Effect of α2-Adrenoreceptors

stimulation after blockade of the current activated by hyperpolarization, if, in 6-week-old rat's isolated heart

Efecto de la estimulación de los receptores adrenérgicos-α2 después del bloqueo de la corriente activada por la hiperpolarización, if, en el corazón aislado la rata de 6 semanas de edad

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Abstract

Adrenergic receptors agonists are widely used to treat cardiovascular diseases. All 3 subtypes of α_2-adrenergic receptors (α_2-AR) have been found in the human heart using the polymerase chain reaction. Radioligand test and immunoblotting revealed α_2-AR in the myocardium of normotensive and also spontaneously hypertensive rats. The evidence demonstrated the interaction of α_2-AR and If in the relief of mechanical and thermal pain. The α_2-AR agonist, clonidine, by inhibiting HCN channels, causes a strong bradycardic effect. The objective of this research is to study the effect of the activation of α_2-AR in the presence of the blockade of If with ZD7288, on the parameters of the isolated rat heart after the formation of adrenergic regulation of the heart. Experiments on isolated hearts were performed *ex vivo*, using the Langendorff-isolated heart preparation. During the study, the coronary flow (CF), heart rate (HR), pressure wave amplitude (PWA), maximum wave rise rate (dP/dt_{max}), maximum wave drop rate (dP/dt_{min}) were studied. The findings demonstrate a role of α_2-AR and If in the adrenergic regulation mechanisms of the function of an isolated heart of 6-week-old rats, since they showed that the blockade of If with ZD7288, caused an increase in heart inotropy, as well as multidirectional effects on the rhythm and blood flow of the isolated rat heart after the formation of adrenergic innervation.

Keywords: α_2-adrenoreceptors, If-currents, isolated heart, rat.

Resumen

Los agonistas de los receptores adrenérgicos se utilizan ampliamente para tratar enfermedades cardiovasculares. Los 3 subtipos de receptores α2-adrenérgicos (α2-AR) se han encontrado en el corazón humano mediante la reacción en cadena de la polimerasa. La prueba de radioligando y la inmunotransferencia revelaron la presencia de α2-AR en el miocardio de ratas normotensas y espontáneamente hipertensas. La evidencia demuestra la interacción de α2-AR y If en el alivio del dolor mecánico y térmico. El agonista α2-AR, la clonidina, al inhibir los canales de HCN, produce un fuerte efecto bradicárdico. El objetivo de esta investigación es estudiar el efecto de la activación de α2-AR después del bloqueo de If, con ZD7288, sobre los parámetros del corazón de rata aislado al completarse la formación de la regulación adrenérgica del corazón. Los experimentos en corazones aislados se realizaron *ex vivo*, utilizando la preparación de corazon aislado de Langendorff. Durante el estudio, se estudió el flujo coronario (FC), la frecuencia cardíaca (FC), la amplitud de la onda de presión (PWA), la tasa de aumento de onda máxima (dP / dtmax), la tasa de caída de onda máxima (dP / dtmin). Los hallazgos demuestran un papel de α2-AR y If en los mecanismos de regulación adrenérgica de la función de un corazón aislado de ratas de 6 semanas, ya que mostró que el bloqueo de If con ZD7288 provocó un aumento de la inotropía cardíaca, así como efectos multidireccionales sobre el ritmo y el flujo sanguineo del corazón de rata aislado al completarse la formación de la inervación adrenérgica

Palabras clave: receptores adrenérgicos α2, corrientes If, corazón aislado, rata

Introduction

The α_2-adrenergic receptor (α_2-AR) agonists are used to treat cardiovascular, psychiatric, and neurological diseases, asthma, glaucoma[1], as well as for anesthesia, sedation, hypotension, and hypothermia[2]. Using DNA microarrays, the expression of three α_2-AR isoforms ($\alpha_{2A/D}$, α_{2B}, α_{2C}-AR) was detected in all mammalian species, including humans, as well as at different stages of cardiogenesis[2].

In mammals, α_{2A}- and α_{2C}-AR isoforms are significantly present in the central nervous system, while all three α_2-AR isoforms are widespread in such structures as epithelial tissue of the intestine, kidneys, vascular smooth muscle cells, membranes of presynaptic adrenergic endings, cell membranes myocardium[3,4]. All 3 subtypes of α_2-AR have been detected in the human heart using the polymerase chain reaction method[5]. Radioligand analysis and immunoblotting revealed the presence of all three subtypes of α_2-AR in the myocardium of normotensive as well as spontaneously hypertensive rats[6].

In the late 1990s, studies were focused on the current activated by hyperpolarization (Ih, If). The research interest in this type of current is due to the biophysical properties and features of regulation of isoforms of HCN channels conducting this current, including their distribution in tissues[7,8]. In the heart, isoforms of HCN channels are distributed in the vascular system[9], as well as in working cardiomyocytes[10].

The evidence indicates the existence of the interaction between α_2-AR and If to reduce mechanical and thermal pain[11]. The α_2-AR agonist, clonidine, by inhibiting HCN channels causes a strong bradycardic effect[12]. It should be noted that the inhibition of HCN channels with clonidine in humans can be applied with rapid intravenous administration during a hypertensive crisis or opioid detoxification[13].

The findings in regard to the ontogenetic features of the formation and development of the cardiovascular system indicate that the age of 6 weeks is of absolute interest, since at this age rats undergo significant changes in the activity of the heart, the minimum heart rate, the completion of adrenergic innervation formation of the heart, and the initiation rat body's work as adult animals[14,15].

The objective of this work is to study the parameters of the isolated rat heart after the formation of adrenergic regulation of the heart after activation of α_2-AR in the presence of If blockade.

Methods

Experiments and procedures on laboratory animals were performed following the principles of good laboratory practice and animal ethical guidelines.

A 25% urethane solution (800 mg/kg) administered intraperitoneally was used as anesthesia. A transverse incision was made at the level of the diaphragm, the chest cavity was opened, and the heart was removed. The isolated heart specimen was washed in the temperature related to the Krebs-Henselite solution (around 2-5ºC). Then, an isolated heart specimen was hooked onto the cannula behind the aorta, fixed, and perfused with Krebs-Henselite solution. The solution was pre-saturated with carbogen (95% O_2, 5% CO_2) for 30 minutes, and then continuously during the experiment. The pH was maintained in the range of 7.3-7.4, the temperature of the perfused solution was maintained at 37°C. Throughout the experiment, the pressure of the perfused solution was maintained at a constant level of 55-60 mmHg. Mechanical activity of the left ventricle was determined using a latex balloon connected through a catheter to a pressure transducer. Signals of pressure developed in the left ventricular cavity were recorded on a computer using LabChart Pro V8.

The obtained curves were used to calculate the coronary flow (CF), heart rate (HR), pressure wave amplitude (PWA), maximum wave rise rate (dP/dt_{max}), and maximum wave drop rate (dP/dt_{min}).

In the experiments, the α_2-AR agonist, clonidine hydrochloride, and the hyperpolarization-activated current blocker ZD7288 were used. There were one group with clonidine alone, one with the blocker alone and a third group with the combination of both. Besides, the used substances are manufactured by Sigma and Tocris.

Paired and unpaired Student's t-test was used for statistical processing of experimental data. Values were considered significant at *p <0.05; **p <0.01 relative to the initial values.

Results and Discussion

To evaluate the interaction of α_2-AR and If, experiments were carried out on with the administration of clonidine hydrochloride (10^{-6}M) in 6-week-old rats pretreated with ZD7288, If blocker (10^{-9}M and 10^{-5}M). The If blocker and the α_2-AR agonist at these concentrations caused significant changes in the functioning of an isolated heart[16,17]. In effect, the application of a α_2-AR agonist in the presence of blockade of If-currents at a concentration of 10^{-9} M led to a significant change in all studied parameters.

Over the course of the experiment it could be observed he PWA gradually increased during the experiment. By the 5th minute of the experiment, its value increased from 23.2±2.2 mm Hg, up to 29.6±2.4 mm Hg (p <0.05). Further, by the 10th minute of observation, the PWA increased to 37.5±2.7 mm Hg. (p<0.01). Maximum PWA 41.1±3 mm Hg (p <0.01) was recorded during the 13th minute of the experiment. At the final minute of observation, the PWA decreased slightly and amounted to 39.6±4.7 mm Hg (p<0.01).

An increase in dP/dt_{max} from 585.2±61 mm Hg/sec to 595±70.3 mm Hg/sec was observed during the first minute. By the 5th minute, dP/dt_{max} continued to increase to 794.5±87.9 mm Hg/sec (p <0.05). During the 10th minute it was 861.2 ± 92.2 mm Hg/sec (p <0.01). Then, by the 15th minute, dP/dt_{max} increased to 954.7±126.2 mm Hg/sec (p <0.01). At the last

minute of the experiment, dP/dtmax was 983±142.6 mm Hg/sec (p <0.01).

Application of an α_2-AR agonist secondary to If blockade led to the increase in dP/dtmin from 480±44.2 mm Hg/sec to 492.51.8 mm Hg/sec. By the 5th minute of the experiment, dP/dt_{min} was 699.9 ± 74.3 mm Hg/sec (p <0.01). During the tenth minute of observation, dP/dt_{min} increased to 783.6±77.7 mm Hg/sec (p <0.01). Further, during observation, dP/dt_{min} continued to increase to 813.1±89.5 mm Hg/sec (p<0.01) and 824±115 mm Hg/sec (p <0.01) during the 15th and 20th minutes, respectively.

Figure 1

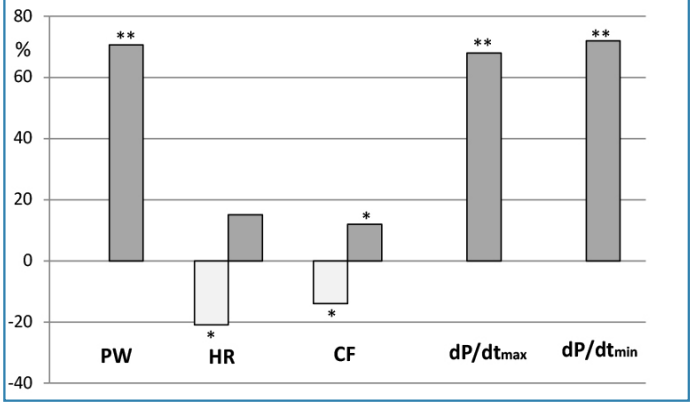

Figure 1. Dynamics of changes in the indices of the isolated heart of 6-week-old rats upon application of a α_2-AR agonist secondary to the blockade of If-currents at a concentration of 10^{-9}M. The ordinate axis is the change in values (%), the abscissa is the performance of an isolated heart - the pressure wave amplitude (PWA), heart rate (HR), coronary flow (CF), the pressure wave rise rate (dP/dt_{max}), the pressure wave drop rate (dP/dt_{min}). *p <0.05; **p <0.01 relative to the initial values.

Heart rate in one group of animals at 1 minute of the experiment showed over time a decrease from 180.2±25.3 bpm to 175.6±23 bpm. By the 5th minute, the heart rate dynamics continued to decrease to 141.6±20.2 bpm (p<0.05). By the 10th minute, the heart rate decreased to 124.6±19 bpm (p<0.05), by the 15th minute the heart rate was 130.8±18.3 bpm (p<0.05). At the final minute of observation, the heart rate decreased to 125.6±18.2 bpm (p<0.05). In the other group, an increase in heart rate was observed already by the 5th minute of the experiment from 179.5±21.6 bpm to 186.4±20 bpm. The maximum increase in heart rate was recorded during the 9th minute of observation - 224.3±67.5 bpm. Further, the heart rate decreased slightly to 217.6±62.4 bpm during the 15th minute and to 207.1±37 bpm during the 20th minute of experimental observation.

The CF change was also multidirectional. In one group, CF decreased during the 1st minute of the experiment from 5.9 ± 1.9 ml/min to 5.7±1.3 ml/min (p <0.05). Then, until 10 minutes, CF remained unchanged. By the 15th minute of experimental observation, CF decreased to 5.3±1.3 ml/min (p <0.05). At the end of the experimental observation, CF decreased to 5.1±1.2 ml/min (p <0.05). In the second group, CF increased from 10.8±2.9 ml/min to 11.4±3 ml/min (p <0.05) by the 5th minute of the experiment. Further, CF did not change. During the 20th minute of observation, CF was 12±2.6 ml/min (p <0.05).

The addition of clonidine hydrochloride against the background of a high concentration (10^{-5}M) of the If blocker caused multidirectional effects of all studied parameters.

The PWA in the first group gradually increased during the experiment. By the 1st minute of the experiment, its value increased from 23.9±5.1 mm Hg up to 24.8 ± 4.9 mm Hg. By the 5th minute, the PWA was 31.6±4.1 mm Hg (p <0.01). The dynamics of the increase in PWA were also observed during the 10th minute - up to 36.3±3.9 mm Hg (p <0.01), and during the 15th minute the value changed to 36.6±3.7 mm Hg (p <0.01). In the final minute, the PWA increased to 37.7±4.6 mm Hg (p <0.01).

There was also the second experimental group, which showed a decrease in PWA. During the 5th minute of the experiment, its value decreased from 23.3±4.7 mm Hg up to 20.9±5.9 mm Hg. Further, until the 10th minute, the values did not change. During the 15th minute of observation, the PWA decreased to 12.7±2 mm Hg. The maximum decrease in PWA was recorded at the 19th minute of the experiment - 11.6±1.7 mm Hg.

Figure 2

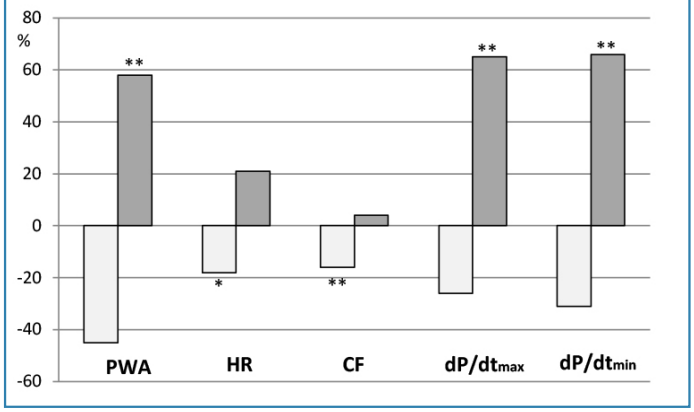

Figure 2. Dynamics of changes in the indices of the isolated heart of 6-week-old rats upon application of a α_2-AR agonist in the presence of If-currents blockade with ZD7288, at a concentration of 10^{-5}M. The ordinate axis is the change in values (%), the abscissa is the performance of an isolated heart - the pressure wave amplitude (PWA), heart rate (HR), coronary flow (CF), the pressure wave rise rate (dP/dt_{max}), the pressure wave drop rate (dP/dt_{min}).

In the first experimental group, dP/dt_{max} during the 1st minute of the experiment increased from 505.6±51.8 mm Hg/sec to 519.3±54 mm Hg/sec. During the 5th minute of observation, dP/dt_{max} increased to 669.2±50.7 mm Hg/sec (p <0.01). By the 10th minute after adding clonidine hydrochloride to the solution secondary to If-blockade, dP/dt_{max} increased to 774.6 ± 53.5 mm Hg /sec (p <0.01). The maximum increase in dP/

dt_{max} was observed during the 17th minute of the experiment, 890.7±84.8 mm Hg/sec (p <0.01). In the second experimental group, a decrease in dP/dt_{max} was from 777.7±266.4 mm Hg/sec to 759.8 ± 363.7 mm Hg/sec, which did not change until the 15th minute. During the 15th-minute dP/dt_{max} was 626.9±416.6 mm Hg/sec. During the last 20th minute of the experiment, dP/dt_{max} decreased to 579.1±372.9 mm Hg/sec.

dP/dt_{min} in one experimental group increased from 380.9±30.7 mm Hg/sec to 399.2±39.4 mm Hg/sec during the 1st minute of observation. By the 5th minute of the experiment, dP/dt_{min} increased to 541.6±22.2 mm Hg/sec (p <0.01). During the 10th and 15th minutes, dP/dtmin increased to 611.1±46 mm Hg/sec (p <0.01) and 656.9±50.6 mm Hg/sec (p <0.01), respectively. During the 20th minute of the experiment, dP/dt_{min} was 634.2±54.3 mm Hg/sec (p <0.01). In another experimental group, dP/dt_{min} decreased from 692.9±319.9 mm Hg/sec to 644.8±275.4 mm Hg/sec. By the 10th minute of observation, dP/dt_{min} decreased to -625.7±355.8 mm Hg/sec. At the 17th minute of the experiment, the α2-AR agonist administered after preliminary If blockade led to the decrease in dP/dt_{min} to 520.4±382.3 mm Hg/sec. During the 20th minute of observation, dP/dt_{min} decreased to 479.2±330.1 mm Hg/sec.

The heart rate changes upon stimulation of α_2-AR with preliminary If blockade were multidirectional. A decrease in heart rate was observed from 128.3±23.1 bpm to 122.5±26.1 bpm in the first minute of the experiment. 5 minutes after the start of α_2-AR stimulation against the background of preliminary If blockade, HR was 108.3±23.2 bpm (p <0.05). By the 10th minute, HR smoothly decreased to 105.3±22.8 bpm (p <0.05) and did not change until the end of the experiment. Also, some animals showed an increase in heart rate from 111.5±18.2 bpm to 160.5±90.4 bpm during the 4th minute of the experiment and a further decrease in heart rate to 152.8±66 bpm during the 15th minute of observation, up to 135.1±46.1 bpm during the 20th minute of observation.

CF in one experimental group increased from 8±3.1 ml/min to 8.54±3.2 ml/min (p <0.05) during the 4th minute of observation. Then CF decreased to 8.36±3.2 ml/min (p <0.05) by the 10th minute of the experiment. During the 15th minute, CF was 8.3±3.1 ml/min. At the end of the experiment, CF was up to 8.4±3.1 ml/min. In the second experimental group of animals, during the 1st minute of the experiment, we recorded a decrease in CF from 5.4±2 ml/min to 5.2±2 ml/min. 5 minutes after the injection of the substance, CF decreased and amounted to 4.8 ± 1.9 ml/min (p <0.01); by the 15th minute of the experiment, CF decreased to 4.4±2 ml/min (p <0.01). By the 20th minute of experimental observation, CF was lower than the initial value and amounted to 4.5±2 ml/min (p <0.01).

Summary

Analysis of the results revealed that stimulation of α_2-AR in the control group led to an increase in contractility by 18%, and administration of an α_2-AR agonist after the If blocker (10^{-9}M) led to the increase in the pressure wave amplitude by 70%. The α_2-AR agonist against the background of the If-blockade (10^{-5}M) led to an increase in the contraction force by 58% and the decrease in the contraction force by 45%. In the control group, clonidine hydrochloride caused bradycardia, reducing the heart rate by 34%. However, stimulation of α_2-AR against the background of If blockade had multidirectional effects on heart rate. The decrease in heart rate during the application of clonidine hydrochloride against the background of ZD7288 blockade at 10^{-9} M and 10^{-5} M was less pronounced - 21% and 18%, respectively. The increase in heart rate was 15% and 21%. There was a change in CF indicators. In the control group, CF decreased by 9%. The introduction of an agonist against the background of If blockade (10^{-9}M and 10^{-5}M) caused a more pronounced negative CF dynamic by 14% and 16%, respectively. But there was also an increase in CF by 12% and 4%.

Conclusions

The development and formation of adrenergic innervation of the heart play an important role in the regulation of ionic currents of cardiomyocytes, the development of the autonomic reaction of the heart[14,15]. Undoubtedly, the study of the mechanism of action of the α_2-AR agonist in the presence of If blockade during the development of adrenergic innervation of the heart is of absolute interest. If-current plays a key role in the autonomic regulation of the heart rate, as well as rhythmic activity in the nervous system. Reviewing the literature data, mechanisms of the possible role of If in the realization of the signaling pathways of α_2-AR are suggested. The activation of α_2-adrenergic receptors on the cell membrane can lead to the activation of G-protein-bound internal K+ rectification channels and inhibition of HCN, which leads to membrane hyperpolarization[16,17]. The evidence showed that such α_2-AR agonists as clonidine and UK14304 led to the decrease in the amplitude of the current activated by hyperpolarization and slowed down the rate of its activation[18]. A direct inhibitory effect of the α_2-AR agonist on If in the mouse heart was also shown[19,20]. Thus, we investigated the age-related mechanisms of adrenergic regulation of the heart of 6-week-old rats. As a result, If-blockade and subsequent stimulation of α_2-AR showed to cause an increase in heart inotropy, as well as multidirectional effects on the rhythm and blood supply of an isolated rat heart at the stage of completion of the formation of adrenergic innervation, in contrast to adult animals with a formed innervation of the heart in which unidirectional increase in inotropy and decrease in heart rate and CF.

Conflict of Interests

The author declares that the provided information has no conflicts of interest.

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