Influence of If-current blockade on rat heart contractility with a model of myocardial infarction

Influencia del bloqueo de la corriente-If en la contractilidad del corazón de rata en un modelo de infarto al miocardio

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Abstract

Hyperpolarization -activated cyclic nucleotide- dependent channels (HCN) are widespread throughout the body and participate in various physiological processes, the most important of which is the generation of spontaneous electrical activity in the heart and the regulation of synaptic transmission in the brain. The physiological role of HCN channels in the working healthy myocardium is still the subject of ongoing research. The objective of this research is to study the effect of I_f blockade with ZD7288, on heart inotropy in rats with a modeled myocardial infarction. Two methods were used, the classical model of reproduction of myocardial infarction in experimental animals, developed in 1960 by Selye, and the isolated heart technique according to Langendorff. The peculiarities of the inotropic function of an isolated heart with a model of myocardial infarction during current blockade activated by hyperpolarization were evaluated. The I_f blocker, ZD7288, at concentrations of 10^{-9} M and 10^{-7} M, led to an increase in the pressure wave amplitude by 43% and 40% ($p \leq 0.05$), respectively, and at a concentration of 10^{-5} M, to a decrease in the pressure wave amplitude by 49% according to Langendorff' technique. The value of the maximum rate of rising of the pressure wave (dP/dt_{max}) increased during perfusion of ZD7288 at concentrations of 10^{-9} M and 10^{-7} M by 31%, and the maximum rate of drop of the pressure wave (dP/dt_{min}) increased by 27% and 32%, respectively. The addition of the drug to the solution (10^{-5} M) caused a decrease in dP/dt_{max} by 44%, and dP/dt_{min} by 41%. Thus, it can be concluded that impaired coronary blood supply is not the only factor in the pathogenesis of the disease typical of human myocardial infarction.

Keywords: a model of myocardial infarction, hyperpolarization-activated currents, isolated heart, rat.

Resumen

Los canales dependientes de nucleótidos cíclicos activados por hiperpolarización (HCN) están diseminados por todo el cuerpo y participan en varios procesos fisiológicos, los más importantes de los cuales son la generación de actividad eléctrica espontánea en el corazón y la regulación de la transmisión sináptica en el cerebro. El papel fisiológico de los canales de HCN en el miocardio sano en funcionamiento sigue siendo objeto de investigación en la actualidad. El objetivo de esta investigación es estudiar el efecto del bloqueo de I_f con ZD7288, sobre la inotropía cardíaca en ratas con un modelo de infarto al miocardio. Se utilizaron dos métodos: el modelo clásico de reproducción del infarto al miocardio en animales de experimentación, desarrollado en 1960 por Selye, y la técnica del corazón aislado según Langendorff. Los resultados mostraron las peculiaridades de la función inotrópica de un corazón aislado con un modelo de infarto de miocardio durante el bloqueo actual activado por hiperpolarización. El bloqueante de la I_f ZD7288, a concentraciones de 10^{-9} M y 10^{-7} M condujo a un aumento en la amplitud de la onda de presión en un 43% y 40% ($p \leq 0.05$), respectivamente; y a una concentración de 10^{-5} M, a una disminución de la amplitud de la onda de presión en un 49%. El valor de la tasa máxima de aumento de la onda de presión (dP/dtmax) incrementó durante la perfusión de ZD7288 a concentraciones de 10^{-9} M y 10^{-7} M en un 31%, y la tasa máxima de caída de la onda de presión (dP/dtmin) fue de un 27% y 32%, respectivamente. La adición del fármaco a la solución (10^{-5} M) provocó una disminución de dP/dtmax en un 44% y de dP/dtmin en un 41%.

Palabras clave: modelo de infarto de miocardio, corrientes activadas por hiperpolarización, corazón aislado, rata.

Introduction

including a hyperpolarization-activated current of mixed ionic nature, known as 'funny current', If, or 'pacemaker current'.

Hyperpolarization-activated cyclic nucleotide-gated channels (HCN) are molecular correlates known as 'funny current' (If) activated by hyperpolarization. These ion channels can conduct sodium and potassium, and their permeability can change when interacting with cyclic nucleotides, mainly cyclic adenosine monophosphate (cAMP). It is known that cesium ions in millimolar concentrations can act as HCN blockers[1]. The proteins that form these channels are sufficiently widespread in the body; their significant contribution to many physiological functions has been shown, the main of which are the myocardial spontaneous electrical activity or intrinsic pacemaker activity, as well as the modulation of interneuronal connections in the brain. The participation of HCN in the regulation of heart rate, stimulation of neurons, integration of dendrites, mechanisms of learning, and memorization, as well as sensory systems, have been studied in detail[2]. Currently, HCN in peripheral tissues remains an attractive research subject.

Dario Di Francesco[1-2] was the first to describe the peculiarity of HCN with some skepticism. Evolutionary and ontogenetic approaches to the study of HCN have provided evidence for a highly conserved and generic origin of this channel, as well as its premature functional and molecular expression in embryonic stem cells[3]. Despite the high interest in these channels, to date, the therapeutic value of specific modulators has been demonstrated only for cardiac pathology. Indeed, the widespread distribution of HCNs in various tissues and their participation in many physiological processes require the discovery of selective substances to prove that these channels can be used as drug targets and to develop new drugs.

The HCN channels belong to the superfamily of pore-loop cation channels. HCN channels are complexes consisting of the assembly of four alpha subunits that are arranged around the central pore. In mammals, four genes encoding HCN subunits were cloned[1-4]. All HCN monomers have the same basic structural scheme, consisting of six alpha-helices, a transmembrane domain, and two cytosolic domains at the ends of the NH and COOH[4]. The structure of HCN channel expression has been studied in several animal and human species at the tissue and single-cell levels. HCN subunits are highly expressed in the central and peripheral nervous system[5]. All four isoforms of HCN channels were found in the heart differently expressed in the departments[6]. The HCN channels are permeable to Na^+ and K^+ and conduct a mixed cation current. Membrane hyperpolarization is necessary and sufficient to activate the HCN channels; cAMP act as co-stimulatory modulators that facilitate voltage-dependent HCN channel activation[6]. The physiological role of HCN channels in the working healthy myocardium is currently subject of research. HCN-mediated current in human atrial and ventricular cardiomyocytes is similar to that obtained in sinus node cells, but its role should diverge, since healthy atrial and ventricular cardiomyocytes have a stable membrane resting potential and do not lead to spontaneous electrical activity [7].

Various experimental and epidemiological studies have identified numerous risk factors, such as hypertension, diabetes, and smoking, in the development of coronary heart disease[8]. An increase in heart rate may favor the progression of myocardial ischemia[8]. Heart rates during ischaemia and reperfusion are possible determinants of reperfusion arrhythmias. Ng[9] used ivabradine, a selective If current inhibitor, to assesse the effects of heart rate reduction during ischaemia-reperfusion on reperfusion ventricular arrhythmias and showed that the antiarrhythmic effect of ivabradine may reflect slower development of ischaemia-induced electrophysiological changes.

The objective of this research is to study the effect of blockade of If-currents on heart inotropy in rats with modeled myocardial infarction.

Material and Methods

The myocardial infarction model reproduction technique

To reproduce myocardial infarction in experimental animals, the classical model developed in 1960 by Selye was used[10].

The experiments were conducted on 18 white outbred rats, weighing 200-250 grams. The animal was anesthetized with ether by placing the rat under a glass cover. After that, the animal limbs were fixed to the operating table with rubber bands. Additional anesthesia may be required during preparation for thoracotomy; if necessary, an ether-soaked gauze pad was placed over the airway of the rat. After opening the chest, anesthesia was no longer applied. To improve the vision of the operating field, lamp lighting was used. The chest of the animal was shaved and treated with an antiseptic. A skin incision was made on the left side of the rat's chest. They spread the pectoral muscles. Blunt curved forceps were then dipped between the fifth and sixth ribs and expanded to about 15 mm. At this time, a rapid heartbeat was observed. Now air enters the chest cavity, and breathing becomes impossible, but this does not pose a serious threat to the life of the rat, since the ligation takes 60-90 seconds. After exteriorization, the heart ventricles were fixed with the thumb and forefinger of the left hand. The anterior branch of the left coronary artery was visually found, to which a ligature (Premiline 6/0, 2xDR12) was placed 0.5-1 mm below its exit from under the auricle of the heart and ligated, the heart was returned to the chest cavity. The muscles were shifted; the skin was sutured and treated with an antiseptic solution. Immediately after shifting the muscles, the animal began to breathe, and in a few minutes after the operation regained consciousness and began to actively move around the cage[10].

The effect of the hyperpolarization-activated current blockade on an isolated heart with modeled myocardial infarction was studied on the 54th day of the formation of heart failure, indicating the adaptation of the cardiovascular system to heart failure caused by ligation of the left coronary artery.

Isolated heart specimen preparation

The *ex vivo* experiments were carried out according to the standard Langendorff isolated heart technique. For anesthesia, a 25% urethane solution (800 mg/kg) was used intraperitoneally. Then the heart was isolated, washed in Krebs-Henselite solution pre-cooled to +4°C. The heart behind the aorta was ligated with a cannula, and retrograde perfusion was performed at a constant pressure of 55-60 mm Hg and a temperature of 37°C using the Langendorff System (Australia). To register the pressure in the left ventricular cavity, a latex balloon connected to a catheter and a pressure transducer was used. The studied parameters of the isolated heart were recorded and processed using the LabChart Pro V8 software. During the experiment, the change in the pressure wave amplitude (PWA), the maximum rate of increase of the pressure wave (dP/dt_{max}), and the maximum rate of drop of the pressure wave (dP/dt_{min}) in response to the addition of ZD7288 (Sigma), a hyperpolarization-activated current blocker, into the perfusion solution were calculated.

Statistical analysis of the results was carried out using paired and unpaired Student's t-tests. Values were considered significant for a value of approximately $p<0.05$.

Results and Discussion

To identify the effects of If blockade in an isolated heart with a model of myocardial infarction, experiments were carried out in the presence of ZD7288 blocker at concentrations of 10^{-9}, 10^{-7}, 10^{-5} M.

As shown in figures 1A 1B and 1C, the application of ZD7288 to a perfused solution at a concentration of 10^{-9} M led to a change in the studied parameters of the heart. The initial values of PWA were 40.2 ± 14.8 mm Hg. During the 4th minute, PWA increased to 45.2 ± 16.7 mm Hg. ($p\leq0.05$). By the 8th minute of the experiment, PWA was 47.8 ± 16.1 mm Hg. During the 15th minute of observation, PWA increased to 49.8 ± 16.5 mm Hg. During the final minute of the experiment, PWA was 57.6 ± 17.2 mm Hg.

dP/dt_{max} before application to the perfused solution ZD7288 was 1082 ± 336 mmHg/sec and increased to 1199 ± 404 mm Hg/sec during the first minute of observation. By the 7th minute of the experiment, dP/dt_{max} increased to 1201 ± 428 mm Hg/sec. During the 12th minute of observation, dP/dt_{max} was 1268 ± 421 mm Hg/sec. During the final minute of observation, dP/dt_{max} decreased to 1419 ± 418 mm Hg/sec.

dP/dt_{min} after injection of the If blocker increased from 713 ± 222 mm Hg/sec up to 764 ± 254 mm Hg/sec. By the 5th minute of observation, dP/dt_{min} was 689 ± 249 mm Hg/sec. Then dP/dt_{min} increased to 720 ± 261 mm Hg/sec and 810 ± 260 mm Hg/sec during the 10th and 15th minutes of observation. By the final minute of observation, dP/dt_{min} increased to 909 ± 267 mm Hg/sec.

The addition of the If blocker to the perfused solution at a concentration of 10^{-7} M caused an increase in PWA from 38.3 ± 10.2 mm Hg up to 42.6 ± 13.2 mmHg by the 5th minute of observation. During the 10th minute of the experiment, PWA increased to 47.8 ± 15 mm Hg. Then PWA continued to increase and by the 15th and 20th minutes reached 50 ± 15.2 mm Hg and 53.5 ± 14.4 mm Hg, respectively ($p\leq0.05$).

Application of ZD7288 caused an increase in dP/dt_{max} from 1053 ± 242 mmHg/sec up to 1207 ± 353 mm Hg/sec by the 5th minute of observation. dP/dt_{max} by the 10th minute of the experiment increased to 1295 ± 413 mm Hg/sec. By the 15th and 20th minutes of the experiment, dP/dt_{max} increased to 1326 ± 413 mm Hg/sec and 1387 ± 393 mm Hg/sec.

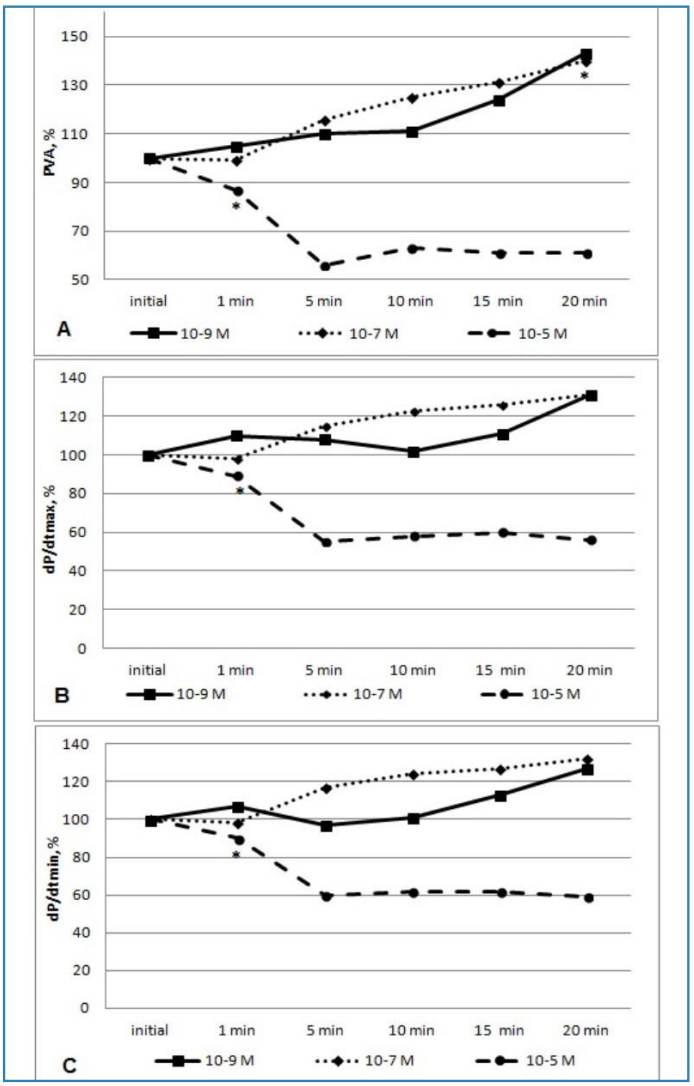

Figure 1A. The effect of I blockade with ZD7288, at concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M on the pressure wave amplitude of rat isolated heart with modeled myocardial infarction. The ordinate is the pressure wave amplitude the abscissa is the experiment time (minutes). The significance is indicated in comparison with the initial values. *p≤0.05.

Figure 1B. The effect of I blockade at concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M on the maximum rate of pressure wave rise (dP/dt_{max}) of rat isolated heart with a myocardial infarction model. The ordinate axis is the maximum rate of rising of the pressure wave (dP/dt_{max}, in %), the abscissa axis is the experiment time (minutes). The significance is indicated in comparison with the initial values. *p≤0.05.

Figure 1C. The effect of I blockade at concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M on the maximum rate of pressure wave drop (dP/dt_{min}) of of rat isolated heart with with a myocardial infarction model. The ordinate axis is the maximum rate of the pressure wave drop (dP/dt_{min}, %), the abscissa axis is the experiment time (minutes). The significance is indicated in comparison with the initial values. *p≤0.05.

Application of the If blocker in an isolated heart with modeled myocardial infarction caused an increase in dP/dt_{min} from 560 ± 112 mm Hg/sec up to 653 ± 184 mm Hg/sec to 5 minutes of the experiment. Then, during the 10th minute of observation, dP/dt_{min} was 692 ± 214 mm Hg/sec. By the 15th minute of observation, dP/dt_{min} increased to 711 ± 216 mm Hg/sec. At the final minute of observation, dP/dt_{min} increased to 743 ± 205 mm Hg/sec.

Meanwile, as shown in figures 1A 1B and 1C, the addition of the If blocker to the perfused solution at a concentration of 10^{-5} M caused a decrease in the studied parameters of an isolated heart with a model of myocardial infarction. In effect, before the addition of ZD7288 to the perfusion solution, PWA was 13.9 ± 4.5 mmHg, and in the first minute after the injection of the substance, it decreased to 12.1 ± 4.5 mm Hg. ($p \leq 0.05$). The minimum decrease in PWA to 7.5 ± 2.8 ml/min was observed during the 4th minute of the experiment. Then, during the 10th minute, PWA slightly increased to 8.81 ± 3.7 ml/min, and during the 15th minutes, up to 9 ± 2.9 ml/min. During the final minute of observation, PWA was 8.5 ± 2.8 ml/min.

The addition of the If blocker to the solution caused a decrease in dP/dt_{max} from 339 ± 112 mm Hg/sec, up to 304 ± 106 mm Hg/sec. ($p \leq 0.05$). During the 5th minute, the lowest dP/dt_{max} value was recorded, 187 ± 26 mm Hg/sec. By the 10th minute of the experiment, dP/dt_{max} increased to 197 ± 31 mm Hg/sec. During the 15th minute, dP/dt_{max} increased to 202 ± 29 mm Hg/sec. During the final minute of the experiment, dP/dt_{max} was 191 ± 24 mm Hg/sec.

After adding the IF blocker to the solution, dP/dt_{min} decreased from 251 ± 61 mm Hg/sec up to 226 ± 60 mm Hg/sec. ($p \leq 0.05$). By the 5th minute, dP/dt_{min} decreased to 159 ± 15 mm Hg/sec. Then, during the 10th and 15th minutes of observation, dP/dt_{min} decreased to 156 ± 23 mm Hg/sec. and 155 ± 19 mm Hg/sec. During the final minute of the experiment, dP/dt_{min} was 149 ± 13 mm Hg/sec.

Figure 2.

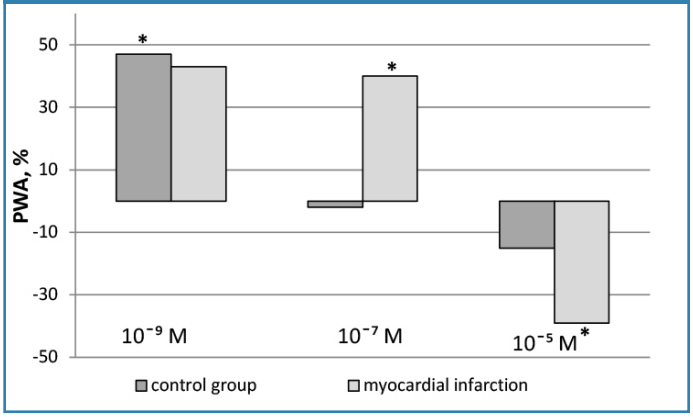

Figure 2. Comparative analysis of the pressure wave amplitude of the Langendorff isolated heart of adult rats of the control group and with modeled myocardial infarction, with If current blocker application, at concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M. The ordinate is the change in the pressure wave amplitude (PWA) (%), the abscissa is the concentration of the ZD7288 blocker (M). The significance is indicated in comparison with the initial values. *$p \leq 0.05$.

During the experiment, the peculiarities of the inotropic function of an isolated heart with a model of myocardial infarction during hyperpolarization-activated If blockade were observed. Application of the If blocker at concentrations of 10^{-9} M caused an increase in the pressure wave amplitude by 43%, and at concentrations of 10^{-7} M – in PWA by 40% ($p \leq 0.05$). Perfusion of the If blocker (10^{-5} M) reduced PWA by 49%. dP/dt_{max} increased upon perfusion of ZD7288 at concentrations of 10^{-9} M and 10^{-7} M by 31%, and dP/dt_{min} increased by 27% and 32%, respectively. The addition of the drug to the solution (10^{-5} M) caused a decrease in dP/dt_{max} by 44%, and dP/dt_{min} by 41%.

A comparative analysis of the results of the study revealed that an isolated heart with a model of myocardial infarction develops a positive inotropic effect in response to adding the ZD7288 blocker at concentrations of 10^{-9} M and 10^{-7} M; while in healthy animals, a positive inotropic effect is observed only when the minimum concentration of the blocker is added. ZD7288 at a concentration of 10^{-5} M decreased the pressure wave amplitude in an isolated heart with a model of myocardial infarction and in a healthy rat heart; however, in a heart with a myocardial infarction model, the decrease in the pressure wave amplitude was more pronounced[10] (Figure 2).

Earlier, in our studies on healthy animals, the effect of If blockade on heart rate and the force of contraction of myocardial strips was shown. The age-related characteristics of the heart rate response[11,12] and inotropy to the hyperpolarization-activated If blockade[13,14] were studied.

Studies aimed at identifying factors causing myocardial infarction suggest that impaired coronary blood supply is not the only factor in the pathogenesis of the disease typical of human myocardial infarction. Myocardial infarction rarely occurs in perfectly healthy young people; it is possible that the development of cardiac muscle pathology is caused by biochemical changes leading to wear and tear of the myocardium and coronary arteries, in particular, chronic atherosclerosis and myocardial fibrosis, which usually precede the development of myocardial infarction in humans. Recent literature shows that the If density in cardiomyocytes changes not only with age but also in pathophysiological hypertrophy [15,16].

Conflict of Interests

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

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