Effect of Alpha2-Adrenoreceptors activation on rat heart chronotropy

Efecto de la activación de los receptores alfa2-adrenérgicos sobre la cronotropía del corazón de rata

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Abstract

A cardiovascular disease is a group of diseases of the heart and blood vessels. The most common cause of death in humans is associated precisely with cardiovascular diseases, and α_2-adrenergic receptors (α_2-AR) are known to be involved. α_2-AR reduce the central sympathetic output and peripheral release of catecholamines and therefore, can prevent sympathetic hyperactivity and hypertension. Therefore, various agonists and antagonists of adrenergic receptors are widely used in the treatment of cardiovascular diseases in medicine, among them clonidine and ST-91. The objective was to compare the effects of clonidine and ST-91, selective agonists of α_2-AR, on the cardiac activity in rats. Stimulation of α_2-AR by ST-91 (0.01 mg/kg) causes a short-term negative chronotropic effect, while stimulation of α_2-adrenergic receptors with clonidine (0.01 mg/kg) led to a negative chronotropic effect throughout the experiment. We hypothesize that the short-term effect of ST-91 on heart rate is associated with the activation of one of the three subtypes of α_2-adrenergic receptors. Identification of the specific subtype due to which this effect was observed is part of future research.

Keywords: heart, α_2-adrenergic receptor, myocardium, heart rate, chronotropy, clonidine, ST-91, agonist, rat.

Resumen

La enfermedad cardiovascular es un grupo de enfermedades del corazón y los vasos sanguíneos. La causa más común de muerte en humanos está asociada precisamente a las enfermedades cardiovasculares, y receptores α2-adrenérgicos (α2-AR) se encuentran involucrados. Los α2-AR reducen eflujo simpático central y la liberación periférica de catecolaminas y, por tanto, pueden prevenir la hiperactividad simpática y la hipertensión. Por tanto, diversos agonistas y antagonistas de los receptores adrenérgicos se utilizan ampliamente en el tratamiento de enfermedades cardiovasculares en medicina, entre ellos la clonidina y el ST-91. El objetivo fue comparar los efectos de la clonidina y ST-91, agonista selectivo de α2-AR, de uso frecuente en medicina, sobre la actividad cardíaca de ratas. La estimulación de α2-AR por el agonista selectivo de ST-91 (0,01 mg/kg) produce un efecto cronotrópico negativo a corto plazo, mientras que la estimulación de los receptores α2-adrenérgicos con clonidina (0,01 mg/kg) condujo a un efecto cronotrópico negativo durante todo el experimento. Posiblemente el efecto a corto plazo de ST-91 sobre la frecuencia cardíaca está asociado con la activación de uno de los tres subtipos de receptores α2-adrenérgicos. La identificación del subtipo específico por el cual se observó este efecto es parte de investigaciones futuras.

Palabras clave: corazón, receptor α2-adrenérgico, miocardio, frecuencia cardíaca, cronotropía, clonidina, ST-91, agonista, rata.

Introduction

Physiological systems of the body are equipped with compensatory feedback mechanisms to prevent excessive use of cellular energy and maintain metabolism, which is necessary to avoid threats, for example, in the case of a "fight or flight" response to stress[1]. Catecholamines are the triggering factor in stress response. Catecholamines (epinephrine, norepinephrine) are released from the adrenal medulla and neuronal synapses and enter the bloodstream[1]. High concentrations of catecholamines in the blood can contribute to arrhythmias. Catecholamines act through adrenergic receptors. Adrenergic receptors mediate various physiological and pharmacological actions of endogenous catecholamines and their synthetic analogs. Heart nerve terminals can also contribute to an increase in the local level of catecholamines and participate both in response to stress and in the basal regulation of the heart and cardiogenesis[2]. Aberrant feedback control of the sympathetic response can lead to a whole range of pathologies[1].

Adrenergic receptors are G-protein coupled receptors. As a result of the study of pharmacological and molecular properties, adrenergic receptors have been subdivided into three types, α1-AR, α_2-AR, and β-AR. These types, in turn, are represented by three (or more) receptor subtypes. α_2-adrenergic receptors are distributed throughout the central nervous system and peripheral tissues. The noradrenergic system and α_2-adrenergic receptors are involved in the regulation of various physiological functions such as excitation, attention, cardiovascular diseases, and activation of the peripheral sympathetic and parasympathetic systems[3].

Cellular reactions accompanied by exposure to catecholamines are associated with cellular signalling pathways related to various heterogeneous complexes of $G_\alpha/G_{\beta\gamma}$ proteins. Currently, 20 G_α, 6 G_β, 12 G_γ proteins are known, which provide almost 1500 combinatorial variants of signal transduction; they are additionally multiplied by various effector isoforms[4]. It is also known that effectors can be regulated either by G_α or $G_{\beta\gamma}$ subunits or by their dual or synergistic effects[5].

Adrenergic receptors use two different signalling cascades to amplify their regulatory signals. β-adrenergic receptors act through $G_{\alpha s}$, and α_2-adrenergic receptors through $G_{\alpha i}$ proteins. Activation of α_2 and β-adrenergic receptors counteract each other by decreasing and increasing the activity of adenylate cyclase, providing homeostatic regulation of intracellular cyclic adenosine monophosphate (cAMP) and its downstream cascades[6].

In contrast to the presynaptic α_1 and β-ARs, which control neuronal activity and neurotransmitter release, α_2-ARs play an overwhelming role. Activation of presynaptic α_2-AR, leads to dissociation of the G_i protein and to the reduction of cAMP levels, the opening of internally rectifying K^+ channels, and inhibition of voltage-driven Ca^{2+} channels that directly affect the exocytosis mechanism[7]. Thus, α_2-ARs have been recognized as short-loop feedback inhibitors for the release of sympathetic and adrenal catecholamines, and in general, they have an inhibitory effect on sympathoadrenergic regulation[8]. Specific α_2-AR agonists are mainly involved in antinociceptive, sedative, central hypotensive, hypothermic, and behavioral effects[9]. One such specific α_2-AR agonist is clonidine, which is used for hypertension. The α_2-adrenergic receptor agonist, clonidine, is widely used in both anesthesia and intensive care. However, clonidine can cause severe hemodynamic side effects such as hypotension and bradycardia[10].

Material and methods

The experiment involved eighteen male and female 20-week-old outbred rats, which were kept in cages in a specially designated room - vivarium. All animals were kept under the same conditions in which the light and temperature were constantly controlled. The animals received proper care: they were fed and watered daily, the cages were cleaned once a week, and washed once a month.

For the experiment, rats were anesthetized with urethane injected intraperitoneally (solution 25%, 800 mg/kg). Urethane does not affect the activity of the rat heart. The effect of anesthesia was determined according to such indicators as blood breathing and the cessation of vibrissae oscillations. The anesthetized rat was placed on the operating table with its limbs fixed, and electrodes were placed to record an electrocardiogram (ECG), the operating field was cut out, which was disinfected with an alcohol solution. Operations were performed under microscope guidance. The selective agonist of α_2-adrenergic receptors ST-91 (Tocris) was used for the experiment, at an effective dose of the agonist is 0.01 mg/kg. The ST-91 preparation was injected into the right femoral vein; an insulin syringe was used for accurate dosing. The signal from the electrograph was sent to the S1-83 oscilloscope and then to the personal computer. The ECG data were subjected to computer-assisted mathematical analysis. As a result of the analysis, we obtained the values of the parameters of the variation pulseogram. In our work, we analyzed the average cardiointerval (Xav, ms), mode (Mo, ms), the amplitude of the mode (AMo, %), variation amplitude (ΔX, ms), rms deviation (δ, ms), as well as conventional units (conventional units), the stress index, the vegetative balance index, the vegetative rhythm index, the indicator of the adequacy of the regulatory processes (regulatory system stress index, vegetative balance index, vegetative rhythm index, regulation adequacy) were determined. These indicators allow us to determine the important mechanisms of regulation of the heart.

Data storage, graphical inference, and statistical analysis were performed using Statistica data analysis software. A value of $p<0.05$ was considered significant.

Results and Discussion

To study the role of α_2-adrenergic receptors in the regulation of cardiovascular activity of the heart, we injected the selective α_2-AR agonist ST-91 into the right femoral vein of rats at

a dose of 0.01 mg/kg of animal weight. This was the minimum dose of the agonist that led to changes in the mean cardiointerval (Xav).

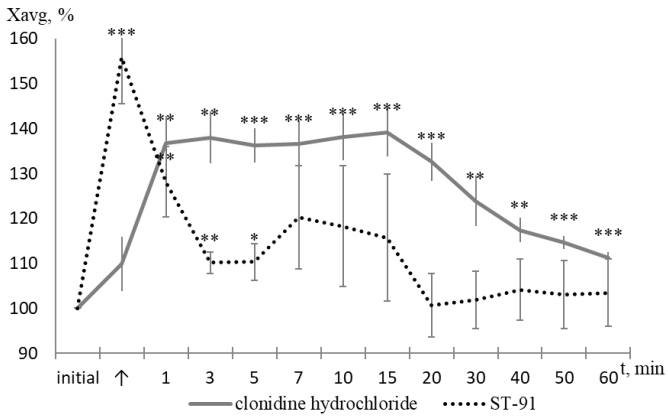
In vivo stimulation of α2-AR with ST-91 causes a short-term negative chronotropic. In effect, α_2-AR activation with ST-91 increases the mean cardio interval (Xav) from 209.88 ± 14.9 ms to 327.13 ± 31.59 ms (p≤0.01) after the agonist administration. By the first minute of observation, Xm is 263.5 ± 14.2 ms (p≤0.05). During the 3rd minute of recording, Xav is 229 ± 12.2 ms, which corresponds to an increase of 10.14 ± 2.4% (p≤0.01) from the initial value. By the 5th minute of registration, the value of Xm does not change. During the 7th and 10th minutes after the administration of the agonist, Xav is 243.88 ± 13.6 ms and 238.6 ± 17.2 ms, respectively. And by the 20th minute of recording, the average cardio interval indicator reaches its initial value and is 208.5 ± 17 ms. Further, during the experiment, no significant changes in Xav were observed (Figure 1).

In the experiment, the α_2-AR agonist ST-91 affects the heart rate and the parameters of autonomic balance. The initial mode was 208.63 ± 14.2 ms. after the start of α_2-AR stimulation, this value increased to 308.75 ± 27.8 ms (p≤0.01), and 1 minute of observations was 254 ± 14.4 ms (p≤0.05). Such indicators as the variation range (Δ X) and the standard deviation (δ) also tended to increase. ΔX increased from 18.63 ± 3.2 ms to 127.63 ± 25.6 ms (p≤0.01), and δ from 21.75 ± 7.44 ms to 1504.38 ± 498.7 ms (p≤ 0.05). Further, the indices and modes and ΔX gradually returned to the initial value. The mode amplitude immediately after the introduction of ST-91 decreased from 14.24 ± 1.92% to 8.25 ± 0.5% (p≤0.05), followed by a gradual recovery of the indicator to the initial value. Other indicators of heart rate variability decreased during the experiment. Vegetative balance index decreased from 1094±318.3 conventional units to 114.38±42.3 conventional units (p≤0.05), regulatory system stress index decreased from 2925.7±94.5 conventional units to 212 ± 76 conventional units (p≤0.05), vegetative rhythm index decreased from 344.63 ± 71.6 conventional units to 44.7±14.4 conventional units (p≤0.01), regulation adequacy decreased from 73.5±13.8 conventional units up to 28.8±4.3 conventional units (p≤0.05). After a decrease in these indicators, the values were restored to the initial level.

In the present work (Figure 1) as reported in our earlier works[10], activation of α_2-AR with clonidine hydrochloride at a dose of 0.01 mg/kg (Tocris), showed a long-lasting increase in the average cardiointerval[10] which persisted throughout the experiment.

Thus, activation of α_2-adrenergic receptors in the whole organism (*in vivo*) with ST-91 and clonidine hydrochloride at doses of 0.01 mg/kg of animal weight leads to a decrease in the cardiac activity of sexually mature rats. At the same time, ST-91 has a short-term decrease in heart rate, and clonidine hydrochloride has a longer-lasting effect on heart contractions.

Figure 1. The effect of activation of alpha2-adrenoceptors on average cardio intervals (Xavg). ***p≤0.001, **p≤0.01, *p≤0.05.



In conclusion, when we compared the effects of two α_2-adrenergic agonists, ST-91 and clonidine, on cardiac activity in rats, we demonstrated that stimulation of α_2-adrenergic receptors with an ST-91 agonist in 20-week-old rats resulted in a short-term decrease in heart rate. At the same time, the analysis of indicators of heart rate variability indicates an increase in the tone of the parasympathetic channel of regulation of the heart. Our previous experiments showed that clonidine reduces heart rate and this bradycardic effect persists throughout the experiment[10]. To date, three different subtypes of α_2-AR have been identified, designated as $\alpha_{2A/D}$, α_{2B}, and α_{2C}[11]. They differ in their pharmacological properties and tissue distribution[11]. To understand the biological significance of each subtype of α_2-AR, genetically engineered mice helped to a large extent[12,13]. All three subtypes of α_2AR are approximately 75% identical in their transmembrane segments, but they perform different physiological functions. $\alpha_{2A/D}$-AR play an important role in the regulation of sympathetic tone and pain perception and also mediates sedative effects in α_2 -agonists. α_{2B}-ARs are responsible for vasoconstriction in some vascular beds. α_{2C}-AR and $\alpha_{2A/D}$-AR regulate the release of catecholamines as presynaptic autoreceptors[14-16].

We hypothesize that the differences in the effects of ST-91 and clonidine are associated with a significant affinity of ST-91 for one of the three subtypes of α_2-AR, which is responsible for short-term cardiac activity at the beginning of the experiment. This assumption requires further research.

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