OLD RATS' ISOLATED HEART REACTION UPON STIMULATION OF ALPHA$_2$-ADRENERGIC RECEPTORS

A.M. Kuptsova[1*], *N.I. Ziyatdinova*[1], *G.F. Shaimardanova*[2], *R.G. Biktemirova*[1], *T.L. Zefirov*[1]

[1] Kazan (Volga Region) Federal University, 18 Kremlyovskaya St., Kazan, Republic of Tatarstan, 420008, Russia;
[2] Kazan Institute of Biochemistry and Biophysics, Federal Research Center Kazan Scientific Center of Russian Academy Sciences, 2/31 Lobachevsky St., Kazan, Republic of Tatarstan, 420111 Russia.

* Corresponding author: anuta0285@mail.ru

Abstract. Aging is a natural and inevitable process that worsens the quality of life and shortens its duration. In the process of aging, the cardiovascular system undergoes significant restructuring, diastolic dysfunction is observed, vascular stiffness increases and vasodilation decreases. α_2-ARs are widely distributed in the body and are involved in the regulation of heart and vascular functions. In pathological conditions, there is an increase in the expression of α_2-AR subtypes, a violation of their functions, and a decrease in the effectiveness of the associated signalling cascades. The study aimed to investigate the effect of the α_2-AR agonist clonidine hydrochloride at concentrations of 10^{-9}-10^{-6} M on the inotropy, chronotropy and coronary flow of the old rats isolated heart. α_2-AR stimulation changes all the studied parameters of the old rats isolated heart. The force of left the ventricular myocardium contraction decreases at concentrations of 10^{-9} and 10^{-8} M, and increases at concentrations of 10^{-7} and 10^{-6} M. The α_2-AR agonist causes a decrease in heart rate in all studied concentrations. Stimulation of α_2-AR in lower concentrations (10^{-9} – 10^{-7} M) reduces coronary flow, and the maximum concentration of clonidine hydrochloride (10^{-6} M) increases.

Keywords: α_2-adrenergic receptors, chronotropy, inotropy, coronary flow, isolated heart, rat.

List of Abbreviations

α_2-AR – α_2-adrenergic receptors
LVP – left ventricular pressure
HR – heart rate
CF – coronary flow

Introduction

Aging is a natural and inevitable process that worsens the quality of life and shortens its duration. With age, a number of changes occur in the heart, which reduce its compensatory capabilities and contribute to the development of the disease (Akasheva *et al.*, 2013).

During aging, the cardiovascular system undergoes significant restructuring, diastolic dysfunction is observed, vascular stiffness increases and vasodilation mediated by NO decreases (North & Sinclair, 2012). Diastolic dysfunction that occurs with aging is the result of a slower rate of cytosolic Ca^{2+} reuptake by the sarcoplasmic reticulum, which slows down cardiac relaxation (Shinmura *et al.*, 2011). At the cellular level, with aging of the myocardium, there is a decrease in mitochondrial function (Tocchi *et al.*, 2015), increased release of reactive oxygen species (Cooper *et al.*, 2013) and impaired Ca^{2+} homeostasis (Janczewski & Lakatta, 2010), as well as a decrease in antioxidant protection and increased activity of ryanodine receptors (Hamilton & Terentyev, 2019). Sensitivity to Ca^{2+} and contractility of the heart muscle decrease with age (Han *et al.*, 2024). The release of Ca^{2+} from the sarcoplasmic reticulum during diastole increases in the aging heart (Zhu X. *et al.*, 2005; Cooper *et al.*, 2013; Hamilton & Terentyev, 2019). A decrease in the amplitude of the transient Ca^{2+} signal and a decrease in the Ca^{2+} content in the sarcoplasmic reticulum were detected in the ventricular myocytes of old rats (Zhu X. *et al.*, 2005). This leads to impaired relaxation and cardiac arrhythmia (Hamilton & Terentyev, 2019). Mitochondria in old cardiomyocytes are usually enlarged with edema, loss of cristae, and even destruction of internal membranes and deficiency of ATP production (Chiao & Rabinovitch, 2015). With age, the expression of the cardiac β-isoform of the myosin heavy chain, troponin I phosphorylation, and cardiac myosin-binding protein C increases during aging, which may also slow down cardiac relaxation and contribute to the mechanism causing diastole disorder (Han *et al.*, 2024).

Using radiotelemetry, it was found that the nightly 12-hour average heart rate in old rats is lower than in adults. The mean values of systolic and diastolic blood pressure between old and adult rats do not differ (Zhang & Sannajust, 2000). Aging causes changes in endothelial cells, which play a crucial role in the regulation of vascular tone (Docherty, 1990).

Alpha$_2$-adrenergic receptors (α_2-AR) are widely distributed in the central and peripheral nervous system, autonomic ganglia, on the presynaptic membrane of adrenergic fibers, on the postsynaptic membrane of cardiomyocytes, in vascular smooth muscles, intestinal and renal epithelium (Maltsev *et al.*, 2014). Three subtypes of α_2-AR are known to exist: α_{2A}- , α_{2B}- , and α_{2C}-AR (Brodde *et al.*, 2006). α_2-AR localized on presynaptic membranes are involved in modulating the release of norepinephrine (Rump *et al.*, 1995), inhibiting the release of acetylcholine from cholinergic synapses, stimulating platelet aggregation and vasoconstriction (Dudek *et al.*, 2015). In the central nervous system, activation of postsynaptic α_2-AR causes a sedative effect, decreases sympathetic activity, and blood pressure (Knaus *et al.*, 2007). α_2-AR plays a special role in the development and course of diseases of the cardiovascular system. In rats with a model of spontaneous arterial hypertension, there is an increase in the expression of α_2-AR subtypes, a violation of their functions, and a decrease in the effectiveness of their associated signaling cascades (Kokoz *et al.*, 2019).

α_2-AR play a leading role in the control of vascular resistance, which increases with increasing age. It is assumed that this increase is associated with an increase in the level of norepinephrine in blood plasma with age, while vasodilation caused by adrenaline in plasma decreases. During aging, the activation of α_2-AR leads to a decrease in cardiac output and tachycardia caused by sympathetic activation, as well as a decrease in renin secretion (Folkow, 1993). The reduced sensitivity of presynaptic α_2-AR and, as a result, increased release of norepinephrine may explain increased plasma levels of norepinephrine in the elderly and, consequently, increased blood pressure with age (Buchholz & Duckles, 1993).

Studies conducted at our department revealed the involvement of α_2-AR in the regulation of heart activity at different stages of postnatal ontogenesis. In studies on adult rats, the activation of α_2-AR causes bradycardia and reduces systolic pressure in rats (Zefirov *et al.*, 2014). α_2-AR blockade reduces heart rate in 1- and 3-week-old rats, and in 6- and 20-week-old rats it has no effect on heart rate (Zefirov *et al.*, 2011). The α_2-AR agonist clonidine hydrochloride has a multidirectional effect on the heart rate and coronary flow of the new born baby rats isolated heart (Zefirov *et al.*, 2021) and reduces the force of contraction, multidirectional changes the heart rate and has a two-phase effect on the coronary flow of the adult rats isolated heart (Ziyatdinova *et al.*, 2018).

Based on the above, the question of the presence and functional significance of α_2-AR in the aging heart of mammals and humans remains relevant. The study aimed to investigate the effect of the α_2-AR agonist clonidine hydrochloride at concentrations of 10^{-9}-10^{-6} M on the inotropy, chronotropy and coronary flow of the old rats isolated heart.

Materials and Methods

The experimental protocol was conducted in compliance with the European Conventions for the Protection of Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and was approved by the Ethics Committee of Kazan Federal University (Minutes No. 39 of December 22, 2022). The study used old white outbred rats aged 24 months (n = 33) (mean weight 300-350 g). The control group consisted of adult young rats 3-4 months old (n = 30), with an established system of the cardiovascular system adrenergic regulation.

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^{-9} M to 10^{-6} 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 (LVP, 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 data is presented as an average value and an error of the average (M ± SEM). The differences were significant at $p < 0.05$.

Results

When clonidine hydrochloride was added to the perfused solution at a concentration of 10^{-9} M, the LVP in the group of old rats decreased from 62.9 ± 3.4 mm Hg to 44.9 ± 4.4 mm Hg ($n = 8$, $p < 0.001$, Fig. 1), by 29%. The heart rate of the isolated heart after administration of the agonist decreased from 187 ± 8.4 bpm to 137.8 ± 5.9 bpm ($p < 0.01$, Fig. 2) at the 20th minute, the reduction was 26%. CF during clonidine perfusion decreased by 50%, from 8.5 ± 0.4 ml/min to 4.3 ± 0.4 ml/min ($p < 0.001$, Fig. 3) by the final 20th minute of observation. In the control group of adult rats, similar changes were observed in response to α_2-AR stimulation: LVP decreased by 21% ($p < 0.05$), heart rate by 10% ($p < 0.05$), CF by 14% ($p < 0.01$) from baseline values ($n = 7$, Fig. 1, 2, 3). The analysis of intergroup differences during α2-AR stimulation at a concentration of 10^{-9} M revealed significant changes in heart rate ($p < 0.05$) and CF ($p < 0.05$, Fig. 2, 3). More pronounced changes were observed in the group of older rats.

Perfusion of the old rats isolated heart with a clonidine hydrochloride solution at a concentration of 10^{-8} M reduced LVP by 28% from 69.7 ± 2.1 mm Hg to 50.7 ± 5.4 mm Hg ($n = 7$, $p < 0.01$, Fig. 1), by the 20th minute of the experiment. By the 20th minute of the experiment, the heart rate decreased from 198.3 ± 9.4 bmp to 157.2 ± 12.3 bmp ($p < 0.05$, Fig. 2), by 20% of the initial value. The CF of the old rats isolated heart decreased with the addition of the α_2-AR agonist by 47% from 7.9 ± 0.7 ml/min to 5 ± 1.4 ml/min ($p < 0.05$, Fig. 3). In the control group of young rats, the agonist in the studied concentration reduced LVP by 20% ($p < 0.05$), heart rate by 22% ($p < 0.05$), and the dynamics of CF change was two–phase - an increase in the 1st minute of the experiment by 33% ($p < 0.05$, Fig. 3), followed by a decrease in CF by 27% ($p < 0.05$) from the initial value ($n = 7$, Fig. 1, 2, 3). Significant changes between young and old animals were observed in changes in coronary flow ($p < 0.05$, Fig. 3), which was observed in a group of young rats, CP increased only in the first minute of the experiment.

Clonidine hydrochloride at a concentration of 10^{-7} M led to a significant increase in LVP by 36%, from 61.7 ± 5.2 mm Hg to 84 ± 8.2 mm Hg ($n = 8$, $p < 0.001$, Fig. 1) at the 20th minute. By the 20th minute of the experiment, the heart rate of the isolated heart decreased from 209.2 ± 6.1 bmp to 173.3 ± 7.6 bmp ($p < 0.001$, Fig. 2). Clonidine hydrochloride reduced the CF of the isolated heart by 15%, from 10.7 ± 0.9 ml/min to 9.2 ± 0.7 ml/min ($p < 0.001$, Fig. 3). In the control group of young rats, the agonist in the studied concentration reduced LVP by 24% ($p < 0.01$), heart rate by 30% ($p < 0.05$), and the dynamics of CF change was also two–phase – an increase in the 1st minute of the experiment by 16% ($p < 0.05$, Fig. 3), followed by a decrease in CF to the initial value ($n = 7$, Fig. 1, 2, 3). Stimulation of α2-AR at a concentration of 10^{-7} M revealed significant changes in LVP ($p < 0.05$), heart rate ($p < 0.05$) and CF ($p < 0.05$, Fig. 1, 2, 3) between young and old rats. At the same time, LVP and CF changed in different directions, and the decrease in heart rate was more pronounced in the group of young animals.

By the final 20th minute of the old rats isolated heart perfusion with clonidine hydrochloride at a concentration of 10^{-6} M, LVP increased from 77.4 ± 7.3 mm Hg to 102.8 ± 5.8 mm Hg ($n = 10$, $p < 0.001$, Fig. 1), by 39%. The addition of the α_2-AR agonist to the perfused solution resulted in a decrease in heart rate from 224.2 ± ± 11.8 bmp to 1987.7 ± 7.87 bmp ($p < 0.05$) at

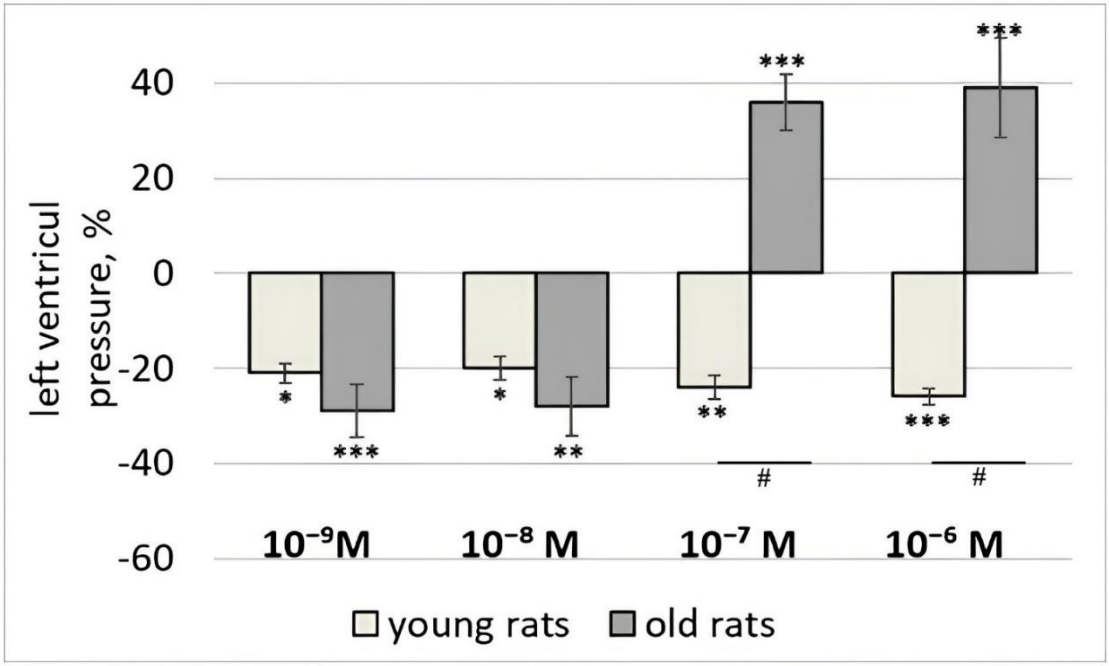

Fig. 1. The dose-dependent effect of clonidine hydrochloride on the left ventricle pressure in the isolated heart of old and young rats. The ordinate axis is the LVP (%), the abscissa axis is the concentration of clonidine hydrochloride (Mol). Note: the confidence is indicated in comparison with the initial values: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; the confidence is indicated in comparison with a group of young rats: # – $p < 0.05$

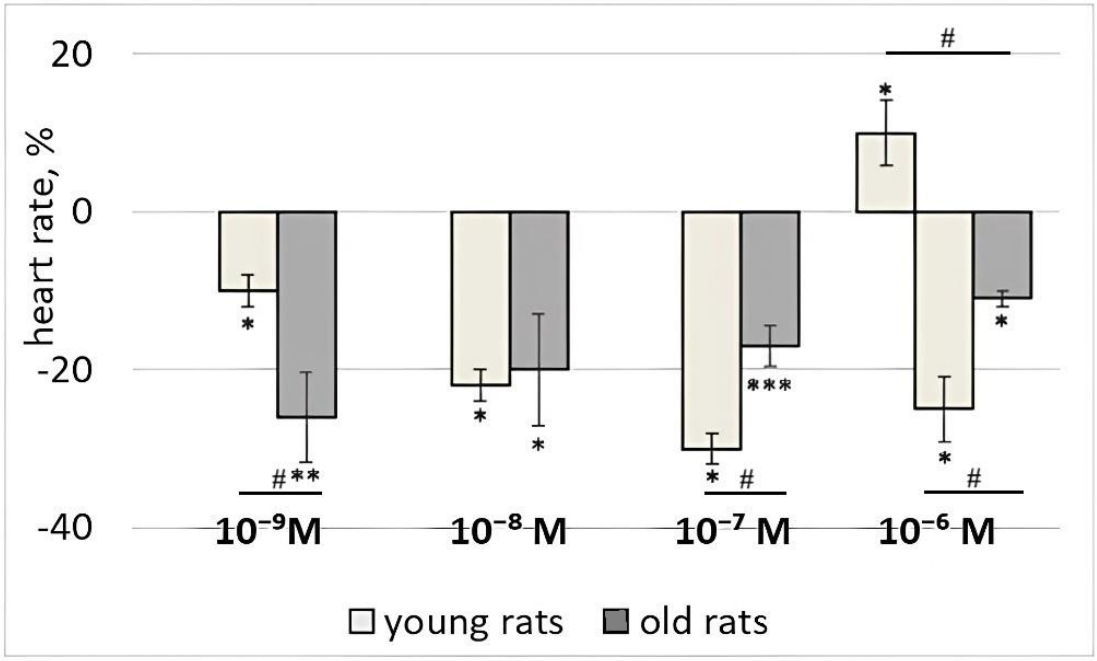

Fig. 2. Dose-dependent effect of clonidine hydrochloride on heart rate in isolated hearts of old and young rats. The ordinate axis is the heart rate (%), the abscissa axis is the concentration of clonidine hydrochloride (Mol). Note: the confidence is indicated in comparison with the initial values: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; the confidence is indicated in comparison with a group of young rats: # – $p < 0.05$

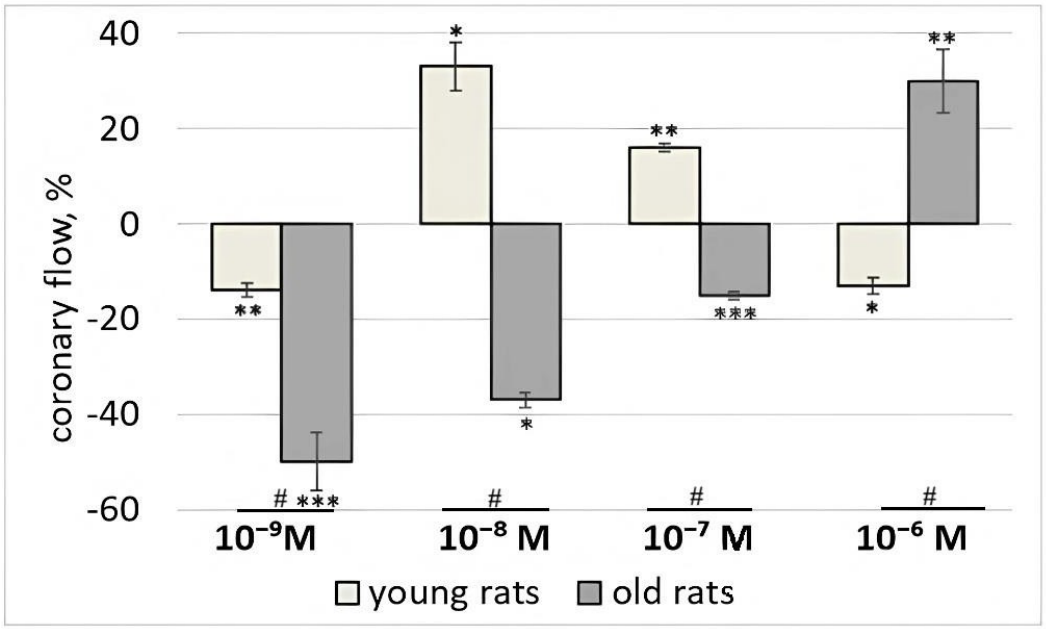

Fig. 3. Dose-dependent effect of clonidine hydrochloride on coronary flow in the isolated heart of old and young rats. The ordinate axis is CF (%), the abscissa axis is the concentration of clonidine hydrochloride (Mol). Note: the confidence is indicated in comparison with the initial values: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; the confidence is indicated in comparison with a group of young rats: # – $p < 0.05$

the 20th minute of the experiment. CF increased by 30% from the initial value by the 20th minute of the experiment with the addition of the α_2-AR agonist from 7.5 ± 0.4 ml/min to 9.8 ± ± 0.7 ml/min ($p < 0.01$, Fig. 3). In the control group of young rats (n = 9), α2-AR stimulation decreased LVP by 26% ($p < 0.001$), CF by 13% ($p < 0.05$), and HR varied in different directions: in some animals (n = 4), it led to a decrease in heart rate by 25% ($p < 0.05$), and in the other part of the animals (n = 5), it increased by 10% ($p < 0.05$, Fig. 1, 2, 3). Clonidine hydrochloride (10^{-6} M) had a significant multidirectional effect on all studied parameters of young and old rats isolated heart. However, in the group of young rats, α2-AR stimulation had both an increase and decrease in heart rate, while in older rats we observed only a decrease in heart rate. A more pronounced decrease in heart rate was observed in the group of young rats.

Discussion

A study conducted to study different concentrations of the α_2-AR agonist on the performance of the old rats isolated heart revealed significant features. In the process of aging, the cardiovascular system undergoes significant restructuring. Dysfunctions associated with aging may result from impaired Ca^{2+} homeostasis (Shinmura *et al.*, 2011; Janczewski & Lakatta, 2010). These changes require more complex adaptation of regulatory targets, including from α_2-AR. This has been demonstrated in our experiments, in which low concentrations of the agonist decrease and high concentrations increase the left ventricles pressure the of the old rats isolated heart. In the group of young animals, the studied concentrations of the agonist decreased LVP, and the maximum decrease in contractility was observed at the maximum concentration of clonidine hydrochloride. These differences may be related to changes in Ca^{2+} sensitivity and cardiac muscle contractility that occur with age (Han *et al.*, 2024).

According to the literature, the nature of heart rate changes during α_2-AR stimulation is ambiguous (Knaus *et al.*, 2007). The multidirectional effect of α_2-adrenergic receptor stimulation on heart rate may be associated with presynaptic and postsynaptic localization of different α_2-AR subtypes. It is known that α_2-AR activation reduces cAMP levels at a low

concentration of agonists, while at a higher concentration of the agonist, α_2-AR stimulation leads to an increase in cAMP levels and, as a result, a change in the effect of the entire cascade of intracellular biochemical reactions (Gyires *et al.*, 2009). The decrease in the heart rate of the isolated heart of old rats had an inverse concentration dependence. The greatest decrease in heart rate was observed when using the minimum concentration, the smallest - at the maximum concentration. In the group of young rats, on the contrary, there was a direct concentration dependence.

Aging causes changes in endothelial cells, which play a crucial role in regulating vascular tone. In the group of old rats, stimulation of α_2-AR at concentrations of 10^{-9} - 10^{-7} M decreased coronary flow, and increased at concentrations of 10^{-6} M. Stimulation of α_2-AR at concentrations of 10^{-8} - 10^{-6} M had a two-phase effect on the coronary flow of the young rats isolated heart, in which it was first increased and then decreased, and the minimum concentration of the agonist decreased the coronary flow. More pronounced changes in the coronary blood supply indicate the involvement of α_2-AR located in vascular smooth muscle cells in the regulation of coronary vascular tone in old rats.

Conclusion

α_2-AR stimulation changes all the studied parameters of the old rats isolated heart. The force of left the ventricular myocardium contraction decreases at concentrations of 10^{-9} and 10^{-8} M, and increases at concentrations of 10^{-7} and 10^{-6} M. The α_2-AR agonist causes a decrease in heart rate in all studied concentrations. Stimulation of α_2-AR in lower concentrations (10^{-9} - 10^{-7} M) reduces coronary flow, and the maximum concentration of clonidine hydrochloride (10^{-6} M) increases.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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