The effect of alpha(1D)-adrenoreceptors selective blockade on the isolated rat heart

El efecto del bloqueo selectivo de los receptores alfa(1D)-adrenérgicos en el corazón de rata aislado

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Abstract

Alpha$_1$-adrenergic receptors (α_1-AR) mediate important adaptive cardiac functions, including physiological hypertrophy, contractility, and protection from multiple injuries, through the activation of various multifactorial signaling cascades. The effects of α_1-AR on chronotropy and coronary circulation of the mammalian heart are poorly understood. The functions regulated by each receptor subtype are also still largely unknown. In addition, scientists often neglect the α_{1D} subtype of the studied receptors due to the low density in cardiomyocytes. The effect of blockade of alpha$_{1D}$-adrenergic receptors with BMY 7378 on the activity indices of the Langendorff heart of 20-, 6-, 3- and 1-week-old rats was studied. BMY 7378 at a concentration of 10^{-8} M had a negative chronotropic effect on isolated hearts in rats starting from 1 week of postnatal development. The inotropic reaction of the isolated heart in 20-week-old rats in response to the blocker was absent; while in 6-week-old rats it had a clear negative character. The α_{1D}-adrenergic receptor blocker, BMY, 7378 (10^{-8} M) causes an increase in the coronary flow of the heart in adult rats and a decrease in 1-week-old rats. At the same time, it does not affect the coronary perfusion of the isolated heart of 3- and 6-week-old rats. An increase in the rate of coronary perfusion in 20-week-old rats indicates a decrease in the tone of the coronary vessels of the heart. In newborn rat pups, BMY 7378 perfusion caused an increase in the tone of the coronary vessels, which led to a decrease in the rate of coronary perfusion. In rats at these stages of postnatal development, α_{1D}-adrenergic receptors may be important in the regulation of the coronary circulation of the heart. The results of the study show that α_1-adrenergic receptors, in addition to providing the effects on coronary vessels, can mediate chrono- and inotropic effects on the rat heart in postnatal ontogenesis.

Keywords: rat, isolated heart, alpha$_{1D}$-adrenergic receptors, BMY7378.

Resumen

Los receptores alfa1-adrenérgicos (α1-AR) median importantes funciones cardíacas adaptativas, incluida la hipertrofia fisiológica, la contractilidad y la protección de múltiples lesiones, a través de la activación de multiples cascadas de señalización. Los efectos de α1-AR sobre la cronotropía y la circulación coronaria del corazón de los mamíferos son poco conocidos. Las funciones reguladas por cada subtipo de receptor también se desconocen en gran medida. Además, los científicos a menudo descuidan el subtipo α_{1D} de los receptores estudiados debido a su baja densidad en los cardiomiocitos. Se estudió el efecto del bloqueo de los receptores adrenérgicos alfa$_{1D}$ con BMY 7378 sobre los índices de actividad del corazón Langendorff de ratas de 20, 6, 3 y 1 semanas de edad. BMY 7378, a una concentración de 10^{-8} M tuvo un efecto cronotrópico negativo en corazones aislados en ratas a partir de 1 semana de desarrollo postnatal. La reacción inotrópica de un corazón aislado en ratas de 20 semanas en respuesta al bloqueador estuvo ausente, mientras que en ratas de 6 semanas tuvo un claro carácter negativo. El bloqueador del receptor adrenérgico α_{1D}, BMY, 7378 (10-8 M), provoca un aumento en el flujo coronario del corazón en ratas adultas y una disminución en ratas de 1 semana de edad. Al mismo tiempo, no afecta la perfusión coronaria del corazón aislado de ratas de 3 y 6 semanas de edad. Un aumento en la tasa de perfusión coronaria en ratas de 20 semanas indica una disminución en el tono de los vasos coronarios del corazón. En crías de rata recién nacidas, la perfusión de BMY 7378 provocó un aumento en el tono de los vasos coronarios, lo que condujo a una disminución de la tasa de perfusión coronaria. En ratas en estas etapas del desarrollo posnatal, los receptores adrenérgicos α_{1D} pueden ser importantes en la regulación de la circulación coronaria del corazón. Los resultados del estudio muestran que los receptores α1-adrenérgicos, además de proporcionar los efectos sobre los vasos coronarios, pueden mediar los efectos crono- e inotrópicos en el corazón de la rata en la ontogénesis posnatal.

Palabras clave: rata, corazón aislado, receptores alfa(1D)-adrenérgicos, BMY7378.

Introduction

The existence of three subtypes of alpha$_1$-adrenergic receptors (α_1-AR) - α_{1A}, $\alpha_{1B,}$ and α_{1D} - has been recognized over the past 35 years; however, the specific functions regulated by each subtype are still largely unknown[1]. Alpha$_1$-adrenergic receptors mediate the most important adaptive functions in the heart, including physiological hypertrophy, contractility, protection from multiple injuries, by activating multiple signaling cascades[2].

The share of α_1-AR in the heart is 10% of the total number of adrenergic receptors[3]. All three subtypes of α_1-AR are found in the heart of rats and mice, and their number significantly increases in weeks 1-2 of postnatal development[4]. In cardiomyocytes of rodents and humans, to a greater extent, there are α_{1A}- and α_{1B}-AR in the ratio of 6:4 - α_{1A}:α_{1B}. In the mouse heart, α_{1B} receptors are present in all cardiomyocytes, while α_{1A}-AR is found in 60% of isolated cardiomyocytes[3]. The quantitative PCR has shown that in the ventricular myocardium of adult rabbits, α_{1B}-AR constitute 99% of the total amount of α_1-AR mRNA; less than 1% of mRNA are α_{1A}- and α_{1D}-AR[5].

In rats of different ages, the α_1-AR agonist, phenylephrine, causes a short-term decrease in heart rate; bradycardia is especially severe in 20- and 6-week-old rats. The blockade of If-currents removes this effect[6].

The effect of phenylephrine is mainly to slow down the heart rate and increase heart rate variability. This change in heart activity is triggered by a baroreflex mechanism, through the parasympathetic system, in response to an increase in blood pressure. A decrease in heart rate upon phenylephrine-induced activation of α_1-AR occurs due to an increase in the duration of cardiomyocytes potential action. Probably, the outgoing K^+ current through the membrane of cardiomyocytes decreases through alpha $_1$-AR[7].

In vivo stimulation of α_1-adrenergic receptors with methoxamine (0.1 mg/kg) also causes short-term bradycardia in rat heart [8]. Methoxamine inhibits myocardial contractility in rats[9] and induces bradycardia in an isolated heart in rats starting from the 3rd week of postnatal development[10-12]. Newborn rat pups have no chronotropic response, but at the same time, the rate of coronary perfusion increases, which indicates a decrease in the tone of the coronary vessels of the isolated heart. This phenomenon may be compensatory, due to which an increase in the blood supply to the heart in rats at this stage of development can be implemented.

Most of the data indicate the involvement of α_{1A}-AR in adaptive effects and cardioprotection, while α_{1D}-AR is present in smooth muscle and endothelial cells of coronary arteries and is involved in the regulation of blood supply to the heart. α_{1D} and α_{1A}-adrenergic receptors in the vascular system play a major role in the control of blood pressure. In addition to smooth muscle contraction, α_1-AR can induce endothelium-dependent vascular relaxation, and activation of the α_{1D}-adrenergic receptor can have a trophic effect on endothelial cells[11-14].

Although new works appear devoted to the study of the role of alpha$_1$-adrenergic receptors in the regulation of cardiac activity both in normal and abnormal conditions, the effects of α_1-AR on chronotropy and coronary circulation of the mammalian heart remain poorly studied. The age aspect of the problem is also practically not studied. In addition, the α_{1D} subtype of the studied receptors, due to the low density in cardiomyocytes, often remains out of sight of researchers. In this regard, the aim of this research was to study the effect of blockade of the α(1D)-subtype of adrenergic receptors on the activity of the isolated heart of rats of different ages.

Methods

The experiments were carried out using the Langendorff heart. For anesthesia, a 25% urethane solution (800 mg/kg) was used intraperitoneally. The study was carried out on the hearts of 40 rats of 20-, 6-, 3- and 1-week old (in four separated groups, each with ten members). The age-related grouping is associated with the characteristics of the maturation of the sympathetic innervation of the heart. The absence characterizes the 1-week old, and the 20-week old is characterized by the complete maturation of the sympathetic innervation of the rat heart. The 3rd week of postnatal development is considered the beginning, and the 6th week is the final stage of maturation of the sympathetic innervation of the heart [12-14].

The isolated heart was first washed with chilled (4-8^{0}C) Krebs-Henseleit solution [8], and then suspended by the aorta on a cannula, and retrograde perfusion was performed with the same solution at a constant pressure of 60-62 mmHg and a temperature of 37^{0}C at the Langendorff System (Australia).

The indicators of the pumping function of the isolated heart of 20- and 6-week-old rats were recorded using a latex balloon placed in the left ventricular cavity and connected through a Teflon catheter with a pressure measurement transducer. The following parameters of the heart were processed: heart rate (HR) and coronary flow (CF). Isolated hearts of 3- and 1-week-old rat pups were examined by recording the electrogram of the heart using atraumatic electrodes applied directly to the heart. The heart rate was calculated and the coronary flow rate was measured. The coronary flow as the volumetric flow rate of coronary perfusion with the working solution was measured in milliliters per minute (mL/min). The studied parameters of the isolated heart were recorded and processed using the LabChart Pro V8 software.

The effects of the alpha$_{1D}$-adrenergic receptor blocker, BMY7378 (Sigma Aldrich, St Louis, MO), were assessed as changes in the studied parameters after the administration of the substance in relation to the initial values. The significance of the data were analyzed by the Student's t-test and a $p<0.05$ was considered significant.

Results and Discussion

The effects of blockade of alpha$_{1D}$-adrenergic receptors with BMY7378, on the activity of the isolated heart of 20-week-old rats

The baseline heart rate in the isolated heart of 20-week-old rats was 184.2±17 bpm. Perfusion of BMY7378, at 10^{-8} M for 3 minutes, did not cause a significant chronotropic response of the isolated heart; by the 7th minute, it led to a decrease in the frequency of contractions to 164±17 bpm ($p<0.05$); bradycardia was 11% (Figure 1).

One minute after BMY7378 perfusion, the coronary flow rate of the heart increased from 3.8±0.2 ml/min to 4.5±0.4 ml/min ($p<0.05$). By the 2nd minute, the coronary flow increased to 4.7±0.5 ml/min ($p<0.05$). The maximum increase in the coronary flow rate up to 4.8±0.5 ml/min ($p<0.05$) was observed during the 3rd minute of perfusion of the alpha$_{1D}$ receptor blocker, the increase in the indicator was 24.7% (Figure 2).

The effects of blockade of alpha$_{1D}$-adrenergic receptors on the activity of the isolated heart of 6-week-old rats.

Figure 1 shows that the chronotropic response of an isolated heart of 6-week-old rats in response to BMY7378 was also negative chronotropic action. The contraction frequency of the isolated heart 1 minute after the injection of the blocker de-

creased from 225.3±23.1 bpm to 203.2±23.0 bpm (p <0.01); after 3 minutes it decreased to 187±23 bpm (p<0.05). Heart rate by the 7th minute decreased to a maximum of 175.4±18 bpm (p<0.05), the effect was 22%.

The baseline values of the coronary flow of the isolated heart of 6-week-old rats were 2.95±0.3 ml/min. After BMY7378 infusion, the coronary flow rate tended to decline (Figure 2).

The effects of blockade of alpha$_{1D}$-adrenergic receptors on the activity of the isolated heart of 3-week-old rats.
The basal contraction rate of the isolated heart of 3-week-old rats was 236±18 bpm. After the start of perfusion with BMY7378 (10^{-8} M) (n=6), there was a tendency towards a decrease in heart rate. By the 7th minute of the action of the blocker, the heart rate decreased to 187.4±17.9 bpm (p<0.05). The negative chronotropic effect of BMY7378 was 20.6% (Figure 1).

The initial values of the rate of coronary perfusion of the heart of 3-week-old rat pups were 2.61±0.12 ml/min (Figure 2). BMY7378 perfusion did not cause significant changes in coronary flow. The CF rate ranged from 2.61±0.12 ml/min to 2.77±0.16 ml/min. There was only a slight upward trend in this indicator.

The effects of blockade of alpha$_{1D}$-adrenergic receptors on the activity of the isolated heart of 1-week-old rats.
The initial contraction rate of the isolated heart of 1-week-old rat pups was 191.5 ± 27 bpm. One minute after the administration of the blocker, the heart rate decreased to 180 ± 25 bpm (p<0.05). Further, changes in the chronotropy of the heart were not significant; only a tendency towards a decline in the frequency of contractions was observed (Figure 1).

Alpha$_{1D}$-AR blockade of the isolated heart of 1-week-old rat pups elicited a relatively rapid coronary vascular response. By the 5th minute of BMY7378 injection, the coronary flow rate decreased from 1.5 ± 0.3 ml/min to 1.06 ± 0.22 ml/min (p <0.05). By the 7th minute of the action of the α_{1D}-AR blocker, the maximum decrease in the coronary flow rate to 0.95 ± 0.2 ml/min (p <0.05) was observed; the change was 37% (Figure 2).

Figure 1. The effect of BMY 7378 (10^{-8}M) on the heart rate of an isolated heart in rats of different ages. The ordinate is the heart rate (HR, %), the abscissa is the time (minute). *p<0.05 and **p<0.01, compared with the initial values.

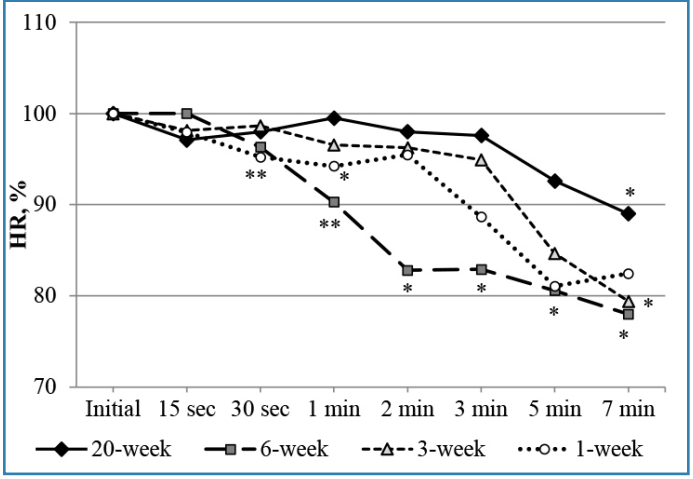
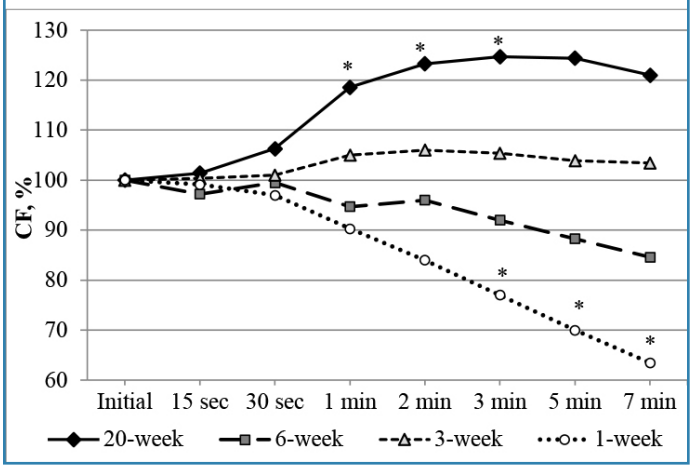


Figure 2. The effect of BMY 7378 (10^{-8}M) on the coronary flow of an isolated heart of rats of different ages. The ordinate is the coronary flow (CF, %), the abscissa is the time (second, minute). *p <0.05 compared with the initial values.



The alpha$_{1D}$-adrenergic receptor blocker, BMY7378, at 10^{-8} M has a negative chronotropic effect on the isolated heart in rats starting from the 1st week of postnatal ontogenesis. A relatively slow chronotropic heart response to the blockade of alpha$_{1D}$-adrenergic receptors was observed in 20-week-old rats. The α_{1D}-adrenergic receptor blocker, BMY7378 (10^{-8} M) causes an increase in the coronary flow of the heart in adult rats and a decrease in it in 1-week-old rats. At the same time, it does not affect the coronary perfusion of the isolated heart of 3- and 6-week-old rats.

Blockade of alpha$_{1D}$-adrenergic receptors causes bradycardia of the isolated heart in all age groups of animals. The frequency of contractions of the isolated heart of newborn rats slightly decreased during the first minutes of the BMY7378 effect. In adult rats, a relatively slow chronotropic heart response was observed in response to the blockade of alpha$_{1D}$-adrenergic receptors. α_{1D}-ARs may be involved in the regulation of the chronotropy of the rat heart at the early stages of maturation of its sympathetic innervation[2-6].

At the same time, adult and newborn rats had the opposite dynamics of the coronary flow rate of an isolated heart. BMY7378 (10^{-8} M) causes an increase in the coronary flow of the heart in adult rats and a decrease in this indicator in newborn rats. At the same time, it does not affect the coronary perfusion of the isolated heart of 3- and 6-week-old rats. An increase in the rate of coronary perfusion of the heart in adult rats, indicating a decrease in the tone of the coronary vessels, was observed against the background of the absence of pronounced changes in the chronotropic response of the heart. In newborn rat pups, BMY7378 perfusion caused an increase in the tone of the coronary vessels, which led to a decrease in the rate of coronary perfusion. In rats at these stages of postnatal development, α(1D)-receptors may be more important in the regulation of the coronary circulation of the heart. These data are also consistent with our previous results[10], which showed that the nonselective agonist of alpha$_1$-adrenergic receptors, methoxamine, has pronounced effects on the coronary perfusion of the heart in 20- and 1-week-old rats. That is, it can be assumed that the effects of non-selective stimulation of alpha1-adrenergic receptors, α_{1D}-receptors play an important role.

This study showed that the effects of blockade of alpha$_{1D}$-AR on the activity of the heart of rats have age-related characteristics. These features can be associated with a different

level of formation of the sympathetic innervation of the heart, which entails a different density and maturity of membrane receptors formations[12-14]. In addition, our results indicate that alpha1-adrenergic receptors, in addition to their effects on coronary vessels, mediate chronotropic effects on the rat heart during postnatal ontogenesis. The mechanisms and age-related characteristics of these influences, requires further research.

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