

Sexual dimorphism in age-related changes in the expression of genes involved into regulation of calcium metabolism in aorta and myocardium of rats

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Abstract

Age is the main risk factor for cardiovascular disease development. Understanding of mechanism underlying the vascular and myocardial ageing is necessary both for prevention and for treatment of age-associated diseases. The research aimed to evaluate age-related changes in the transcriptional activity of genes involved in the regulation of Ca^{2+} signalling – IP₃R, RyR, and modulators of their activity CaM and Epac in blood vessels and myocardium of rats of both sexes. Gene expression was evaluated according to the mRNA content of the test protein relative to the β -actin mRNA content. It is proven that rats of both sexes develop unidirectional changes in the expression of genes encoding IP₃R and RyR2, and different ones in the expression of protein genes modulating their activity – CaM and Epac depending on the sex of animals. In the vessels of old rats (24 months) of both sexes the relative level of mRNA for IP₃R type 2 and 3 was reduced and not changed for IP₃R1, and significantly increased for RyR2 receptors compared to these indicators in young rats (4 months). In the aorta of old females the relative mRNA content for CaM and Epac1 was reduced and not changed for Epac2. On the contrary, the expression of Epac1 and Epac2 was increased by 67% and 50% respectively compared to similar indicators in young rats (4 months) and rests unchanged for CaM which indicates gender differences in the violation of subtle mechanisms for modulating the activity of RyR2 and IP₃R in blood vessels. Old rats showed significant changes in myocardium. In older males, the expression of RyR2, IP₃R1, 2, 3 increases in the left ventricle, RyR2 and IP₃R1 – in the left atrium, and RyR2 and IP₃R3 – in the right atrium. Unlike males, the mRNA content for RyR2, IP₃R1, 2, 3 was significantly lower in the left ventricle of females than in young animals. High levels of IP₃R1 and IP₃R3 expression were detected in the right atrium of senior females, and IP₃R3 expression was detected in the left atrium. The expression of IP₃R2 was unchanged in all parts of the female heart. In the myocardium of old rats of both sexes the expression of CaM and Epac2 proteins increased significantly. The revealed age differences in the transcriptional activity of genes involved in the regulation of intracellular Ca^{2+} signalling at the level of IP₃R and RyR2-mediated mechanisms suggest that with the increasing life expectancy males are significantly more likely to develop myocardial hypertrophy and heart rhythm disorders than females.

Introduction

Every year the world's elderly population keeps growing. As the life expectancy increases, so does the leading cause of risk worldwide that is the risk of cardiovascular diseases developing [1, 2]. With age, the structural and electromechanical properties of smooth muscle cells and cardiomyocytes change in the blood vessels and heart. The precursors or manifestations of such transformations are changes in the expression levels of genes of proteins involved in the regulation of intracellular signaling and functional activity of cells [3-7]. Knowing the extent to which age-related changes contribute to the adaptation or development of cardiovascular diseases is extremely important. It is shown that in the process of physiological aging, the integrity of the myocardial structure is more preserved in the female body, which may be one of the reasons for the difference in life expectancy between women and men [8]. In elderly people the frequency and severity of atrial fibrillation increases, which plays a critical role in the genesis of sudden cardiac death. According to the results of an epidemiological study, the occurrence of atrial fibrillation (AF) in elderly men is higher than in women [7, 9-11]. It is considered that it is associated with earlier damage to the coronary arteries in men. Women are diagnosed with AF at an older age than men but they have a high risk of developing AF-related complications. Experiments on rats also demonstrated that age-related structural and functional changes in blood vessels and myocardium in males develop earlier than in females [12]. It is supposed that it is associated with a deficiency of testosterone, which can cause arrhythmogenic leakage of Ca^{2+} ions from the sarcoplasmic reticulum (SR) [13].

Maintaining intracellular calcium homeostasis is crucial for normal cell function, since Ca^{2+} plays central role in regulating the processes of cell excitation, contraction, transcription, and proliferation. The most important role in the generation, distribution and regulation of intracellular calcium signals is played by Ca^{2+} – selective intracellular channels – inositol-1,4,5-trisphosphate (IP₃R) and ryanodine (RyR) receptors embedded in the sarco/endoplasmic reticulum (SR/ER) membrane. They regulate numerous physiological and pathophysiological processes in the body [14-16]. Disorders of vascular tone and heart rate are primarily associated with IP₃R and RyR2-induced dysregulation of intracellular calcium metabolism [17-19].

Three isoforms of the IP₃Rs (IP3R1,2,3) and RyRs (RyR1,2,3) receptors were identified [20-22]. There is regional heterogeneity in the expression of IP₃Rs and RyRs isoforms in blood vessels and myocardium, which seems to be important in the implementation of various physiological functions, such as excitability, contractility, and gene expression [18, 23-25]. Localization of these receptors on the SR/ER membrane is also isoform and is tissue-specific [14, 26].

It was shown that the most highly expressed isoform in vascular smooth muscle cells (SMCs) is IP₃R. It is then followed with significant heterogeneity in expression by IP₃R3 and IP3R2, in cardiomyocytes – by RyR2. The activity of IP₃Rs and RyRs is stimulated by many endogenous molecules that act through G-protein coupled receptors (GPCR) and is modulated by Ca²⁺, calmodulin (CaM), protein kinases A and G (PKA and PKG), active oxygen forms, and others [27-32]. Agonists acting through the Gs-protein activate adenylate cyclase leading to an increase in the concentration of cyclic adenosine monophosphate (cAMP), activation of PKA and cAMP-binding Epac proteins (Exchange Proteins Directly Activated by cAMP), subsequent phosphorylation of IP₃Rs and RyRs, and changes in their functional activity [16, 33]. In order to understand the mechanisms underlying biological aging and to identify possible risk factors for the development of age-related cardiovascular diseases the study featured changes in the transcriptional activity of IP₃Rs, RyRs genes and modulators of their activity – the signal proteins CaM and Epac in the blood vessels and myocardium of rats depending on the sex of animals.

The aim of the study was to evaluate age-related changes in the transcriptional activity of protein genes involved in the regulation of Ca²⁺ signaling - IP₃Rs, RyRs receptors and modulators of their CaM and Epac activity in the blood vessels and myocardium of rats of both sexes.

Materials And Methods

The work was performed on mongrel male and female rats at the age of 4 months and 24 months. Animal welfare conformed to the order of Ministry of Health of the Russian Federation No. 708n "On establishment of rules of laboratory practice" dated from 23.08.2010 and ethical standards outlined in the good laboratory practice (GLP) Declaration of Helsinki (2000).

The rats included in the study were randomized into 4 groups: Group 1 – males aged 4 months (young, n = 10); group 2 males at the age of 24 months (old, n = 8); group 3 – females at the age of 4 months (young, n = 14); group 4-females aged 24 months (old, n = 10). Anesthetized rats (25% urethane solution, 4 ml/kg) were decapitated, the chest was opened, fragments of the heart and aorta were extracted, blood was washed off in a saline solution of sodium chloride at 4°C, after which the tissues were placed in a solution of RNAlater (Ambion, USA) and stored until RNA was isolated at -20°C. Pieces of tissue from the atria, left ventricle, and aorta were ground in liquid nitrogen. RNA extraction from tissues was performed using a GeneJET™ kit (Thermo Fisher Scientific Inc., USA) according to the manufacturer's protocol. The concentration of total RNA in the samples was determined using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., USA). The isolated total RNA was treated with DNase I (Thermo Fisher Scientific Inc., USA) to prevent genomic DNA contamination. cDNA synthesis was performed using the RevertAid™ H Minus first Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA) according to the manufacturer's protocol. The β-actin gene was used as a reference gene. The level of expression of the β-actin gene between the compared groups (females 4 and 24 months; males 4 and 24 months) remained unchanged in our experiments. Changes in the expression level of the target gene were assessed by changes in the mRNA level of the studied β-actin gene/mRNA. Negative controls were set according to generally accepted standards for negative controls during quantitative PCR: to exclude contamination of reagents, the reaction was started without cDNA fragments; to exclude cDNA contamination of genomic DNA, the reaction was started with a total RNA sample.

Table 1. Sequences of primers used in the work.

Protein	Upstream primer	Downstream primer	Primer with a fluorescent probe
RyR2	CAGCAGACTGGAGAGACTGG	CCTTCTTTGATGATATTCAGAG	FAM-GTCCTCTGGTATCCTGGAGGTC-BHQ1
IP ₃ R3	AACTCAACGAGGGCTTCATC	CATTGTTGGGAACATCGC	FAM-GACCGCAGGTTTGTTCATCCAG-BHQ1
IP ₃ R2	CATAGACACAGAGGAAGAGAG	CACACGATGGCAAAGGCT	FAM-CATGCTGAAAATTGGGACCTGC-BHQ1
IP ₃ R1	CCCTGACTTTGAGGAAGAATG	CACAGAGACCAGAGAGTACACC	FAM-AGCCCTCAGTGGACCCTGATC-BHQ1
CaM	GGTGGATGCTGATGGCAAT	GTATCTCCTCCTCGCTGTCTG	FAM-CATTGACTTCCCAGAGTTCCTGAC-BHQ1
Epac1	GCTCCCTGGACTTCAGAGTG	CAAGAGATGACGGTACAGGA	FAM-CTGGAGCAGGCCACCACAGAG-BHQ1
Epac2	GGACGCCTGTTTGCCTG	CATCTGGTACGCCAAGTCT	FAM-CGACTCACTGACTCCCTTGCCA-BHQ1

Statistics. Preliminary processing of the results was performed using software incorporated into the device. Further processing was performed in Microsoft Excel using the 2- $\Delta\Delta$ Ct algorithm. The statistical significance of discrepancies was determined using the Student's t-test. The discrepancies were considered significant at $p < 0.05$.

Results And Discussion

Aorta. The level of mRNA of ryanodine (RyR2) and Inositol-1,4,5-trisphosphate (IP₃R1, IP₃R2, IP₃R3) receptors as well as regulatory proteins – CaM, Epac1 and Epac2 - were evaluated in the aortic tissues of male and female rats. Analysis of the results shows that the aorta of old rats of both sexes (24 months) decreases the level of mRNA for IP₃R2 (up to 63% and 61%, respectively in females and males) and IP₃R3 (up to 62% and 67%, respectively in females and males) and doesn't change the expression of IP₃R2-type receptors in comparison with similar data in young rats (4 months). It was found that in old rats regardless of the sex of animals the content of mRNA for RyR2 in blood vessels increased by more than 1,5 times (up to 153% in males and up to 185% in females) (Fig. 1a).

However, there are significant sexual discrepancies in nature of age-related changes in the expression of CaM genes and Epac proteins. It was proven that in old females the level of mRNA in the aorta decreases for CaM and Epac1 (by 30% and 32%, respectively) and doesn't change for Epac2 in comparison with similar indicators in the vessels of young females (Fig.1b). In contrast to females, the expression of Epac1 and Epac2 proteins increases in the vessels of old male rats and doesn't change for CaM (Fig. 1b).

IP₃Rs function in vessels.

IP₃Rs play a central role in generating and maintaining myogenic vascular tone. However, it is still unknown exactly what contribution each of the three IP₃Rs subtypes makes to the complex transmission of Ca²⁺ signals in the cell. The role of IP₃R vessels in the development of hypertension remains unclear and data available rests contradictory. It is supposed that in hypertension increased sensitivity to phenylephrine is caused by an increase in the expression of IP₃R1 in vascular SMCs as a result of Ca²⁺ - dependent activation of the transcription factor CN-NFAT (calcineurin-Nuclear factor of activated T-cells) [34]. At the same time, activation of IP₃R1 in cerebral arteries located in close proximity to Ca²⁺-activated high-conductivity potassium channels (BKCa) has been shown to increase the sensitivity of these channels to Ca²⁺. According to the authors, this mechanism can limit IP₃R-induced vasoconstriction in the cerebral arteries [35].

Experiments on HeLa cell culture that express comparable amounts of IP₃R1 and IP₃R3 showed that suppression of IP₃R1 subtype expression by RNA interference leads to a significant decrease in total Ca²⁺ signals and the termination of Ca²⁺ oscillations [17]. In contrast, suppression of IP₃R3 expression leads to more stable and prolonged fluctuations in intracellular Ca²⁺ concentration than in the control. Similar effects of IP₃R3 knockout were obtained on COS-7 cells, which primarily express IP₃R3 [17]. These results allowed the authors to suggest that IP₃R1 and IP₃R3 cells have opposite effects on the generation of intracellular Ca²⁺ oscillations.

Experiments on rat aortic SMCs culture showed that proliferating cells express mainly IP₃R2 and IP₃R3 but during their development the expression of these types of receptors decreases, and IP₃R1, on the contrary, increases [36, 37]. It was shown that IP₃R1 is distributed throughout the cytoplasm, IP₃R2 is closely related to the nucleus and to the plasma membrane, and IP₃R3 is distributed mainly around the nucleus. It is shown that each isoform of IP₃Rs can lead to activation of apoptosis [38]. However, the IP₃R3 isoform plays a special role in inducing apoptosis primarily by transmitting Ca²⁺ signals to mitochondria, since IP₃R3 has a special tendency to form subcellular clusters with mitochondria, which leads to the formation of associated signaling microdomains. Interestingly, IP₃R3 can not only activate, but also inhibit apoptosis, and this process is implemented by an Akt-IP₃R3-dependent mechanism [39].

We determined that one of the manifestations of age-related vascular changes is an imbalance in the expression of IP₃Rs: the expression of type 2 and 3 receptors decreases and IP₃R1 doesn't change. These changes in the expression of IP₃Rs subtypes in aging vessels of animals of both sexes, apparently, can be critical for various forms of Ca²⁺ signal transmission in both SMCs and endothelial cells and affect the contractile properties of vessels. In addition, it can be assumed that a significant decrease in the expression of IP₃R2 and IP₃R3 types in the aorta of old rats of both sexes is a manifestation of an age-related decrease in the proliferative and apoptotic activity of SMCs.

RyRs function in vessels.

RyRs function in SMC is less clear. Although RyRs in smooth muscles generate local Ca²⁺ sparks that are similar to those observed in striated muscles, however, the contribution of these changes to the depolarization-induced global increase in intracellular Ca²⁺ ([Ca²⁺]_i) concentration is quite limited. Unlike cardiac or skeletal muscles, RyR-dependent Ca²⁺ - induced calcium release (CICR) isn't necessary for vasoconstriction as the concentration of Ca²⁺ in vascular SMC causes vascular contraction that is lower than required for RyR-mediated CICR activation [40]. In vascular SMC RyR-mediated Ca²⁺ sparks are accompanied by activation of BKCa that leads to a decrease in myogenic tone and vasodilation. At the same time, it is shown that BKCa and RyR channels play different roles in the regulation of myogenic and neurogenic tone: while BKCa and RyR channels work together to counteract myogenic vasoconstriction, BKCa channels counteract neurogenic vasoconstriction, and RyR channels, on the contrary, enhance it [41]. The authors suggest that at the level of neurogenic vasoconstriction of resistive arteries, RyRs act in conjunction with voltage-dependent L-type Ca²⁺ channels.

It was determined that in vessels of old females and males, the level of mRNA RyR2 increases by 1.9 and 1.5 times respectively compared to similar indicators in young rats. An increase in the expression of RyR2 and a decrease in IP₃R2 and IP₃R3 in the aorta of rats of both sexes caused by aging indicate significant changes in intracellular Ca²⁺ metabolism but it isn't clear yet how they affect the regulation of myogenic and neurogenic vascular tone in a whole organism. Moreover, the expression of the most important regulators of channel protein activity – CaM and Epac – changes during vascular aging. We found out that within a high expression of RyR2 in the aorta of old females, there is a significant decrease in the expression of CaM and Epac1, while in the vessels of males the level of mRNA for CaM remains unchanged but the expression of Epac1 and Epac2 proteins increases.

For RyRs calcium is the main physiological activity regulator [30, 42]. With a moderate increase in [Ca²⁺]_i the excitation of RyRs is mediated through activation of CaMK-dependent channel phosphorylation or by interaction of Ca²⁺ with the Ca²⁺ -

binding protein S100A which competes with CaM for binding to RyRs. Vasodilators that act on receptors associated with the Gs protein activate adenylate cyclase, which leads to an increase in cAMP and subsequent activation of PKA- and Epac-mediated phosphorylation of RyRs, which leads to an increase in the frequency of RyR-dependent local calcium signals, followed by activation of BKCa-channels and vasodilation. At a high level of $[Ca^{2+}]_i$, RyR activity, in contrast, is inhibited through mechanisms including Ca^{2+} -binding sorbin proteins (SORC) or CaM [16].

Thus, considering aging, unidirectional changes in the expression of genes for channel proteins IP_3 Rs and RyR2 develop in the aorta of rats, and changes in the expression of proteins that modulate their activity – CaM and Epac indicating age-related changes in intracellular calcium metabolism. It can be assumed that a high level of Epac1 and Epac 2 expression in the vessels of old male rats negatively affects the activity of IP_3 Rs and positively affects RyRs, since Epac-induced phosphorylation leads to a decrease in the activity of the former and an increase in the latter. A decrease in CaM expression in the vessels of older females may lead to a decrease in its inhibitory effect on IP_3 Rs and RyRs, and Epac1 may lead to a decrease in its activating effect on RyR2 and inhibiting on IP_3 Rs. Apparently, one of the aging vessels signs is a violation of the goodness of fit modulation mechanisms of the most important ion channels involved in the regulation of $[Ca^{2+}]_i$, which have a certain specificity in female and male rats. The detected changes can make a significant contribution to age-initiated vascular pathology at the level of their contractile, proliferative and apoptotic activity. However, it is possible that some of them may be a manifestation of compensatory mechanisms aimed at preserving Ca^{2+} -homeostasis in aging vessels.

Heart. It was determined that in old male rats, the level of mRNA RyR2 increases in the left ventricle and both atria (Fig. 2). In older males, the content of mRNA in the left ventricle increases for all three types of IP_3 Rs, in the left atrium only for IP_3R1 -type, in the right atrium – increases for IP_3R3 -type, but the level of mRNA for IP_3R2 -type decreases compared to control animals (Fig. 2).

Other age-related changes in the expression of the RyR2 and IP_3 Rs genes were detected in the hearts of old females. It was found that the expression of RyR2, IP_3R1 , and IP_3R3 decreases in the left ventricle during aging in females in contrast to males, while the level of mRNA for IP_3R3 -type increases in the left atrium, and for IP_3R1 – and IP_3R3 - type receptors in the right atrium (Fig. 2). the level of expression of IP_3R2 -type mRNA in the hearts of old females does not differ from the indicators in the hearts of young rats.

While aging, the expression of CaM and Epac2 increases significantly in the left ventricle and atria of rats of both sexes as evidenced by a higher level of their mRNA compared to similar indicators in young animals (Fig. 3). In older males, the mRNA level for Epac1 remains unchanged in the heart, while in females, the mRNA level of this protein decreases in the left ventricle and, on the contrary, increases in the left atrium (Fig. 3).

It is known that the aging heart is characterized by a reduced response to sympathetic stimulation, a high risk of arrhythmias and sudden cardiac death [43-45]. Age-related electrical instability and contractile dysfunction of the myocardium are primarily associated with impaired Ca^{2+} -homeostasis. However, specific molecular mechanisms underlying the altered regulation of Ca^{2+} metabolism in the aging heart remain poorly understood. It is shown that older people have reduced functional activity of the sinoatrial node, that is believed to be associated with an age-related decrease in the expression of the main Ca^{2+} - conducting channel SR-RyR2, as well as with changes in the expression of other ion channels – an increase in $Na(v)1.5$, $Na(v)\beta1$ and $Ca(v)1.2$ and a decrease in $K(v)1.5$ and HCN1 [46].

RyRs function in heart.

It is supposed that RyR2 receptors are the key intracellular structures that implement the effect of the sympathetic nervous system on the myocardium. Our research has shown that in older males, the expression of RyR2 increases significantly in the left ventricle and atria, while in females, on the contrary, it decreases in the left ventricle and doesn't change in the atria. Along with a change in the expression of RyR2 in the aging hearts of rats of both sexes, a high level of expression of the

mRNA proteins CaM and Epac2 was detected. Changes in the transcription level of these genes can lead to excessive activation of RyR2 and to serious violations of calcium metabolism in cardiomyocytes [25, 47]. It was found that an excessive increase in the activity of RyR2 as a result of their oxidation, nitrosylation or hyperphosphorylation contributes to the development of heart failure and the occurrence of ventricular arrhythmias [48, 49]. It is known that activation of β -adrenoreceptors (β -AR) can trigger cardiac arrhythmias through cAMP-dependent mechanisms by increasing Ca^{2+} leakage from SR to diastole. cAMP can activate both PKA and Epac proteins. In studies using genetic modifications of Epac1, Epac2, RyR2, and CaMKII, it has been shown that activation of Epac and PKA can equally contribute to the occurrence of β -AR-induced arrhythmias and Ca^{2+} leak from SR. It has been proved that the β 1-AR induced increase in arrhythmogenic Ca^{2+} emissions is mediated by Epac2 [50]. The arrhythmogenic effect of Epac2 has been demonstrated in numerous studies in recent years [47, 51]. It was found that the β -AR-cAMP-Epac2-PI3K-Akt-NOS1-CaMKII signaling cascade mediates increased (pathological) Ca^{2+} leak from SR to diastole, while the cAMP-PKA pathway mainly implements inotropic and lucitropic effects of β -AR [52].

In cardiomyocytes, CaM regulates the activity of RyR2 by direct interaction with them, as well as through CaMKII, which phosphorylate both RyR2 and IP_3Rs [53]. CaM reduces the probability of opening RyR2 at submicromolar (diastolic) and micromolar (systolic) concentrations of Ca^{2+} . CaM reduces the probability of opening RyR2 at submicromolar (diastolic) and micromolar (systolic) concentrations of Ca^{2+} . Recent years data have been obtained on the key role of CaM in the mechanisms of development of heart hypertrophy. *In vitro* and *in vivo* experiments have shown that the suppression of CaM gene expression by microRNA has a pronounced antihypertrophic effect [54]. It was determined that point mutations in the CaM-binding domain of RyR2 (W3587A/ L3591D/F3603A) in mice lead to severe heart hypertrophy, a sharp decrease in left ventricular inotropic function, and early death of animals [55, 56]. Our data on the high level of expression of RyR2, CaM and Epac2 in the hearts of old animals suggest that males are much more likely to develop myocardial hypertrophy and ventricular arrhythmias than females (Fig. 2, 3).

IP_3Rs heart functions.

If the role of RyR2 as a receptor regulating the release of Ca^{2+} from SR, necessary for the implementation of electromechanical coupling of cardiomyocytes is obvious, there is still no clear understanding of IP_3Rs contribution to this process. Under physiological conditions cardiomyocytes, in contrast to non-excitabile cells, have a low level of Gq-induced IP_3 generation and a weak IP_3R response [18]. The role of IP_3Rs in pathophysiology, especially in arrhythmogenesis and myocardial hypertrophy, is more obvious. Currently, it is proved that in the global increase in $[\text{Ca}^{2+}]_i$ in cardiomyocytes, the share of IP_3R -induced release of Ca^{2+} from SR is insignificant, which makes their contribution to the regulation of the process of electro-mechanical coupling of cardiomyocytes minimal. It is considered that an important condition for IP_3Rs activation is their localization in the subsarcolemmal zone meaning in the immediate vicinity of the IP_3 generation site [18].

This study identifies sexual dimorphisms of age-related changes in the expression level of $\text{IP}_3\text{R1}$ – $\text{IP}_3\text{R3}$ type receptors in the left ventricle of the hearts of old rats. Thus, in old males, the expression of $\text{IP}_3\text{R1}$, $\text{IP}_3\text{R2}$, and $\text{IP}_3\text{R3}$ in the left ventricle was significantly higher, while in females, on the contrary, it was lower ($\text{IP}_3\text{R1}$ and $\text{IP}_3\text{R3}$) than in the hearts of young rats.

$\text{IP}_3\text{R1}$ is localized in the heart mainly in conducting and $\text{IP}_3\text{R2}$ in contractile cardiomyocytes and $\text{IP}_3\text{R2}$ is located mainly in the perinuclear membrane region in ventricular cardiomyocytes [57, 58]. Atrial cells express a greater number of IP_3Rs compared to ventricular cardiomyocytes. They are mostly dominated by type 1 and type 2 IP_3Rs [59]. In atria the amount of $\text{IP}_3\text{R2}$ is 6-10 times greater than in ventricles, and in SR they are in close proximity to the sarcolemma in close proximity to the RyRs [57, 58]. It has been shown that agonist-induced activation of phospholipase C (PLC) in atrial cardiomyocytes causes local Ca^{2+} sparks that can be transmitted to nearby RyR2 and amplify the CICR response, and thus IP_3Rs can affect the process of electro-mechanical coupling in atria [60]. It is assumed that IP_3R -induced activation of RyR2, other ion channels and exchangers, in particular Ca^{2+} - I-type channels and $\text{Na}^+/\text{Ca}^{2+}$ - exchanger, can lead to atrial fibrillation [59].

Taking into account the literature data on the predominant localization of IP₃R1 type in conducting cardiomyocytes, it can be assumed that an increase in the expression of this receptor isoform in the left ventricle and left atrium of old male rats is a risk factor for arrhythmias. In addition, a high level of IP₃R2 expression in the left atrium can also provoke atrial fibrillation, since their role in arrhythmogenesis has been proven [61, 62].

It was determined that the GPCR/IP₃R axis modulates not only heart rhythm disorders but also the hypertrophy development. There is strong evidence that IP₃R2 is involved in the development of myocardial hypertrophy under conditions of increased expression of these receptors and/or their over-activation as a result of agonist-induced signaling from GPCR [63], and these mechanisms are implemented at the level of nuclear membranes.

In recent years, more and more attention has been paid to studying the features of Ca²⁺ signal transmission in the nuclei of cardiomyocytes and their role in the regulation of gene transcription [64, 65]. The transcription of genes and cell growth is influenced by nuclear Ca²⁺ signals, which differ from cytosolic Ca²⁺ signals. The regulation of intranuclear concentration of Ca²⁺ ([Ca²⁺]_{nuc}) is carried out mainly through passive diffusion of ions from the cytoplasm. However, there is an additional source of Ca²⁺ in the nucleus that is completely independent of the cytosolic one: the nuclear envelope (NE), which is a cell compartment with its own perinuclear Ca²⁺ storage [66]. In NE, proteins that regulate Ca²⁺ traffic, including IP₃R, are expressed that address both the nucleoplasm and the cytoplasm [67]. Specific GPCR stimuli can increase [Ca²⁺]_{nuc} via IP₃R-mediated release of Ca²⁺ from NE regardless of the concentration of Ca²⁺ ions in the cell cytosol ([Ca²⁺]_{cyt}) [68]. In contrast to the global increase in [Ca²⁺]_{cyt}, it is currently believed that the local increase in [Ca²⁺]_{nuc} plays a central role in the regulation of gene expression in cardiomyocytes.

The structural organization of Ca²⁺ signal transmission in the nucleus may include both receptors and IP₃ generation on the T-channel membranes of ventricular cardiomyocytes located in the immediate vicinity of the nucleus, and GPCR complexes localized on NE [64, 65]. It is known that IP₃R2-type ventricular cardiomyocytes are concentrated in NE [69]. Specific GPCR stimuli, such as endothelin 1, angiotensin II, and others, can increase [Ca²⁺]_{nuc} independently of [Ca²⁺]_{cyt} through IP₃R2-mediated release of Ca²⁺ from NE [68]. IP₃R2 localized on NE are associated with CaM, CaMKII. Activation of CaMKII-dependent nuclear signaling triggers the phosphorylation of class II histone deacetylases (HDAC) and the export of nuclear HDACs, which leads to the activation of MEF2-dependent transcription of various genes (myocyte enhancer factor-2) [70]. The CaN-NFAT transcriptional pathway is also activated. The presence of local Ca²⁺-dependent mechanisms of IP₃R2-mediated regulation of nuclear pore permeability for transcription factor traffic in adult rat cardiomyocytes suggests that IP₃R2 is involved in the development of pathological left ventricular hypertrophy in old males. This assumption is supported by the high level of expression of IP₃R2, as well as CaM in cardiomyocytes of the left ventricle of rats at the age of 24 months compared with similar rates in young rats.

In contrast to males, older females are highly likely to develop atrial fibrillation, since the level of IP₃R1 expression significantly increases in the right atrium of females during aging. An increase in IP₃R is also observed in atrial cardiomyocytes in patients with chronic atrial fibrillation [71, 72]. Unlike males, in older females, the risk of developing ventricular arrhythmias, as well as their hypertrophy, is minimal, since they have a low level of expression of RyR2, IP₃R1 and IP₃R3 in the left ventricle and unchanged for IP₃R2 type.

It has been determined that the IP₃R3 receptor isoform is involved in the development of various human diseases [73]. However, the role of IP₃R3 in the regulation of cardiac activity in normal and pathological conditions has been less studied. It is known that IP₃R3-dependent Ca²⁺ signaling is necessary for NO-induced differentiation of cardiomyocytes obtained from embryonic stem cells [74]. Based on the above data on the important contribution of IP₃R3 to the modulation of the generation of Ca²⁺ oscillations [17], it can be assumed that a significant increase in the expression of these receptors in

cardiomyocytes of rats of both sexes in combination with altered expression of RyR2, IP3R1 and IP₃R2 indicates a transition to a new level of regulation of intracellular calcium exchange in the hearts of old rats.

Conclusions

Aging is a natural process of withering of the functional activity of the body at the level of all systems and organs which leads to reprogramming of the gene expression profile primarily aimed at maintaining vital activity in the conditions of changing needs of the body, i.e. they are adaptive. The most important role in these processes is played by cytosolic and nuclear Ca²⁺ signaling. Along with it, taking into consideration the data obtained, there is every reason to believe that in the process of aging of the body, significant changes occur in the SMCs and cardiomyocytes at the level of regulation of intracellular calcium metabolism that can make a significant contribution to the development of age-associated cardiovascular diseases. The revealed sexual-age dimorphisms in the transcriptional activity of genes involved in the regulation of intracellular Ca²⁺ signaling at the level of IP₃Rs - and RyR2-mediated mechanisms suggest that with increasing life expectancy, males are significantly more likely to develop myocardial hypertrophy and heart rhythm disorders than females. Age-related changes in the expression level of RyR2 and IP₃Rs genes and modulators of their activity – CaM and Epac in the aorta can also negatively affect vascular contractility in old age.

Abbreviations

AF – atrial fibrillation

CICR – Ca²⁺ - induced calcium release

BKCa – Ca²⁺-activated high-conductivity potassium channel

SORC – Ca²⁺-binding sorbin proteins

CN-NFAT – calcineurin-Nuclear factor of activated T-cells

CaM – calmodulin

cAMP - cyclic adenosine monophosphate

Epac proteins – Exchange Proteins Directly Activated by cAMP

GLP – good laboratory practice

HDAC – histone deacetylase

IP3R – inositol-1,4,5-trisphosphate

NE – nuclear envelope

PLC – phospholipase C

PKA and PKG – protein kinases A and G

RyR – ryanodine

SR/ER – sarco/endoplasmic reticulum

SR – sarcoplasmic reticulum

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the recommendations of the Ethical Committee of the Institute of General Pathology and Pathophysiology (the project approval protocol Number 5 of June 07, 2019) and was approved by the Ethical Committee (final approval protocol Number 1a of October 02, 2020). All participants signed informed consent after a complete explanation of the study in accordance with the Helsinki Declaration of 1964 with all subsequent amendments.

Consent for publication

Not applicable

Availability of data and material

Please contact author for data requests.

Competing interests

The authors declare that they have no competing interests."

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Authors' contributions

Each author accounts for a 25% contribution to research. All authors read and approved the final manuscript.

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References

1. Lakatta E.G. So! What's aging? Is cardiovascular aging a disease? 2015. *J. Mol. Cell Cardiol.* 83: 1–13.
2. de Almeida AJPO, Ribeiro TP, de Medeiros IA. 2017. Aging: Molecular pathways and implications on the cardiovascular system. *Oxid. Med. Cell. Longev.* **2017**: 7941563.
3. Sheydina A., Riordon D.R., Boheler K.R. 2011. Molecular mechanisms of cardiomyocyte aging. *Clin. Sci. (Lond)*. 121 (8): 315–329.
4. Thijssen D.H., Carter S.E., Green D.J. 2016. Arterial structure and function in vascular ageing: Are you as old as your arteries? *J. Physiol.* 594 (8): 2275–2284.
5. Saeed Y., Temple I.P., Borbas Z., Atkinson A., Yanni J., Maczewski M., Mackiewicz U., Aly M., Logantha S.J.R.J., Garratt C.J., Dobrzynski H. 2018. Structural and functional remodeling of the atrioventricular node with aging in rats: The role of

- hyperpolarization-activated cyclic nucleotide-gated and ryanodine 2 channels. *Heart Rhythm*. 15 (5): 752–760.
6. Wirth A., Wang S., Takefuji M., Tang C., Althoff T.F., Schweda F., Wettschureck N., Offermanns S. 2016. Age-dependent blood pressure elevation is due to increased vascular smooth muscle tone mediated by G-protein signaling. *Cardiovascular Research*. 109: 131–140.
 7. Lin Y.K., Chen Y.A., Lee T.I., Chen Y.C., Chen S.A., Chen Y.J. 2018. Aging modulates the substrate and triggers remodeling in atrial fibrillation. *Circ. J.* 82 (5): 1237–1244.
 8. Olivetti G., Giordano G., Corradi D., Melissari M., Lagrasta C., Gambert S.R., Anversa P. 1995. Gender differences and aging: Effects on the human heart. *J. Am. Coll. Cardiol.* 26 (4): 1068–1079.
 9. Magnani J.W., Moser C.B., Murabito J.M., Nelson K.P., Fontes J.D., Lubitz S.A., Sullivan L.M., Ellinor P.T., Benjamin E.J. 2012. Age of natural menopause and atrial fibrillation: The framingham heart study. *Am. Heart J.* 163 (4): 729–734.
 10. Magnani J.W., Moser C.B., Murabito J.M., Sullivan L.M., Wang N., Ellinor P.T., Vasani R.S., Benjamin E.J., Coviello A.D. 2014. Association of sex hormones, aging, and atrial fibrillation in men: The framingham heart study. *Circ. Arrhythm. Electrophysiol.* 7 (2): 307–712.
 11. Kavousi M. 2020. Differences in epidemiology and risk factors for atrial fibrillation between women and men. *Front. Cardiovasc. Med.* 7: 3.
 12. Al-Ghuri S., Deussen A.J., Galli R., Muders M.H., Zatschler B., Neisser A., Müller B., Kopalani I. 2017. Sex-specific differences in age-dependent progression of aortic dysfunction and related cardiac remodeling in spontaneously hypertensive rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 312 (5): R835–R849.
 13. Tsuneda T., Yamashita T., Kato T., Sekiguchi A., Sagara K., Sawada H., Aizawa T., Fu L.T., Fujiki A., Inoue H. 2009. Deficiency of testosterone associates with the substrate of atrial fibrillation in the rat model. *J. Cardiovasc. Electrophysiol.* 20 (9): 1055–1060.
 14. Ghosh D., Syed A.U., Prada M.P., Nystoriak M.A., Santana L.F., Nieves-Cintrón M., Navedo M.F. 2017. Calcium channels in vascular smooth muscle. *Adv. Pharmacol.* 78: 49–87.
 15. Sun M.Y., Geyer M., Komarova Y.A. 2017. IP3 Receptor signaling and endothelial barrier function. *Cell Mol. Life Sci.* 74 (22): 4189–4207.
 16. Tykocki N.R., Boerman E.M., Jackson W.F. 2017. Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles. *Compr. Physiol.* 7 (2): 485–581.
 17. Hattori M., Suzuki A.Z., Higo T., Miyauchi H., Michikawa T., Nakamura T., Inoue T., Mikoshiba K. 2004. Distinct roles of inositol 1,4,5-trisphosphate receptor types 1 and 3 in Ca²⁺ signaling. *Biol. Chem.* 279 (12): 11967–11975.
 18. Woodcock E.A., Matkovich S.J. 2005. Ins(1,4,5)P₃ receptors and inositol phosphates in the heart-evolutionary artefacts or active signal transducers? *Pharmacol. Ther.* 107(2): 240–251.
 19. Greiser M. 2017. Calcium signalling silencing in atrial fibrillation. *J. Physiol.* 595 (12): 4009–4017.
 20. Foskett J.K., White C., Cheung K.H., Mak D.O. 2007. Inositol trisphosphate receptor Ca²⁺ release channels. *Physiol. Rev.* 87: 593–658.
 21. Lanner J.T., Georgiou D.K., Joshi A.D., Hamilton S.L. 2010. Ryanodine receptors: Structure, expression, molecular details and function in calcium release. *Cold Spring Harb. Perspect. Biol.* 2 (11): a003996.
 22. Mak D.O., Foskett J.K. 2015. Inositol 1,4,5-trisphosphate receptors in the endoplasmic reticulum: A single-channel point of view. *Cell Calcium.* 58: 67–78.
 23. Grayson T.H., Haddock R.E., Murray T.P., Wojcikiewicz R.J., Hill C.E. 2004. Inositol 1,4,5-trisphosphate receptor subtypes are differentially distributed between smooth muscle and endothelial layers of rat arteries. *Cell Calcium.* 36: 447–458.
 24. Narayanan D., Adebisi A., Jaggar J.H. 2012. Inositol trisphosphate receptors in smooth muscle cells. *Am. J. Physiol. Heart. Circ. Physiol.* 302 (11): H2190–H2210.
 25. Meissner G. 2017. The structural basis of ryanodine receptor ion channel function. *J. Gen. Physiol.* 149 (12): 1065–1089.

26. Zhao G., Adebisi A., Blaskova E., Xi Q., Jaggar J.H. 2008. Type 1 inositol 1,4,5-trisphosphate receptors mediate UTP-induced cation currents, Ca²⁺ signals, and vasoconstriction in cerebral arteries. *Am. J. Physiol. Cell Physiol.* 295: C1376–C1384.
27. Serysheva I.I. 2014. Toward a high-resolution structure of IP₃R channel. *Cell Calcium.* 56 (3): 125–132.
28. Rossi A.M., Taylor C.W. 2004. Ca²⁺ regulation of inositol 1,4,5-trisphosphate receptors: Can Ca²⁺ function without calmodulin? *Mol. Pharmacol.* 66 (2): 199–203.
29. Prole D.L., Taylor C.W. 2019. Structure and Function of IP₃ Receptors. *Cold Spring Harb. Perspect. Biol.* 11 (4): a035063.
30. Seo M.D., Enomoto M., Ishiyama N., Stathopoulos P.B., Ikura M. 2015. Structural insights into endoplasmic reticulum stored calcium regulation by inositol 1,4,5-trisphosphate and ryanodine receptors. *Biochim. Biophys. Acta.* 1853 (9): 1980–1991.
31. Amador F.J., Stathopoulos P.B., Enomoto M., Ikura M. 2013. Ryanodine receptor calcium release channels: Lessons from structure-function studies. *FEBS J.* 280 (21): 5456–5470.
32. Van Petegem F. 2015. Ryanodine receptors: Allosteric ion channel giants. *J. Mol. Biol.* 427: 31–53.
33. Dobrev D., Wehrens X.H. 2014. Role of RyR2 phosphorylation in heart failure and arrhythmias: Controversies around ryanodine receptor phosphorylation in cardiac disease. *Circ. Res.* 114 (8): 1311–1319.
34. Abou-Saleh H., Pathan A.R., Daalis A., Hubrack S., Abou-Jassoum H., Al-Naeimi H., Rusch N.J., Machaca K. 2013. Inositol 1,4,5-trisphosphate (IP₃) receptor up-regulation in hypertension is associated with sensitization of Ca²⁺ release and vascular smooth muscle contractility. *J. Biol. Chem.* 288 (46): 32941–32951.
35. Zhao G., Neeb Z.P., Leo M.D., Pachuau J., Adebisi A., Ouyang K., Chen J., Jaggar J.H. 2010. Type 1 IP₃ receptors activate BKCa channels via local molecular coupling in arterial smooth muscle cells. *J. Gen. Physiol.* 136: 283–291.
36. Tasker P.N., Michelangeli F., Nixon G.F. 1999. Expression and distribution of the type 1 and type 3 inositol 1,4,5-trisphosphate receptor in developing vascular smooth muscle. *Circ. Res.* 84: 536–542.
37. Tasker P.N., Taylor C.W., Nixon G.F. 2000. Expression and distribution of InsP(3) receptor subtypes in proliferating vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 273: 907–912.
38. Mendes C.C., Gomes D.A., Thompson M., Souto N.C., Goes T.S., Goes A.M., Rodrigues M.A., Gomez M.V., Nathanson M.H., Leite M.F. 2005. The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca²⁺ signals into mitochondria. *J. Biol. Chem.* 280 (49): 40892–40900.
39. Marchi S., Marinello M., Bononi A., Bonora M., Giorgi C., Rimessi A., Pinton P. 2012. Selective modulation of subtype III IP₃R by Akt regulates ER Ca²⁺ release and apoptosis. *Cell Death. Dis.* 3 (5): e304.
40. Guerrero-Hernández A., Gómez-Viquez L., Guerrero-Serna G., Rueda A. 2002. Ryanodine receptors in smooth muscle. *Front. Biosci.* 7: d1676-1688.
41. Krishnamoorthy G., Sonkusare S.K., Heppner T.J., Nelson M.T. 2014. Opposing roles of smooth muscle BK channels and ryanodine receptors in the regulation of nerve-evoked constriction of mesenteric resistance arteries. *Am. J. Physiol. Heart Circ. Physiol.* 306 (7): H981–H988.
42. Van Petegem F. 2012. Ryanodine receptors: Structure and function. *J. Biol. Chem.* 287 (38): 31624–31632.
43. de Lucia C., Eguchi A., Koch W.J. 2018. New insights in cardiac β-adrenergic signaling during heart failure and aging. *Front. Pharmacol.* 9: 904.
44. Hamilton S., Terentyev D. 2019. Altered intracellular calcium homeostasis and arrhythmogenesis in the aged heart. *Int. J. Mol. Sci.* 20 (10): E2386.
45. Federico M., Valverde C.A., Mattiazzi A., Palomeque J. 2020. Unbalance between sarcoplasmic reticulum Ca²⁺ uptake and release: A first step toward Ca²⁺ triggered arrhythmias and cardiac damage. *Front. Physiol.* 10, 1630.
46. Tellez J.O., Mczewski M., Yanni J., Sutyagin P., Mackiewicz U., Atkinson A., Inada S., Beresewicz A., Billeter R., Dobrzynski H., Boyett M.R. 2011. Ageing-dependent remodelling of ion channel and Ca²⁺ clock genes underlying sino-atrial node pacemaking. *Exp. Physiol.* 96 (11): 1163–1178.

47. Ruiz-Hurtado G., Morel E., Domínguez-Rodríguez A., Llach A., Lezoualc'h F., Benitah J.P., Gomez A.M. 2013. Epac in cardiac calcium signaling. *J. Mol. Cell. Cardiol.* 58: 162–171.
48. Marx S.O., Marks A.R. 2013. Dysfunctional ryanodine receptors in the heart: New insights into complex cardiovascular diseases. *J. Mol. Cell. Cardiol.* 58: 225–231.
49. Houser S.R. 2014. Role of RyR2 phosphorylation in heart failure and arrhythmias: Protein kinase A-mediated hyperphosphorylation of the ryanodine receptor at serine 2808 does not alter cardiac contractility or cause heart failure and arrhythmias. *Circ. Res.* 114 (8): 1320–1327.
50. Pereira L., Cheng H., Lao D.H., Na L., van Oort R.J., Brown J.H., Wehrens X.H., Chen J., Bers D.M. 2013. Epac2 mediates cardiac β 1-adrenergic-dependent sarcoplasmic reticulum Ca^{2+} leak and arrhythmia. *Circulation.* 127 (8): 913–922.
51. Schmidt M., Dekker F.J., Maarsingh H. 2013. Exchange protein directly activated by cAMP (epac): A multidomain cAMP mediator in the regulation of diverse biological functions. *Pharmacol. Rev.* 65 (2): 670–709.
52. Pereira L., Bare D.J., Galice S., Shannon T.R., Bers D.M. 2017. β -Adrenergic induced SR Ca^{2+} leak is mediated by an Epac-NOS pathway. *J. Mol. Cell. Cardiol.* 108: 8–16.
53. Urrutia J., Aguado A., Muguruza-Montero A., Núñez E., Malo C., Casis O., Villarroel A. 2019. The crossroad of ion channels and calmodulin in disease. *Int. J. Mol. Sci.* 20 (2): E400.
54. Zhou X., Sun F., Luo S., Zhao W., Yang T., Zhang G., Gao M., Lu R., Shu Y., Mu W., Zhuang Y., Ding F., Xu C., Lu Y. 2017. Let-7a is an antihypertrophic regulator in the heart via targeting calmodulin. *Int. J. Biol. Sci.* 13 (1): 22–31.
55. Arnáiz-Cot J.J., Damon B.J., Zhang X.H., Cleemann L., Yamaguchi N., Meissner G., Morad M. 2013. Cardiac calcium signalling pathologies associated with defective calmodulin regulation of type 2 ryanodine receptor. *J. Physiol.* 591 (17): 4287–4299.
56. Yamaguchi N., Chakraborty A., Huang T-Q., Xu L., Gomez A.C., Pasek D.A., Meissner G. 2013. Cardiac hypertrophy associated with impaired regulation of cardiac ryanodine receptor by calmodulin and S100A1. *Am. J. Physiol. Heart Circ. Physiol.* 305 (1): H86–H94.
57. Lipp P., Laine M., Tovey S.C., Burrell K.M., Berridge M.J., Li W., Bootman M.D. 2000. Functional InsP_3 receptors that may modulate excitation-contraction coupling in the heart. *Curr. Biol.* 10 (15): 939–942.
58. Kockskämper J., Zima A.V., Roderick H.L., Pieske B., Blatter L.A., Bootman M.D. 2008. Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. *J. Mol. Cell. Cardiol.* 45 (2): 128–147.
59. Kim J.C., Son M.J., Subedi K.P., Li Y., Ahn J.R., Woo S.H. 2010. Atrial local Ca^{2+} signaling and inositol 1,4,5-trisphosphate receptors. *Prog. Biophys. Mol. Biol.* 103 (1): 59–70.
60. Zima A.V., Blatter L.A. 2004. Inositol-1,4,5-trisphosphate-dependent Ca^{2+} signalling in cat atrial excitation-contraction coupling and arrhythmias. *J. Physiol.* 555 (3): 607–615.
61. Ju Y.K., Woodcock E.A., Allen D.G., Cannell M.B. 2012. Inositol 1,4,5-trisphosphate receptors and pacemaker rhythms. *J. Mol. Cell. Cardiol.* 53 (3): 375–381.
62. Kapoor N., Tran A., Kang J., Zhang R., Philipson K.D., Goldhaber J.I. 2015. Regulation of calcium clock-mediated pacemaking by inositol-1,4,5-trisphosphate receptors in mouse sinoatrial nodal cells. *J. Physiol.* 593 (12): 2649–2663.
63. Nakayama H., Bodi I., Maillet M., De Santiago J., Domeier T.L., Mikoshiba K., Lorenz J.N., Blatter L.A., Bers D.M., Molkentin J.D. 2010. The IP_3 receptor regulates cardiac hypertrophy in response to select stimuli. *Circ. Res.* 107 (5): 659–666.
64. Wu X., Zhang T., Bossuyt J., Li X., McKinsey T.A., Dedman J.R., Olson E.N., Chen J., Brown J.H., Bers D.M. 2006. Local InsP_3 -dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J. Clin. Invest.* 116 (3): 675–682.
65. Kockskämper J., Seidlmayer L., Walther S., Hellenkamp K., Maier L.S., Pieske B. 2008. Endothelin-1 enhances nuclear Ca^{2+} transients in atrial myocytes through $\text{Ins}(1,4,5)\text{P}_3$ -dependent Ca^{2+} release from perinuclear Ca^{2+} stores. *J. Cell. Sci.* 121 (2): 186–195.

66. Ljubojevic S., Radulovic S., Leitinger G., Sedej S., Sacherer M., Holzer M., Winkler C., Pritz E., Mittler T., Schmidt A., Sereinigg M., Wakula P., Zissimopoulos S., Bisping E., Post H., Marsche G., Bossuyt J., Bers D.M., Kockskämper J., Pieske B. 2014. Early remodeling of perinuclear Ca²⁺ stores and nucleoplasmic Ca²⁺ signaling during the development of hypertrophy and heart failure. *Circulation*. 130 (3): 244–255.
67. Echevarría W., Leite M.F., Guerra M.T., Zipfel W.R., Nathanson M.H. 2003. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. *Nat. Cell. Biol.* 5 (5): 440–446.
68. Ljubojevic S., Bers D.M. 2015. Nuclear calcium in cardiac myocytes. *J. Cardiovasc. Pharmacol.* 65 (3): 211–217.
69. Zima A.V., Bare D.J., Mignery G.A., Blatter L.A. 2007. IP₃-dependent nuclear Ca²⁺ signalling in the mammalian heart. *J. Physiol.* 584 (2): 601–611.
70. Bossuyt J., Helmstadter K., Wu X., Clements-Jewery H., Haworth R.S., Avkiran M., Martin J.L., Pogwizd S.M., Bers D.M. 2008. Ca²⁺/calmodulin-dependent protein kinase I δ and protein kinase D overexpression reinforce the histone deacetylase 5 redistribution in heart failure. *Circ. Res.* 102 (6): 695–702.
71. Liang X., Xie H., Zhu P.H., Hu J., Zhao Q., Wang C.S., Yang C. 2009. Enhanced activity of inositol-1,4,5-trisphosphate receptors in atrial myocytes of atrial fibrillation patients. *Cardiology*. 114 (3): 180–191.
72. Yamada J., Ohkusa T., Nao T., Ueyama T., Yano M., Kobayashi S., Hamano K., Esato K., Matsuzaki M. 2002. Up-regulation of inositol 1,4,5-trisphosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. *J. Cardiol.* 39: 57–58.
73. Mangla A., Guerra M.T., Nathanson M.H. 2020. Type 3 inositol 1,4,5-trisphosphate receptor: A calcium channel for all seasons. *Cell Calcium*. 85: 102132.
74. Wei W., Huang W., Yue J. 2016. Requirement of IP₃ receptor 3 (IP₃R3) in nitric oxide induced cardiomyocyte differentiation of mouse embryonic stem cells. *Exp. Cell Res. Actions*. 346 (1): 9–16.

Figures

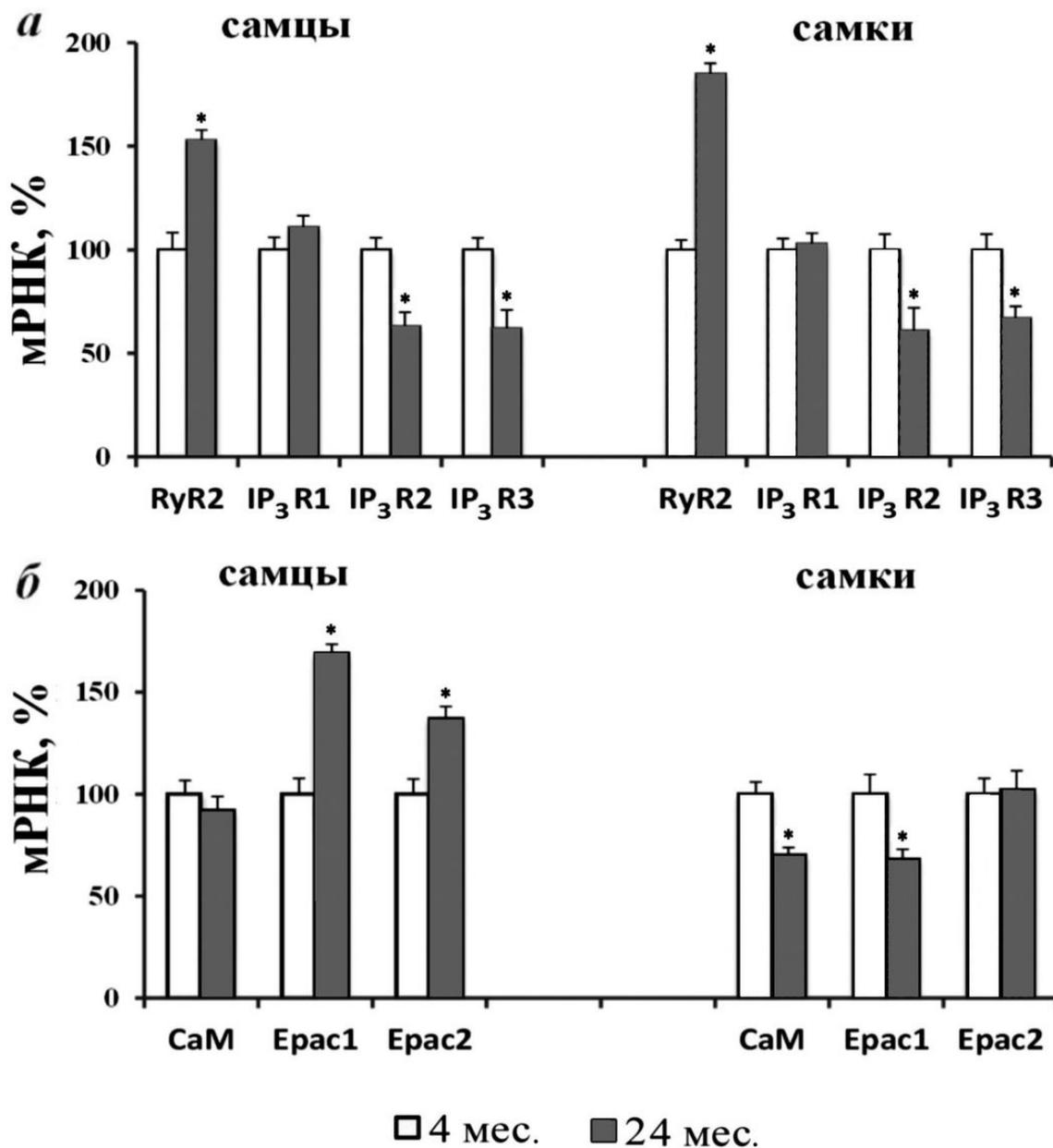


Figure 1

to the article by L.M. Kozhevnikova and al. Age-related changes in the level of mRNA of ryanodine (RyR2), inositol-1,4,5-trisphosphate receptors (IP3R1, IP3R2, IP3R3) (a), calmodulin (CaM) and proteins Epac1, Epac2 (b) in the aorta of male and female rats. The expression values for each studied gene were normalized to the expression value of the reference gene, β -actin, in the same tissue sample. The mRNA content of each test protein is expressed as a percentage of the average value of the same protein content in the group of young male or female rats, taken as 100%. * - $p < 0.01$ compared with rats aged 4 months.

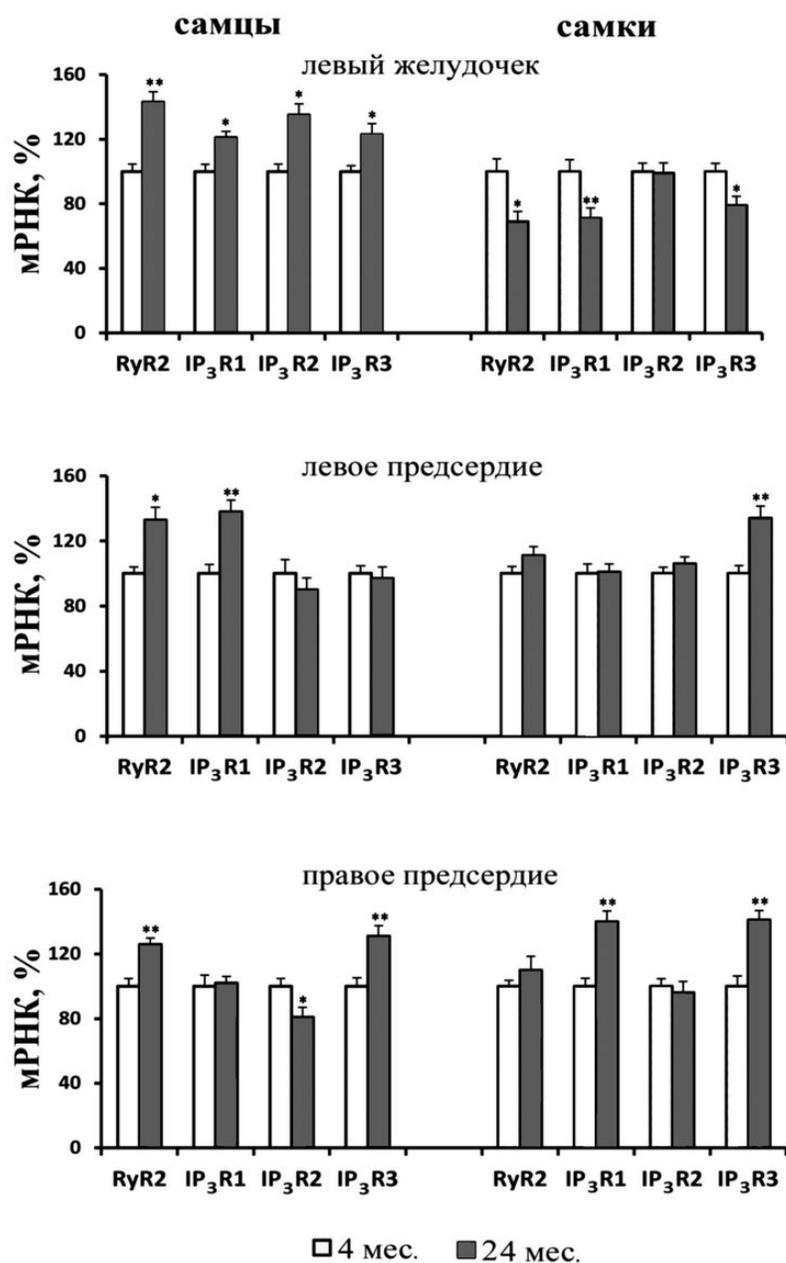


Figure 2

to the article by L.M. Kozhevnikova and al. Age-related changes in the level of mRNA of ryanodine (RyR2) and Inositol-1,4,5-trisphosphate receptors (IP3R1, IP3R2, IP3R3) in the left ventricle, left and right atria of the heart of male and female rats. The expression values for each studied gene were normalized to the expression value of the reference gene – β -actin in the same tissue sample. The mRNA content of each test protein is expressed as a percentage of the average content of the same protein in a group of young male or female rats, taken as 100%. * – $p < 0.05$; ** - $p < 0.01$ compared to rats aged 4 months.

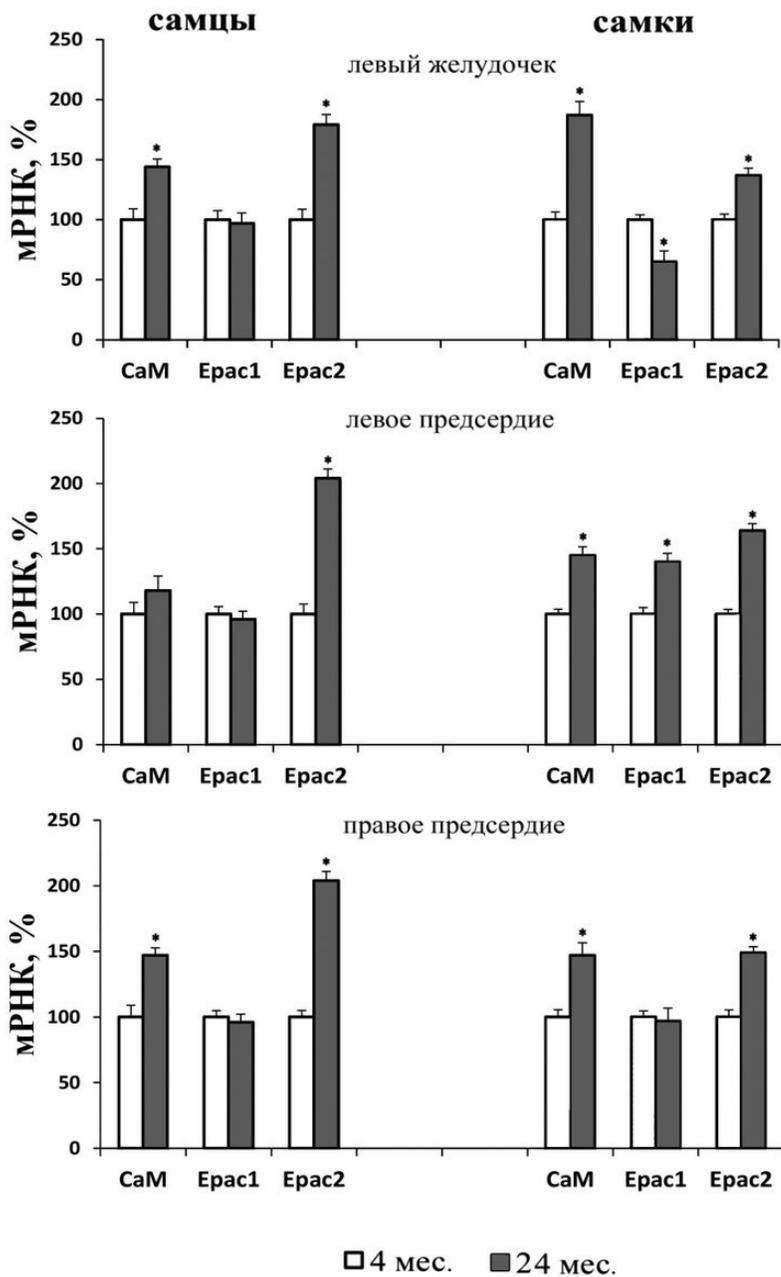


Figure 3

to the article by L.M. Kozhevnikova and al. Age-related changes in the level of calmodulin mRNA (CaM) and Epac1 and Epac2 proteins in the left ventricle, left and right atria of the heart of male and female rats. The expression values for each studied gene were normalized to the expression value of the reference gene- β -actin in the same tissue sample. The mRNA content of each test protein is expressed as a percentage of the average value of the same protein in a group of young male or female rats, taken as 100%. * $p < 0.01$ compared to rats at the age of 4 months.