©Borgis

*Urszula Mackiewicz, Joanna Kołodziejczyk, Bohdan Lewartowski

Cellular mechanisms of diastolic dysfunction in the heart failure

Komórkowe mechanizmy zaburzeń rozkurczu w niewydolności serca

Department of Clinical Physiology, Medical Center of Postgraduate Education, Warszawa
Head of Department: prof. Andrzej Beręsewicz, MD, PhD

Key words

heart failure, diastolic dysfunction, Ca^{2+} handling, titin, extracellular matrix

Słowa kluczowe

niewydolność serca, zaburzenia rozkurczu, obieg Ca^{2+}, titina, macierz zewnątrzkomórkowa

S u m m a r y

The relaxation of cardiac muscle is the complicated process comprising two stages: active and passive. Active part of relaxation includes ATP-dependent Ca^{2+} removal from the cytoplasm and dissociation of Ca^{2+} from contractile apparatus followed by separation of contractile proteins – myosin and actin. Ca^{2+} removal from the cytoplasm depends on activity and expression of the two main Ca^{2+} transporters: sarcoplasmic reticulum Ca^{2+}-ATPase and located in extracellular membrane Na^{+}/Ca^{2+} exchanger. The rate of Ca^{2+} dissociation from the contractile apparatus is determined by Ca^{2+} sensitivity of troponin C, which, in turn, depends on the phosphorylation level of troponin I. Passive stage of relaxation process comprises the restoration of cardiomyocyte resting length and subsequent cardiomyocyte stretching through blood flowing into the heart. These processes are determined by the stiffness of titin, the important cytoskeletal protein, as well as by the collagen composition of the extracellular matrix. The titin stiffness is regulated by the phosphorylation level of its elastic domains. Detailed investigations on the cardiomyocyte and the tissue levels reveled disturbances at the each stage of relaxation process in the heart failure patients. It is especially truth for patients with preserved ejection fraction and the diastolic dysfunction as a dominant contributor to symptoms. The effective therapy of these patients requires improvement of diastolic function throughout targeting disturbed stages of the relaxation process.

S t r e s z c z e n i e

Rozkurcz mięśnia sercowego jest złożonym kilkuetapowym procesem. W jego przebiegu wyróżniamy rozkurcz czynny i rozkurcz bierny. Rozkurcz czynny obejmuje usuwanie jonów Ca^{2+} z cytoplazmy i odłączenie się jonów Ca^{2+} od aparatu kurczliwego, po którym następuje separacja białek kurczliwych – miozyny i aktyny. Szybkość usuwania jonów Ca^{2+} z cytoplazmy zależy od aktywności i ekspresji ATP-azy wapniowej siateczki sarkoplazmatycznej oraz zlokalizowanego w błonie komórkowej wymiennika Na^{+}/Ca^{2+}. Z kolei odłączenie Ca^{2+} od aparatu kurczliwego zależy od wrażliwości troponiny C na jony Ca^{2+}, która jest regulowana przez stopień ufosforylowania troponiny I. Rozkurcz bierny obejmuje przywrócenie długości spoczynkowej kardiomiocytów oraz ich spoczynkowe rozciąganie kardiomiocytów przez napływającą do serca krew. O sprawności tego etapu decydują sztywność białka cytoszkieletu – titiny, zależna od fosforylacji jej sprężystych domen, oraz skład kolagenowy macierzy zewnątrzkomórkowej oplatającej kardiomiocyty. Szczegółowe badania na poziomie komórkowym pokazują, że każdy z wymienionych etapów rozkurczu może być zaburzony w niewydolności serca. Dotyczy to przede wszystkim chorych z rozkurczową postacią niewydolności serca, u których frakcja wyrzucania lewej komory jest zachowana, a objawy są wynikiem zaburzeń rozkurczu. Skuteczna terapia tych pacjentów wymaga znalezienia metod korekty zaburzonych etapów rozkurczu.

Address/adres:

*Urszula Mackiewicz
Department of Clinical Physiology
Medical Center of Postgraduate Education
ul. Marymoncka 99/103, 01-813 Warszawa
tel. +48 (22) 569-38-42
urszula.mackiewicz@cmkp.edu.pl

INTRODUCTION

Heart failure (HF) in the developed countries is diagnosed in 0.2% of population between 35 and 64 years of age. The morbidity increases with age and in the group of people over 80 exceeds 10%. The most frequent cause of HF (about 70% of cases) is ischemic heart disease, including myocardial infarction and hypertension (1-3).

HF and its symptoms, such as dyspnea, exercise intolerance and edema, were for many years connected

to impaired systolic function of the left ventricle (LV) and inadequate organ perfusion. Lately, however, it has been highlighted that almost always systolic dysfunction is **accompanied by diastolic disturbances.** Moreover, in over 50% of patients with symptomatic HF there are only diastolic dysfunction and ejection fraction (EF) is preserved or slightly lowered (EF > 45-50%). The research showed that diastolic dysfunction is more correlated with severity of HF symptoms and patients' hospitalization than systolic dysfunction. Isolated diastolic dysfunction in HF occur more often in older people, in patients with hypertension, obese patients and patients with type II diabetes (4-6).

Apart from intensive therapy based on multicenter studies long term prognosis in patient with HF is poor (over 50% of patient die in 4 years) (7). Ineffectiveness of treatment concerns, first of all, patients with diastolic dysfunction and preserved EF. In the last few decades survival in this group of patients did not improve (8).

One of the causes of such situation may be the fact that practically in all multicenter trials, testing effectiveness of HF therapy, patients with isolated diastolic dysfunction (FW > 45-50%) were not included in the studies.

Only a few trials were planned for patients with isolated diastolic dysfunction. They revealed that in patients with preserved EF ACE inhibitors, beta-blockers and aldosterone receptor antagonist – spironolacton did not reduce the mortality and HF hospitalization (9-13). On the other hand, statins significantly reduced mortality in patients with HF and preserved EF and did not in patients with lowered EF (14, 15). The only study which showed that the tested intervention was equally effective in patient with preserved or only mildly decreased EF and with significantly lowered EF was the study with third generation beta-blocker – nebivolol (16). The results of the above mentioned studies suggest that treatment bringing benefits to patients with lowered EF is not effective in patient with isolated diastolic dysfunction and that the pathomechanism of these two HF types is different.

The amount of patients with HF will increase due to lengthening of life time and higher survival of patients with myocardial infarction. Due to community ageing and obesity epidemic there will probably be higher percentage of patients with isolated diastolic dysfunction.

Finding new effective methods of treatment aimed to improve diastolic function of LV requires deep understanding of the pathomechanism of diastolic disturbances in HF.

During diastole cardiac muscle relax. Cardiac muscle relaxation is a complex multistage process. In its course there is an active and passive component. The active relaxation is an energy consuming process comprising ATP – dependent elimination of Ca^{2+} from cytoplasm, and Ca^{2+} disconnecting from the contractile apparatus which results disconnecting between the main contractile proteins – myosin and actin. Passive relaxation involves restitution of resting sarcomere length thanks to elastic recoil generated by cytoskeleton protein titin and resting stretching of cardiomyocytes by blood flowing into the heart. The efficiency of this phase is determined by susceptibility to stretch of the titin and extracellular matrix surrounding cardiomyocytes (fig. 1) (17). **Detailed research on the cellular level of heart muscle show that each of the mentioned stages of relaxation process can be disturbed in HF. Modern therapy of patients with HF should be directed to improvement of both relaxation stages.**

DISTURBANCES OF ACTIVE RELAXATION PROCESS IN HEART FAILURE

Intracellular Ca^{2+} handling

The first stage of relaxation process, called active diastole, begins with Ca^{2+} ions elimination from cytoplasm. Before contraction there is an increase in the concentration of Ca^{2+} ions in cytoplasm (from about 0.1 μM to 1 μM). This increase is an effect of Ca^{2+} influx from the extracellular space through voltage activated L type calcium channels. This influx opens calcium channels of the sarcoplasmatic reticulum (SR), also called ryanodine receptors (RyRs). This phenomenon is called calcium induced calcium release (fig. 2). When Ca^{2+} concentration in cytoplasm increases over 1 μM contractile apparatus protein, troponin C, binds Ca^{2+} ions. After binding Ca^{2+} troponin C changes it's conformation which enables interaction between main contractile proteins: myosin and actin, slipping of actin filaments between myosin ones and as a result cell shortening (contraction) (fig. 2).

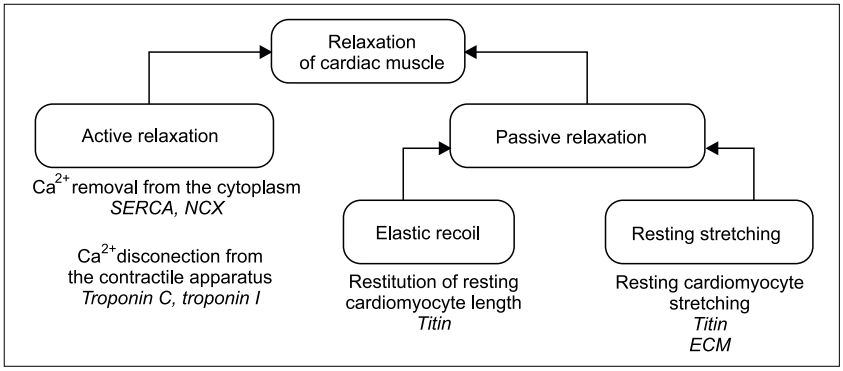

Fig. 1. Stages of cardiac muscle relaxation.
SERCA – sarco-endoplasmic reticulum calcium ATPase; NCX – Na^{+}/Ca^{2+} exchanger; ECM – extracellular matrix

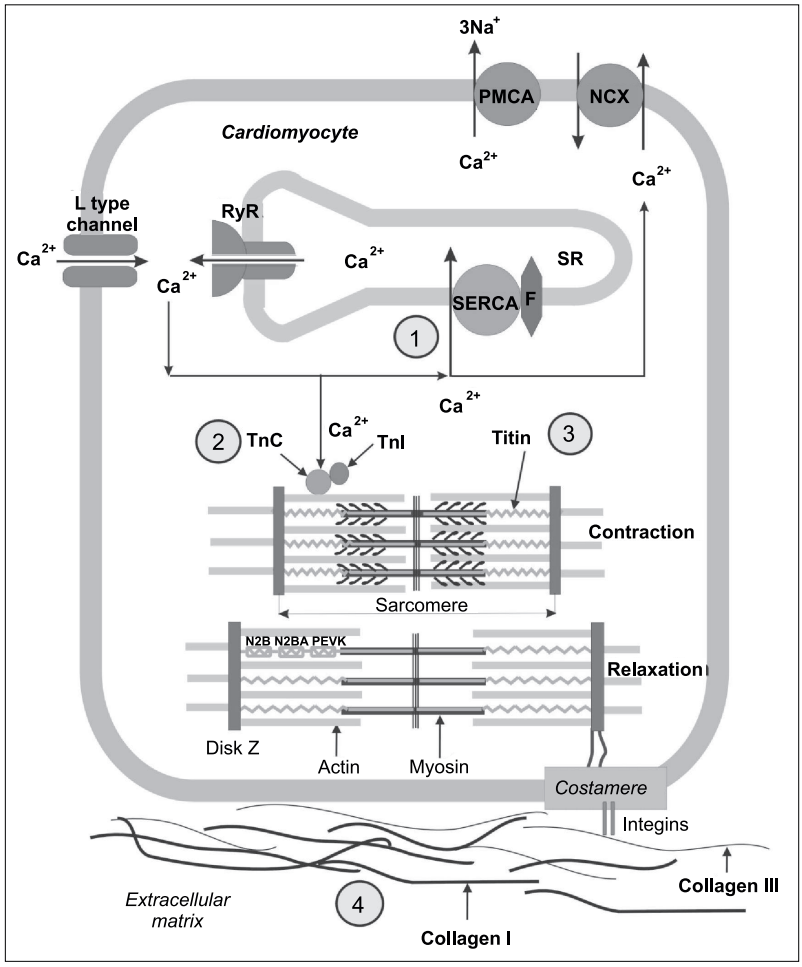

Fig. 2. Cellular mechanisms of cardiac muscle relaxation. After contraction Ca^{2+} ions are transported to sarcoplasmatic reticulum (SR) by calcium ATPase (SERCA) regulated by fosfolamban (F) and removed to extracellular space by Na^+/Ca^{2+} exchanger (NCX) and membrane ATPase (PMCA) (1). As a result of the decrease of Ca^{2+} ions concentration in cytoplasm they are released from troponin C (2) which leads to disconnecting of constrictile proteins – actin and myosin. Energy collected in elastic domains (PEVK, N2B, N2BA) of titin molecule generates elastic recoil, which leads to resting sarcomere length restoration (3). Sarcomere stretching over its resting length depends on titin susceptibility to stretch (3) as well as the expression of collagen I and III in the extracellular matrix (4).

Cardiomyocyte relaxation requires Ca^{2+} removing from cytoplasm. It consecutively enables disconnecting of Ca^{2+} from troponin C, disconnecting actin and myosin and relaxation. Three ion transporters are responsible for removal of Ca^{2+} from the cytoplasm after contraction (1) located in SR membranes, sarco-endoplasmatic reticulum calcium ATPase (SERCA), which transports Ca^{2+} ions to SR, and (2) two proteins located in the cell membrane: Na^+/Ca^{2+} exchanger (NCX) and plasma membrane calcium ATPase (PMCA), which remove Ca^{2+} ions from the cell (fig. 2) (18).

In human cardiomyocytes SERCA is responsible for removal of about 70-80% of Ca^{2+} ions from the cytoplasm. That makes it a protein which determines the rate of active stage of relaxation process at the highest degree. Transport of Ca^{2+} ions to SR is energy consuming. SERCA uses about 15% of cardiomyocyte ATP. SERCA is inhibited by the protein localized in SR membranes – phospholamban. Phospholamban phosphorylation decrease SERCA inhibition. Phospholamban is phosphorylated by two kinases: protein kinase A (PKA) activated by stimulation of β-1 adrenergic receptors and by Ca^{2+} and calmodulin dependent kinase (CAMKII), activated by increase in the heart rate (which means also under catecholamine stimulation). In turn phosphatases PP1, PP2A and PP2B (calcineurin) are responsible for phospholamban dephosphorylation. Stimulation of Gq protein coupled receptors (such as angiotensin II or endothelin-1 receptors) or increase in wall stress in the heart muscle activates these phosphatases (19). The final level of phospholamban phosphorylation (and SERCA activity) depends on the resultant of kinases and phosphatases activity.

NCX localized in the cell membrane is the main route of Ca^{2+} eflux. It removes about 20% of Ca^{2+} ions activating contraction (PMCA is responsible for removal of about 1-4% of Ca^{2+} ions). NCX is not able to utilize ATP. It takes the energy required to Ca^{2+} transport from sodium gradient (during one Ca^{2+} ion removal from the cell three Na^+ ions are transported into the cell) (18). All factors leading to lowering transmembrane sodium gradient (such as inhibition of Na^+/K^+ pump by digitalis glycosides or ion imbalances like hypokalaemia

or hypomagnesaemia inhibit NCX activity and lead to intracellular Ca^{2+} accumulation).

SERCA activity and at a lower degree NCX determine the rate of Ca^{2+} ions removal from cytoplasm and thus the rate of their disconnecting from troponin C. This last process is additionally regulated by troponin C sensitivity to Ca^{2+} ions, which depends on the level of troponin I phosphorylation. This subunit of troponin is phosphorylated by three kinases: by PKA (similarly as phospholamban), by activated by nitric oxide (NO) and natriuretic peptydes protein kinase G (PKG) and by protein kinase C (PKC) activated under activation of Gq protein coupled receptors troponin I phosphorylation by PKA and PKG lowers troponin C sensitivity to Ca^{2+} ions which makes relaxation easier. On the other hand, phosphorylation by PKC has a reverse effect (20).

Summing up, the rate of the active part of relaxation depends on the rate of Ca^{2+} ions removal from cytoplasm and on the rate of Ca^{2+} ions disconnecting from troponin C. The first process mostly depends on SERCA activity regulated by the level of phospholamban phosphorylation. The second one depends on the troponin C Ca^{2+} sensitivity, which is regulated by the level of troponin I phosphorylation.

Neurohumoral regulation of intracellular Ca^{2+} handling

Regulation of intracellular Ca^{2+} handling and thus cardiomyocytes contraction-relaxation cycle by the sympathetic nervous system is, beside Frank-Starling mechanism, a main mechanism adjusting heart muscle to increased work in conditions of physical and psychical stress (fight or flight response). Activation of sympathetic system causes noradrenalin release from the sympathetic nerve terminals and adrenalin from the medulla of adrenal glands. Catecholamines, by activating beta-adrenergic receptors (mainly beta-1), lead to, in turn, activation of Gs protein, adenylate cyclase synthetizing cAMP and to activation of cAMP-dependent PKA. In addition, catecholamines cause increase in the heart rate which leads to CaMKII activation (21).

Activation of PKA and CaMKII by phosphorylation of a few Ca^{2+} handling proteins leads to increase in contraction strength as well as the rate of cardiomyocytes relaxation. The objects of phosphorylation are: L type Ca^{2+} channels, RyRs, phospholamban and troponin I.

Phosphorylation of L type Ca^{2+} channels increases Ca^{2+} influx and the amount of Ca^{2+} ions released from SR. RyRs phosphorylation increases their sensitivity to Ca^{2+} and makes their opening easier. Phospholamban phosphorylation increases SERCA activity, Ca^{2+} ions transport to SR and the amount of Ca^{2+} collected in the SR. Troponin I phosphorylation decreases troponin C sensitivity to Ca^{2+} ions and makes their disconnecting from protein easier, which promote relaxation.

Higher Ca^{2+} influx, higher Ca^{2+} sensitivity of RyRs and higher activity of SERCA are the main elements of positive inotropic catecholamine effect. Increased SERCA activity and decrease in troponin C Ca^{2+} sensitivity make relaxation easier and are the main elements of positive lusitropic catecholamine effect (22).

Ca^{2+} handling disturbances in the heart failure

In HF, in animal models as well as in human explanted hearts, there are numerous disturbances of expression and function of proteins engaged in intracellular Ca^{2+} handling. The most frequent observation is the decrease in expression and activation of SERCA, excessive increase in RyRs Ca^{2+} sensitivity to and increase in NCX expression.

Decrease in SERCA expression in explanted hearts may even reach 50%. Moreover, there is also a decrease level of phospholamban phosphorylation and thus higher inhibition of SERCA (23, 24). Lowered level of phospholamban phosphorylation in HF is not fully understood. On the one hand, due to intensive catecholamine stimulation activation of PKA and CaMKII kinases increases, which should lead to increase in phospholamban phosphorylation. On the other hand, however, the increase in the level of angiotensin II results in AT1 receptors activation and subsequent PKC activation which is the main activator of PP1 phosphatase. Additionally, in HF (particularly in its diastolic form) activity of PP2B phosphatase (calcineurin) increases (25, 26). It seems that the resultant effect is the predominance of phosphatases effect over kinase, which leads to decrease in phospholamban phosphorylation, decrease in SERCA activity and finally lowering the rate of relaxation. In addition, the decrease in SE CA expression and activity leads to lowering of the $SERCA^{2+}$ content and to the decrease in contraction amplitude.

The following element of cellular remodeling in HF is the increase of RyRs Ca^{2+} sensitivity. It results in spontaneous opening of channels even at the resting Ca^{2+} concentration (so called Ca^{2+}-leak) (27). This phenomenon has a lot of detrimental effects. First of all, it leads to the increase in resting Ca^{2+} ions concentration in cytoplasm, which prevents complete disconnection of Ca^{2+} from troponin C and impede relaxation process. Secondly, the SR Ca^{2+} content decreases which results in lowering of contraction strength. Thirdly, diastolic Ca^{2+}-leak from SR has a pro-arrhythmic effect because it activates NCX. NCX generates depolarizing current that evokes afterdepolarizations, which may induce premature beats and arrhythmias (28). Increase in Ca^{2+}-leak is caused by excessive RyRs phosphorylation by PKA and CaMKII. In addition, this effect is intensified by disconnecting phosphatases from RyRs. This is a different situation than phospholamban, where active effect of phosphatases prevailed over kinase activity and decided about the decrease in its phosphorylation (29). These observations highlight the fact of a very complicated regulation of Ca^{2+}-handling proteins and complexity of their disorders in HF.

The next change in Ca^{2+} handling observed in HF is the increase in NCX expression. In extreme cases

in the explanted hearts there was an increase in the NCX expression even by 100%. It is probably a change that is aimed to compensate decrease in SERCA expression. However, this compensation is slight. The time constant of the rate of Ca^{2+} removal from the cytoplazm realized by NCX is about 8-10 times lower than by SERCA (30). In addition, the increase in NCX expression leads to decrease in contraction strength because Ca^{2+} ions transported by NCX leave the cell and do not enter SR.

The above disturbances in expression and activity of the main proteins involved in Ca^{2+} handling are the cause of cardiomycytes systolic as well as diastolic dysfunction in an filing heart.

The Ca^{2+} handling disorders in HF at least partly can be explained by the excessive catecholamine stimulation. One of the pathomechanism of HF are the disturbed sympathetic – parasympathetic balance. The activity of sympathetic system increases while parasympathetic one decreases. The level of catecholamines in the plasma of the patients with advanced HF increase even by 100% and in the heart muscle tissue even 50 times. Additionally, the increase in catecholamine level and beta-1 receptors activation in the juxtaglomerular complex cells of the kidneys leads to renin production and consequently increased production of angiotensin II and aldosterone (RAAS system activation) (31). In literature the fact that excessive catecholamine stimulation in HF, increasing the degree of RyRs phosphorylation, which leads to excessive RyRs activity and spontaneous release of Ca^{2+} ions from the SR is well documented (29, 32, 33). Beta-blockers lower the spontaneous Ca^{2+} release from the SR by lowering the degree of RyRs phosphorylation leading at the same time to the increase of SERCA expression (34). It is also proved that the increase in mRNA NCX expression correlates positively with the increase in catecholamine level in the blood of patient suffering from HF (35).

Ca^{2+} sensitivity of contractile apparatus in heart failure

Disturbances in activity and expression of Ca^{2+} handling proteins in HF lead to slower removal of Ca^{2+} ions from cytoplasm. Moreover, in patients with HF, particularly with its diastolic form, there is an increase in troponin C Ca^{2+} sensitivity, which additionally lowers the rate of disconnecting Ca^{2+} from troponin C and cardiomyocyte relaxation. The cause of increase in troponin C sensitivity to Ca^{2+} ions is a fall in the level of troponin I phosphorylation. In explanted hearts there is troponin I hypophosphorylation by PKA (probably like in case of low phospholamban phosphorylation which results from increased activity of phosphatases) and by PKG (20). In biopsies of patients with HF and preserved EF there is a decrease in PKG activity and an increase in sensitivity of contractile system to Ca^{2+} (36). Also in experimental model in dogs with hypertension and HF there is troponin I hypophosphorylation, decrease in PKG activity, increase in PKC and phosphatases activity and increase Ca^{2+} sensitivity of contractile system (37). It is probably a mechanism compensating the decrease in SR Ca^{2+} content. Indeed, the increase in troponin C sensitivity to Ca^{2+} allows better use of Ca^{2+} present in the cytoplasm before contraction (activity of levosimendan, medicine that makes troponin C more sensitive to Ca^{2+}, applied in acute HF, is based on the similar idea) (38). However, the adverse effect of this compensating mechanism is making release of Ca^{2+} from troponin C more difficult, which impairs relaxation.

Summing up, the decrease in SERCA expression and activity and spontaneous release of Ca^{2+} from SR by RyRs slows down their elimination from cytoplasm and impairs active relaxation of the heart muscle in HF. This effect is magnified by the increase in troponin C Ca^{2+} sensitivity.

DISTURBANCES OF PASSIVE RELAXATION PROCESS IN HEART FAILURE

The role of titin in passive relaxation

The second phase of relaxation process is a passive relaxation which is bringing back the resting length of sarcomeres after disconnecting myosin and actin. At this stage of relaxation the major role plays a sarcomere protein – titin. It is the biggest of the known proteins with molecular mass 3-4.2 MDa. One end of titin cell is located in Z disc and the other in M line in the center of sarcomere (fig. 2). In titin molecule there are two different functional parts: rigid, built from immunoglobin-like elements, binding with myosin and elastic – located between Z disc and the beginning of myosin filament, in which elastic domains are built in between rigid Ig elements. These are PEVK sequence, N2B domain and N2BA domain. In human heart there are two isoforms of titin: N2B isoform (does not contain N2BA domain) and N2BA isoform (containing three elastic domains). N2BA isoform thanks to additional elastic element is more susceptible to stretching and isoform N2B is more rigid. In a healthy heart the ratio of isoforms N2BA/N2B is about 0.6 (39, 40).

During contraction elastic domains of titin collect energy, which, after myosin and actin disconnection, is used to elastic recoil leading to sliding actin filaments out from between myosin ones, which restores resting sarcomere length (fig. 2). The strength of this recoil has a significant meaning for relaxation rate because during isovolumetric relaxation it produces suction force, which enables fast ventricles filling after the atrioventricular valves open. The recoil strength restoring the resting cardiomyocyte length is the bigger, and at the same time the relaxation faster, the more rigid titin is. Probably the more rigid isoform N2B (40%) is responsible for the proper recoil dynamics and a larger content of isoform N2BA (60%) makes cardiomyocytes more susceptible to resting stretching during blood inflow into ventricles.

The last stage of relaxation is passive cardiomyocytes stretching. When pressure in the ventricle falls down to the level of the pressure in the atria,

atrioventricular valves open and the phase of ventricular filling begins. Before ventricle contraction atrial muscles constrict and blood is forced into ventricles, which additionally stretches cardiomyocytes over their resting length. According to Frank-Sterling mechanism resting cardiomyocyte stretching before contraction allows optimal setting of actin and myosin filaments, which increases the range of cardiomyocyte shortening (constriction strength). **Ventricle muscle susceptibility to stretching during blood influx depends on titin susceptibility to stretching and on susceptibility of extracellular matrix, which from the outside restricts cardiomyocytes resting stretching** (41). The extracellular matrix composition and its role in the development of diastole disorders in HF are described in the following chapters.

Regulation of the mechanical titin properties

Titin stiffness and susceptibility to stretching depends on the level of phosphorylation of its elastic domains (N2B, N2BA i PEVK). Regulation of titin stiffness by phosphorylation of its domains is complex and involves four kinases. Some kinases phosphorylate two different domains in one titin molecule and each of these phosphorylations has an opposite effect on its stiffness. Biological meaning of some elements of this regulation is not fully understood. It is known that domains N2B and N2BA are phosphorylated by PKA and PKG, which, increase titin susceptibility to stretching. Additionally domains N2B and PEVK are phosphorylated by CaMKII, activated by the increase in the heart rate. Phosphorylation of N2B by CaMKII increases titin susceptibility and PEVK phosphorylation decreases it. PEVK domain is also phosphorylated by PKC, which decreases titin susceptibility (42). It seems that phosphorylation of N2B and N2BA domains is aimed to increase titin susceptibility and phosphorylation of PEVK sequence to make titin more rigid.

Physiological role of increase in titin susceptibility by PKA and CaMKII dependent phosphorylation of N2B and N2BA domains is clear. Increase in titin susceptibility makes diastolic filling easier, which is very significant during sympathetic activation and increase in the heart rate. Phosphorylation of N2B domain by PKG is probably one of the mechanisms (beside PKG dependent troponin I phosphorylation) which by the NO support relaxation in cardiomyocytes.

Whereas, functional role of lowering diastolic titin susceptibility by PKC and CaMKII dependent PEVK phosphorylation is not clear enough. Maybe phosphorylations increasing titin susceptibility to stretching are important to ensure cardiomyocytes susceptibility to resting stretching and those which increase titin stiffness are to preserve proper elastic recoil during passive relaxation. It is a fact that lower titin stiffness makes it more susceptible to stretching during ventricle filling but, on the other hand, it impairs the recoil during passive relaxation. Perhaps this complex pattern of phosphorylation in one titin molecule is aimed to realize its complex and dual function in the diastole.

Changes in titin expression and phosphorylation in heart failure

Elastic recoil during relaxation and cardiomyocyte susceptibility to stretching may be regulated as a result of change in proportion of titin isoforms in cardiomyocytes. It is a rather long process including days or weeks. In addition, a faster regulation is possible, which is a result of changes in the level of titin isoforms phosphorylation (40, 42).

In humans HF there are changes in titin isoforms proportion as well as in the level of their phosphorylation. In a healthy heart the isoforms ratio N2BA/N2B = 0.6. The pattern of changes in isoform proportion seems to depend on the type of HF. In patients with systolic HF N2BA/N2B ratio increases and reaches 0.65-1. It means that there is a relative increase in more susceptible isoform N2BA. It could indicate higher cardiomyocyte susceptibility to stretching in this group of patients; however, the examinations of isolated cardiomyocytes do not confirm that. Cardiomyocyte stiffness is higher and results probably from the decrease in the N2B domain phosphorylation and increase in PEVK domain phosphorylation. These changes in phosphorylation make the titin more stiff and they are responsible for decreased cardiomyocytes susceptibility to stretching in systolic HF (apart from changes in titin isoforms ratio in favor of more susceptible isoform) (43, 44).

In patients with diastolic HF N2BA/N2B ratio decreases and may reach even as low value as 0.2. It means that relative content of stiff isoform N2B increases. In addition, there are similar changes in titin phosphorylation like in systolic HF (increase in PEVK phosphorylation by PKC and CaMKII and decrease in N2B and N2BA phosphorylation by PKA and PKG) (45-47). These observations show that in patients with diastolic HF the change in expression of particular isoforms as well as decrease in their phosphorylation lower cardiomyocyte susceptibility to stretching. Detailed knowledge of titin phosphorylation pattern and its correction could be particularly beneficial in the group of patients with diastolic HF. N2B/N2BA phosphorylation by PKG deserves particular attention because its disturbances seem to play important role in HF pathophysiology, particularly in diastolic form. In HF an inflammation process and oxidative stress decrease NO bioavailability, which leads to decrease in PKG expression and N2B and N2BA domains phosphorylation. This leads to decrease in titin susceptibility and thus cardiomyocytes' (48). PKG is activated by cyclic GMP (cGMP). cGMP is synthesized by guanylate cyclase. Guanylate cyclase is activated by NO and natriuretic peptides. cGMP is degraded by phosphodiesterase type 5 (PDE5), which is inhibited by sildenafil. PDE5 inhibition causes increase in cGMP content in the cell, increase in PKG activity and N2B and N2BA domains phosphorylation. This may lead to increase in diastolic cardiomyocytes susceptibility to stretching (49). In addition, sildenafil may increase troponin I phosphorylation by PKG and by that lower troponin C Ca^{2+} sensitivity.

PDE5 inhibition with sildenafil for the first time enabled integration in diastolic susceptibility of the left ventricle in clinical conditions. Guazzi et al. published very good results of a year-therapy with sildenafil in patients with diastolic HF and pulmonary hypertension (50). The key change was the improvement of diastolic susceptibility of the left ventricle and the quality of life. Larger, multicenter research of sildenafil effectiveness in diastolic HF (RELAX) did not bring such promising results. The authors did not find differences between the groups of patients with diastolic HF treated by sildenafil and placebo. There was no improvement either in the quality of life or in the diastolic parameters of the left ventricle (50). Further research on large groups of patients is necessary to determine whether sildenafil is effective in treatment of diastolic HF.

The role of extracellular matrix in passive relaxation

Titin susceptibility decides about passive cardiomyocytes susceptibility, and thus the whole heart muscle, to stretching by blood influx during diastolic filling. It was estimated that about 50% of passive tension generated in heart muscle during relaxation depends on titin and the rest 50% on collagen network of the extracellular matrix (ECM) (41). ECM is a mechanical support for cardiomyocytes. It holds them in the correct position, determines the shape and size of the heart. The matrix protects cardiomyocytes against excessive stretching during blood influx into the heart (52).

ECM is a network of connective tissue elements surrounding cardiomyocytes and connecting with their membranes through proteins called integrins (fig. 2). Thanks to that the contractile strength generated in the contractile apparatus is transferred to the matrix and the information about tension generated in the cardiac wall from the matrix to cardiomyocytes. Collagen fibers are the main component of ECM. Moreover ECM consists of elastin, fibronectin and laminin responsible, among others, for contact of ECM with cardiomyocyte integrins.

Collagen is synthesized by fibroblasts. Only 5 (I, III, IV, V and VI) out of 18 known isoforms are present in the heart muscle, while I and III dominate and are about ~75-80% and ~15-20% of the whole collagen respectively. Collagen I creates thick (50-150 nm) parallel fibers resistant to stretching and difficult to deform. Collagen III creates a net of thinner, more elastic fibers and more susceptible to stretching than collagen I. Collagen III is a main component of the part of ECM which directly contact with cardiomyocytes (about 60-70%). It means that this part of ECM is easily stretched during cardiomyocyte contraction and relaxation. On the other hand, structures of ECM surrounding bundles and layers of cardiomyocytes are built mainly from collagen I and thus more stiff, which ensures stability of the heart cavities geometry and makes moving of cardiomyocytes in the bundles and the bundles in layers impossible (52).

Fibroblasts synthesize collagen as well as proteolytic enzymes degrading collagen so called metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Total amount of collagen is a resultant of collagen synthesis by fibroblasts and the expression and activity of MMPs and TIMPs. Degradation dominates when activation and expression of MMPs increases relatively to activity and expression of TIMPs (53, 54). Balance between synthesis and degradation of collagen enables the process of ECM rebuilding in the course of myocardium growing and repair. The total amount of collagen and the collagen I to III ratio, particularly in the area of ECM surrounding cardiomyocytes, decide greatly about diastolic susceptibility of the heart muscle.

Extracellular matrix remodeling in heart failure

Remodeling of ECM in HF is a heterogeneous process and to a great extend depends on the type of destructive factors. In diastolic HF (often caused by pressure overload) there is mainly an increase in collagen synthesis. The amount and size of collagen fibers increase. There is also an increase in synthesis and activity of MMPs as well as their tissue inhibitors. However, finally the MMPs/TIMPs ratio for most MMPs is lowered (55-57). Thus accumulation of collagen exceeds its degradation. In patients with diastolic HF developing due to aorta stenosis collagen accumulation increases several times (56). Intensive fibrosis and thickening of collagen fibers concerns all parts of ECM also this surrounding vessels. It is accompanied by the increase in left ventricle and vessels' walls stiffness. Left ventricle function worsening and the decrease in coronary reserve correlate well with the increase in the total collagen content (58). The probable stimulus to increased synthesis and accumulation of collagen is the activation of neurohumoral systems, mainly RAAS system. ACE inhibitors, angiotensin II and aldosterone receptor antagonists decrease fibrosis and improve left ventricle function.

In the systolic HF, often caused by volume overload, the total content of collagen is often normal and the changes concern mainly its structure. In the total collagen amount relative content of elastic collagen III increases. Additionally, there are disturbances during collagen molecule linking during fibers creation (so called cross-linking). Thus collagen fibers (particularly type I) become less stiff. As a result of these changes the ECM elasticity increases, which favors ventricle dilation (59).

CONCLUSIONS

Cardiac muscle relaxation is a complex multistage process. It's rate depends on: (a) the rate of Ca^{2+} removal from cytoplasm after contraction, which is determined mainly by SERCA expression and activity, (b) troponin C Ca^{2+} sensitivity regulated by troponin I phosphorylation, (c) titin stiffness dependent on the phosphorylation of its elastic domains and (d) collagen expression and composition in extracellular matrix which is the resultant of synthesis and degradation intensity. Modern therapy

of HF, particularly its diastolic form, should be directed to improvement of all stages of the cardiac muscle relaxation process. The present therapy of HF, which involves drugs limiting the effects of excessive neurohormonal stimulation, is potentially directed to improvement of some stages of relaxation process.

Beta-blockers lowering phosphorylation of RyRs decrease spontaneous Ca^{2+} release from SR and diastolic Ca^{2+} concentration. There are also reports about improvement of SERCA function in patients treated with beta-blockers (60, 61). SERCA activity may be improved also under the treatment by ACE inhibitors or sartans which may prevent PP1 phosphatase activation and thus increase phospholamban phosphorylation (62). In addition, RAAS inhibitors decrease collagen synthesis increasing extracellular matrix susceptibility to stretching during diastole (63). Despite these facts currently applied treatment is not optimal for patients with diastolic HF. Some hopes in the treatment of this form of HF are laid in the interventions decreasing titin stiffness and lowering troponin C Ca^{2+} sensitivity by increasing titin and troponin I phosphorylation by PKG (50). In addition, there are researches upon more effective methods of Ca^{2+} handling improvement by direct increase in SERCA activity. There is ongoing trial on the effectiveness of a new drug (istaroxime), which is a direct SERCA activator independent from the level of phospholamban phosphorylation (64). Expectations are also connected with gene therapy, which is supposed to deliver a SERCA gene to cardiomyocytes by the viral vector, to increase the protein expression. Phase I/II of CUPID trial has just finished, in which a intracoronarily delivered SERCA gene was well tolerated and safe for the patients (65). There is phase II of this trial planned. In the pre-clinical phase there are promising studies on cardiomyocyte transfection with genes coding proteins increasing SERCA activity – S100A1 and SUMO-1 (66, 67).

It is possible that only effective improvement of SERCA, decrease in titin stiffness, lowering Ca^{2+} sensitivity of contractile apparatus together with anti-fibrosis interventions will bring improvement in treatment of diastolic dysfunction in HF.

BIBLIOGRAPHY

1. Cowie MR, Mostead A, Wood DA et al.: The epidemiology of heart failure. Eur Heart J 1997; 18: 208-225.
2. Dickstein K, Cohen-Solal A, Filippatos G et al.: ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J 2008; 29: 2388-2442.
3. Hole T, Hall C, Skjaerpe T: N-terminal proatrial natriuretic peptide predicts two-year remodelling in patients with acute transmural myocardial infarction. Eur Heart J 2004; 25: 416-423.
4. Paulus WJ, Tschope C, Sanderson JE et al.: How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007; 28: 2539-2550.
5. Gulec S, Ertas F, Tutar E et al.: Exercise performance in patients with dilated cardiomyopathy: relationship to resting left ventricular function. Int J Cardiol 1998; 65: 247-253.
6. Skaluba SJ, Litwin SE: Mechanisms of exercise intolerance: insights from tissue Doppler imaging. Circulation 2004; 109: 972-977.
7. Mosterd A, Hoes AW: Clinical epidemiology of heart failure. Heart 2007; 93: 1137-1146.
8. Owan TE, Hodge DO, Herges RM et al.: Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 2006; 355: 251-259.
9. Yusuf S, Pfeffer MA, Swedberg K et al.: Effects of candesartan in patients with chronic hear failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. Lancet 2003; 362: 777-781.
10. Cleland JG, Tendera M, Adamus J et al.: Perindopril for elderly people with chronic hearnfailure: the PEP-CHF study. The PEP investigators. Eur J Heart Fail 1999; 1: 211-217.
11. Lam CS, Carson PE, Anand IS et al.: Sex differences in clinical characteristics and outcomes in elderly patients with heart failure and preserved ejection fraction: the Irbesartan in Heart Failure with Preserved Ejection Fraction (I-PRESERVE) trial. Circ Heart Fail 2012; 5: 571-578.
12. Hernandez AF, Hammill BG, O'Connor CM et al.: Clinical effectiveness of beta-blockers in heart failure: findings from the OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure) Registry. J Am Coll Cardiol 2009 Jan 13; 53(2): 184-192.
13. Fukuta H, Sane DC, Brucks S, Littre WC: Statin Therapy May Be Associated With Lower Mortality in Patients With Diastolic Heart Failure: a preliminary raport. Circulation 2005; 112: 357-363.
14. Kjekshus J, Apetrei E, Barrios V et al.: Rosuvastatin in older patients with systolic heart failure. N Engl J Med 2007; 357: 2248-2261.
15. Edelmann F, Gelbrich G, Duvinage A et al.: Differential interaction of clinical characteristics with key functional parameters in heart failure with preserved ejection fraction – Results of the Aldo-DHF trial. Int J Cardiol 2013 Nov 30; 169(6): 408-417.
16. Ghio S, Magrini G, Serio A et al.: Effects of nebivolol in elderly heart failure patients with or without systolic left ventricular dysfunction: results of the SENIORS echocardiographic sub-placebo-controlled crossover study. Eur Heart J 2006; 27(5): 562-568.
17. Opie LH: Mechanisms of Cardiac Contraction and Relaxation. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 8th ed., Saunders Elsevier 2007.
18. Bers DM, Gua T: Calcium signaling in cardiac ventricular myocytes. Ann NY Acad Sci 2005: 1047: 86-98.
19. Neumann J, Eschenhagen T, Jones LR et al.: Increased expression of cardiac phosphatases in patients with end-stage heart failure. J Mol Cell Cardiol 1997; 29: 265-272.
20. Marston SB, de Tombe PP: Troponin phosphorylation and myofilament Ca^{2+}-sensitivity in heart failure: increased or decreased? J Mol Cell Cardiol 2008; 45: 603-607.
21. Mackiewicz U, Klemenska E, Beręsewicz A: Receptory beta-adrenergiczne w zdrowym i niewydolnym sercu. Kardiologia Polska 2007; 65: 294-302.
22. Lohse MJ, Engelhardt S, Eschenhagen T: What is the role of beta-adrenergic signaling in heart failure? Circ Res 2003; 93: 896-906.
23. Hasenfuss G, Pieske B: Calcium cycling in congestive heart failure. J Mol Cell Cardiol 2002; 34: 951-969.
24. Yano M, Ikeda Y, Matsuzaki M: Altered intracellular Ca^{2+} handling in hart failure. J Clin Invest 2005; 115: 556-564.
25. Huang B, Wang S, Qin D et al.: Diminished basal phosphorylation level of phospholamban in the postinfarction remodeled rat ventricle: role of beta-adrenergic pathway, G(i) protein, phosphodiesterase, phosphatases. Circ Res 1999; 85: 848-855.
26. Gupta RC, Mishra S, Rastogi S et al.: Cardiac SR-coupled PP1 activity and expression are increased and inhibitor 1 protein expression is decreased in failing hearts. Am J Physiol Heart Circ Physiol 2003; 285: H2373-H2381.
27. Marx SO, Marks AR: Dysfunctional ryanodine receptors in the heart: new insights into complex cardiovascular diseases. Mol Cell Cardiol 2013; 58: 225-231.
28. Nattel S, Maguy A, LeBouter S, Yeh Y-H: Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. Physiol Rev 2007; 87: 425-456.

29. Marx SO, Reiken S, Hisamatsu Y et al.: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 2000; 101: 365-376.
30. Mackiewicz U, Lewartowski B: Temperature dependent contribution of Ca^{2+} transporters to relaxation in cardiac myocytes: important role of sarcolemmal Ca^{2+}-ATPase. J Physiol Pharmacol 2006; 57: 3-15.
31. Rundqvist B, Elam M, Bergmann-Sverrisdottir Y et al.: Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. Circulation 1997 Jan 7; 95(1): 169-175.
32. Marks AR, Reiken S, Marx SO: Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? Circulation 2002; 105(3): 272-275.
33. Ai X, Curran JW, Shannon TR et al.: Ca^{2+}/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. Circ Res 2005; 97: 1314-1322.
34. Sun YL, Hu SJ, Wang LH et al.: Comparison of low and high doses of carvedilol on restoration of cardiac function and calcium-handling proteins in rat failing heart. Clin Exp Pharmacol Physiol 2005 Jul; 32(7): 553-560.
35. Schillinger W, Schneider H, Minami K et al.: Importance of sympathetic activation for the expression of Na^{+}-Ca^{2+} exchanger in end-stage failing human myocardium. Eur Heart J 2002 Jul; 23(14): 1118-1124.
36. van Heerebeek L, Hamdani N, Falcão-Pires I et al.: Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation 2012; 126(7): 830-839.
37. Hamdani N, Bishu KG, von Frieling-Salewsky M et al.: Deranged myofilament phosphorylation and function in experimental heart failure with preserved ejection fraction. Cardiovasc Res 2013; 97(3): 464-471.
38. Nawarskas JJ, Anderson JR: Levosimendan: a unique approach to the treatment of heart failure. Heart Dis 2002; 4(4): 265-271.
39. LeWinter MM, Granzier H: Cardiac Titin – A Multifunctional Giant. Circulation 2010 May 18; 121(19): 2137-2145.
40. Martin M, LeWinter MD, Granzier HL: Cardiac Titin and Heart Disease. Journal of Cardiovascular Pharmacology 2014 March; 63(3): 207-212.
41. Granzier HL, Irving TC: Passive tension in cardiac muscle: contribution of collagen, titin, microtubules and intermediate filaments. Biophys J 1996; 68: 1027-1044.
42. Hidalgo CG, Chung CS, Saripalli C et al.: The multifunctional Ca^{2+}/calmodulin-dependent proteinkinase II delta (CaMKII phosphorylates cardiac titin's spring elemets. J Mol Cell Cardiol 2013; 54: 90-97.
43. Neagoe C, Kulke M, del Monte F et al.: Titin isoform switch in ischemic human heart disease. Circulation 2002; 106: 1333-1341.
44. Nagueh SF, Shah G, Wu Y et al.: Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. Circulation 2004; 110: 155-162.
45. van Heerebeck L, Borbely A, Nissen HW et al.: Myocardial structure and function differ in systolic and diastolic heart failure. Circulation 2006; 113: 1966-1973.
46. Borbely A, van der Velden J, Papp Z et al.: Cardiomyocyte stiffness in diastolic heart failure. Circulation 2005; 111: 774-781.
47. Hamdani N, Krysiak J, Kreussner MM et al.: Crucial role for Ca^{2+}/calmodulin-dependent protein kinase-II in regulating diastolic stress of normal and failing hearts via titin phosphorylation. Circ Res 2013 Jan, ahead of print.
48. van Heerebeek L, Franssen CPM, Hamdani N et al.: Molecular and cellular basis for diastolic dysfunction. Curr Heart Fail Rep 2012; 9: 293-302.
49. Takimoto E: Cyclic GMP-dependent signaling in cardiac myocytes. Circulation J 2012; 76: 1819-1825.
50. Guazzi M, Vincenzi M, Arena R, Guazzi MD: Pulmonary hypertension in heart failure with preserved ejection raction: a target of phosphodisterase-5 inhibition in a 1-year study. Circulation 2011a; 124: 164-174.
51. Redfield MM, Chen HH, Borlaug BA et al.: Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction. A randomized clinical trial. JAMA 2013; 309: 1268-1277.
52. Clark KA, McElhinny AS, Beckerle MC, Gregorio CC: Striated muscle cytoarchitecture: an intricate web of form and function. Annu Rev Cell Dev Biol 2002; 18: 637-706.
53. LeGrice I, Pope A, Smaill B: The architecture of the heart: myocyte organization and the cardiac extracellular matrix. [In:] Villarreal FJ (ed.): Interstitial fibrosis in heart failure. NY: Springer, New York 2005: 5-21.
54. Spinale FG, Janicki JS, Zile MR: Membrane-Associated Matrix Proteolysis and Heart Failure. Circ Res 2013; 112: 195-208.
55. Marchesia C, Dentalia F, Nicolinia E et al.: Plasma levels of matrix metalloproteinases and their inhibitors in hypertension: a systematic review and meta-analysis. J Hypertension 2012; 30: 3-16.
56. Polyakova V, Hein S, Kostin S et al.: Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. J Am Coll Cardiol 2004; 44(8): 1609-1618.
57. Fielitz J, Leuschner M, Zurbrügg HR et al.: Regulation of matrix metalloproteinases and their inhibitors in the left ventricular myocardium of patients with aortic stenosis. J Mol Med 2004; 82(12): 809-820.
58. López B, González A, Querejeta R et al.: Alterations in the pattern of collagen deposition may contribute to the deterioration of systolic function in hypertensive patients with heart failure. J Am Coll Cardiol 2006 Jul 4; 48(1): 89-96. Epub 2006 Jun 12.
59. Mann DL, Spinale FG: Activation of matrix metalloproteinases in the failing human heart: breaking the tie that binds. Circulation 1998; 98(17): 1699-1702.
60. Reiken S, Wehrens XH, Vest JA et al.: Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. Circulation 2003; 107(19): 2459-2466.
61. Doi M, Yano M, Kobayashi S et al.: Propranolol prevents the development of heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. Circulation 2002; 105(11): 1374-1379.
62. Satoh S, Ueda Y, Suematsu N et al.: Beneficial effects of angiotensin--converting enzyme inhibition on sarcoplasmic reticulum function in the failing heart of the Dahl rat. Circ J 2003; 67(8): 705-711.
63. Lijnen PJ, Petrov VV: Role of intracardiac renin-angiotensin-aldosterone system in extracellular matrix remodeling. Methods Find Exp Clin Pharmacol 2003; 25(7): 541-564.
64. Shah SJ, Blair JE, Filippatos GS et al.: Effects of istaroxime on diastolic stiffness in acute heart failure syndromes: results from the Hemodynamic, Echocardiographic, and Neurohormonal Effects of Istaroxime, a Novel Intravenous Inotropic and Lusitropic Agent: a Randomized Controlled Trial in Patients Hospitalized with Heart Failure (HORIZON-HF) trial. Am Heart J 2009; 157: 1035-1041.
65. Jessup M, Greenberg B, Mancini D et al.: Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Investigators.Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca^{2+}-ATPase in patients with advanced heart failure. Circulation 2011 Jul 19; 124(3): 304-313.
66. Pleger ST, Shan C, Ksienzyk J et al.: Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. Sci Transl Med 2011; 3(92): 92-104.
67. Tilemann L, Lee A, Ishikawa K et al.: SUMO-1 Gene Transfer Improves Cardiac Function in a Large-Animal Model of Heart Failure. Sci Transl Med 2013, in press.

received/otrzymano: 09.04.2014
accepted/zaakceptowano: 03.06.2014