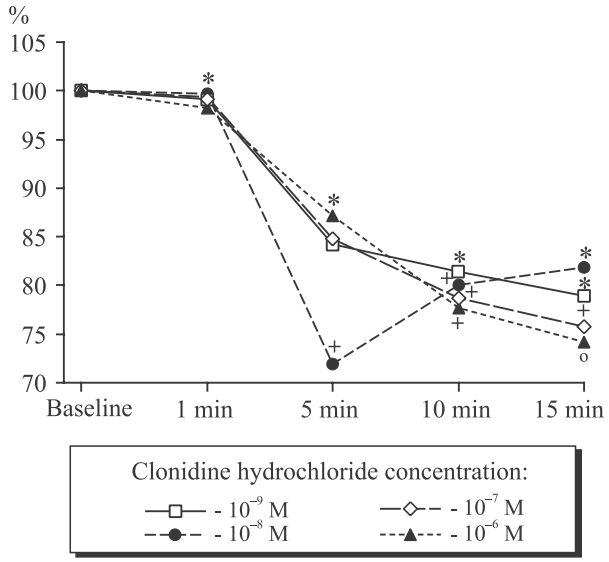

Fig. 1. The dose-dependent effect of clonidine hydrochloride on LVP in the isolated rat heart. *p<0.05, +p<0.01, °p<0.001 in comparison with the initial values.

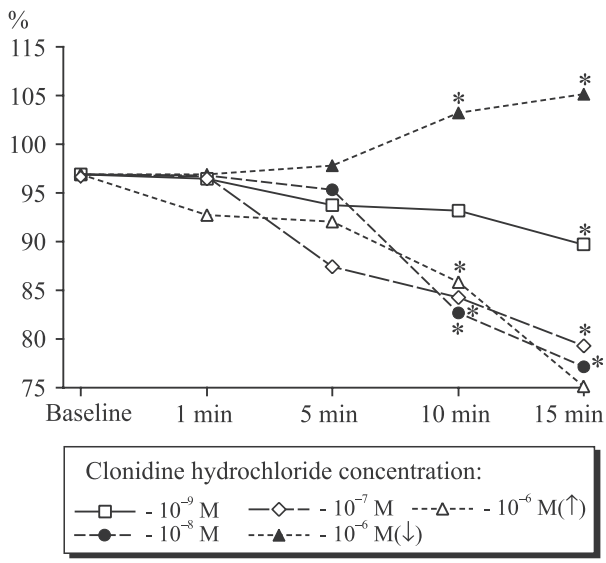

Fig. 2. The dose-dependent effect of clonidine hydrochloride on HR in the isolated rat heart. *p<0.05 in comparison with the initial values.

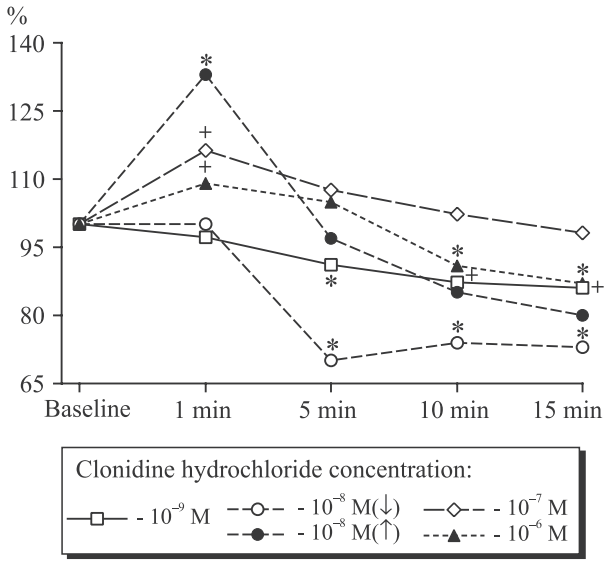

Fig. 3. The dose-dependent effect of clonidine hydrochloride on CF in the isolated rat heart. *$p<0.05$, +$p<0.01$ in comparison with the initial values.

Fig. 1). By minute 7 after clonidine addition, HR decreased from 187.3±9.8 to 160.1±8.4 bpm ($p\leq0.05$). The maximum decrease in HR (23% from the initial value) was observed at minute 15 of the experiment: 145.2±9.1 bpm ($p\leq0.05$, Fig. 2). The effect of the α_2-AR agonist on CF in the isolated heart was not unidirectional (Fig. 3). In some rats, CF increased from 3.5±0.9 up to 4.7±0.9 ml/min, *i.e.* by 33% ($p\leq0.05$) 1 min after drug administration and then tended to initial value 3.7±0.6 ml/min. In others, CF gradually decreased by 27%, from 4.0±0.2 to 2.9±0.2 ml/min ($p\leq0.05$) by observation minute 15.

Clonidine hydrochloride in a concentration of 10^{-7} M significantly reduced LVDP by 24%, from 40.2±2.7 to 30.5±3.2 mm Hg ($p\leq0.01$) at minute 15. By minute 9 of the experiment, HR decreased from 165.0±6.2 to 141.8±4.9 bpm ($p\leq0.05$). The maximum decrease in HR (by 20%, $p\leq0.05$) was observed at minute 15 of the experiment (Fig. 2). At infusion minute 1, CF of the isolated heart increased by 16%, from 3.9±0.2 to 4.5±0.2 ml/min ($p\leq0.01$), then this index recovered.

By minute 15 of perfusion with 10^{-6} M of clonidine hydrochloride, the LVDP decreased by 26% from 24.9±1.8 to 18.5±1.9 mm Hg ($p\leq0.001$). Addition of the α_2-AR agonist into the perfused solution resulted in differently directed changes in HR. In one group of animals, HR increased from 148.0±16.6 up to 162.0±12.9 bpm ($p\leq0.05$) at minute 15. In another group of animals, HR decreased from 167.7±26.3 to 145.9±24.4 bpm ($p\leq0.05$) at minute 10. With addition of the α_2-AR agonist, CF increased at minute 1 of the experiment from 3.8±0.09 up to 4.1±0.09 ml/min ($p\leq0.01$), *i.e.* by 9%. Then, at minute 10, CF gradually decreased to 3.4±0.1 ml/min ($p\leq0.05$). At minute 15, CF reduction was 13% ($p\leq0.01$) of the initial value.

These results suggest that nonselective α_2-AR agonist clonidine hydrochloride changes all studied parameters of the isolated heart. We previously studied the effect of clonidine hydrochloride on the chronotropic and inotropic heart functions [15]. Stimulation of α_2-AR in rats *in vivo* was followed by bradycardia and decreased systolic BP. In *in vitro* experiments on adult rats, a dose-dependent decrease in the contraction force of atrial and ventricular strips was demonstrated. *Ex vivo* study on Langendorff-isolated heart confirmed previous data obtained on the whole body and on isolated myocardium strips, which is indicative of direct action of clonidine hydrochloride on cardiac performance.

All tested concentrations of clonidine hydrochloride reduced the contractility of myocardium of the isolated heart, the maximum effect was observed for the concentration of 10^{-8} M. Addition of the agonist induced opposite changes in CF. Stimulation of α_2-AR with concentrations of 10^{-8} M, 10^{-7} M, and 10^{-6} M exerted a two-phase effect on CF with initial increase and subsequent decrease (Fig. 3). The results of this study are consistent with the data showing that nonselective α_2-AR activation leads to a two-phase change in BP: after a short hypertensive phase, which is more pronounced after quick intravenous injection, BP falls below the baseline. It is possible that the two-phase change in BP is mediated by two different α_2-AR subtypes: α_{2B}-AR are responsible for the initial hypertensive phase, while the prolonged hypotension is mediated by α_{2A}-AR [13]. This assumption is consistent with published data on the different localization of different α_2-AR subtypes in different heart cells. The α_2-AR agonist reduced HR in the isolated heart. However, the maximum clonidine hydrochloride concentration (10^{-6} M) in one group of animals caused a HR decrease, but in the other group produced its elevation (Fig. 2). The opposite effects of α_2-AR stimulation on HR and CF can be associated with presynaptic and postsynaptic localization of different α_2-AR subtypes. In addition, the positive and negative dynamics of CF and HR in the isolated heart upon clonidine hydrochloride administration may be concentration-dependent. It is known that α_2-AR activation causes a decrease in cAMP level at low agonist concentration, while at higher agonist concentration, α_2-AR stimulation caused an increase in cAMP level and, as a consequence, modulated the effect of the entire intracellular biochemical cascade [3].

This study was supported by Russian Foundation for Basic Research (project Nos. 15-04-05384 and 17-04-00071).

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