Posttranslational titin isoform modifications in perinatal diastolic dysfunction

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Abstract in English:

In newborns, the background of diastolic dysfunction (leading potentially to heart failure with preserved ejection fraction, HFPEF) is unknown. Diastolic heart failure emerges as disturbed coupling between cardiac ventricular relaxation and vascular compliance. Earlier, we have pointed out the significance of cardiomyocyte passive force increase (Fpassive = tension determined in Ca2+-free solution) in the pathogenesis of adult HFPEF. One of the most important determinants of Fpassive is the giant sarcomeric protein, titin. The starting point of this project was the hypothesis, that physiological and pathological adaptation processes to the extrauterine life involves posttranslational modifications (phosphorylation and oxidation) of differentially expressed titin isoforms and of other myofibrillar proteins, and that these changes together coordinate Fpassive of newborns. Results of these investigations shed new light on posttranslational titin isoform modifications in the developing heart, extended our understanding on the modifications of cardiovascular function during oxidative stress, and helped to elucidate the mechanisms of novel cardiotonic agents.

Abstract in Hungarian:

Az újszülöttkori diasztolés diszfunkció háttere, mely akár diasztolés szívelégtelenséghez (HFPEF) is vezethet, jelenleg még ismeretlen. A diasztolés szívelégtelenség a szív kamrai relaxáció és az érfal tágulékonyságának kóros kapcsolatrendszeréből származik. Korábbi vizsgálatainkban a szívizomsejtek fokozott passzív erőkomponensének (Fpasszív = a Ca2+-mentes oldatban mért erő) jelentőségére hívtuk fel a figyelmet a felnőttkori HFPEF létrejöttében. Az Fpasszív egyik legfontosabb meghatározója a nagy méretű szarkomerikus titin molekula. Jelen vizsgálataink kiindulási pontja az a hipotézis volt, miszerint az élettani és kóros adaptációs mechanizmusok a születést követően olyan poszttranszlációs (foszforilációs és oxidatív) módosulásokat idéznek elő az eltérő szerkezettel kifejezett titin izoformáiban és más miofibrilláris fehérjékben, melyek együttesen határozzák meg az Fpasszív értékét az újszülöttek szívében. Eredményeink új megvilágításba helyezték a titin izoformák születést követő poszttranszlációs módosulásait, hozzájárultak ahhoz, hogy a szív-érrendszert érő oxidatív stressz következményeit pontosabban megértsük, és segítettek abban, hogy több újszerű szívtámogató gyógyszer hatásmechanizmusát feltárjuk.

The following investigations were performed during the 4 year of the OTKA/NKFIH grant:

2014:

1*/ Balogh A, Santer D, Pásztor ET, Tóth A, Czuriga D, Podesser BK, Trescher K, Jaquet K, Erdodi F, Edes I, Papp Z.: Myofilament protein carbonylation contributes to the contractile dysfunction in the infarcted LV region of mouse hearts., Cardiovasc Res. 101(1):108-19. doi: 10.1093/cvr/cvt236, 2014.*

Original article

To determine the mechnical and biochemical consequences of oxidative myofilament protein alterations in association with protein phosphoryaltion left ventricular (LV) murine cardiomyocytes were investigated in a post-myocardial infarction model. Permeabilized murine cardiomyocytes from the remaining anterior and a remote non-infarcted inferior LV area were compared with those of non-infarcted age-matched controls. Myofilament phosphorylation, sulfhydryl (SH) oxidation, and carbonylation were also assayed. Ca(2+) sensitivity of force production was significantly lower in the anterior wall (pCa50: 5.81 ± 0.03, means ± SEM, at 2.3 μm sarcomere length) than that in the controls (pCa50: 5.91 ± 0.02) or in the MI inferior area (pCa50: 5.88 ± 0.02). The level of troponin I phosphorylation was lower and that of myofilament protein SH oxidation was higher in the anterior location relative to controls, but these changes did not explain the differences in Ca(2+) sensitivities. On the other hand, significantly higher carbonylation levels, [e.g. in myosin heavy chain (MHC) and actin] were observed in the MI anterior wall [carbonylation index (CI), CIMHC: 2.06 ± 0.46, CIactin: 1.46 ± 0.18] than in the controls (CI: 1). In vitro Fenton-based myofilament carbonylation in the control cardiomyocytes also decreased the Ca(2+) sensitivity of force production irrespective of the phosphorylation status of the myofilaments. Furthermore, the Ca(2+) sensitivity correlated strongly with myofilament carbonylation levels in all investigated samples. Our data suggested that post-MI myocardial remodelling involves increased myofibrillar protein carbonylation and decreased Ca(2+) sensitivity of force production, leading potentially to contractile dysfunction in the remaining cardiomyocytes of the infarcted area.

2/ Csató V, Pető A, Koller Á, Édes I, Tóth A, Papp Z: Hydrogen peroxide elicits constriction of skeletal muscle arterioles by activating the arachidonic acid pathway., PLoS One. 2014 Aug 5;9(8):e103858. doi: 10.1371/journal.pone.0103858. eCollection 2014.

Original article

The consequences of oxidative protein changes on vascular fucntions were also studied. To this end the molecular mechanisms of the vasoconstrictor responses evoked by hydrogen peroxide (H2O2) have been investigated. Changes in diameter of isolated, cannulated and pressurized gracilis muscle arterioles (GAs) of Wistar-

Kyoto rats were determined under various test conditions. H2O2 (10-100 μM) evoked concentration-dependent constrictions in the GAs, which were inhibited by endothelium removal, or by antagonists of phospholipase A (PLA; 100 μM 7,7-dimethyl-(5Z,8Z)-eicosadienoic acid), protein kinase C (PKC; 10 μM chelerythrine), phospholipase C (PLC; 10 μM U-73122), or Src family tyrosine kinase (Src kinase; 1 μM Src Inhibitor-1). Antagonists of thromboxane A2 (TXA2; 1 μM SQ-29548) or the non-specific cyclooxygenase (COX) inhibitor indomethacin (10 μM) converted constrictions to dilations. The COX-1 inhibitor (SC-560, 1 μM) demonstrated a greater reduction in constriction and conversion to dilation than that of COX-2 (celecoxib, 3 μM). H2O2 did not elicit significant changes in arteriolar Ca(2+) levels measured with Fura-2. These data suggested that H2O2 activates the endothelial Src kinase/PLC/PKC/PLA pathway, ultimately leading to the synthesis and release of TXA2 by COX-1, thereby increasing the Ca(2+) sensitivity of the vascular smooth muscle cells and eliciting constriction in rat skeletal muscle arterioles.

3/ Kovács Á, Papp Z., Nagy L.: Causes and pathophysiology of heart failure with preserved ejection fraction., Heart Fail Clin. 2014 Jul;10(3):389-98. doi: 10.1016/j.hfc.2014.04.002., 2014

Review article

We have overviewed the causes and pathophysiology of heart failure with preserved ejection fraction in the above review article, where the following points were addressed:

The pathophysiology of heart failure with preserved ejection fraction (HFPEF) is driven by interactions among age-dependent and gender-dependent characteristics of ventricular-arterial coupling and various predisposing comorbidities and risk factors. Ventricular diastolic dysfunction is central in the pathogenesis of HFPEF caused by an increased ventricular stiffness and is responsible for limited exercise tolerance. At tissue, cellular, and molecular levels, concentric myocardial hypertrophy, alterations in extracellular matrix and fibrosis, expressional changes, and posttranslational modifications of titin leading to increased cardiomyocyte passive stiffness (Fpassive) as well as perturbations of intracellular Ca2+ handling have been implicated. Further phenotyping of patients with HFPEF and preclinical studies in animal models of HFPEF may bring further insights into the pathogenesis of the complex syndrome of HFPEF.

4/ Papp Z, Borbély A, Paulus WJ. CrossTalk opposing view: The late sodium current is not an important player in the development of diastolic heart failure (heart failure with a preserved ejection fraction). J Physiol. 2014 Feb 1;592(3):415-7. doi: 10.1113/jphysiol.2013.264242.

Rebuttal from Zoltan Papp, Attila Borbely and Walter J. Paulus: Papp Z, Borbély A, Paulus WJ. J Physiol. 2014 Feb 1;592(3):421-2. doi: 10.1113/jphysiol.2013.268904.

Review article

This crosstalk article analyzed the potential involvement of the late sodium current in the development of diastolic heart failure.

2015:

5/ Papp Z, van der Velden J, Borbély A, Édes I, Stienen GJM.: Altered myocardial force generation in end-stage human heart failure, ESC Heart Fail. 2014 Dec;1(2):160-165. doi: 10.1002/ehf2.12020.

Original article

This study aimed to elucidate the molecular background of increased Ca2+ sensitivity of force production in cardiomyocytes of end-stage human heart failure. Ca2+-activated isometric force and the cross-bridge specific rate of force redevelopment (ktr) were determined in Triton-skinned myocytes from end-stage failing and non-failing donor hearts. Measurements (control: pH 7.2, 0 mM inorganic phosphate (Pi)) were performed under test conditions that probed either the Ca2+-regulatory function of the thin filaments (pH 6.5), the kinetics of the actin-myosin cross-bridge cycle (10 mM Pi), or both (pH 6.5, 10 mM Pi). The control maximal Ca2+-activated force (Fo) and ktrmax did not differ between failing and non-failing myocytes. At submaximal [Ca2+], however, both force and ktr were higher in failing than in donor myocytes. The difference in the Ca2+ sensitivities of force production was preserved when the thin filament regulatory function was perturbed by acidosis (pH 6.5) but was abolished by cross-bridge modulation (i.e. by Pi) both at pH 7.2 and at pH 6.5. Pi induced a larger reduction in force but a smaller increase in ktr in the failing myocytes than in the non-failing myocytes at submaximal [Ca2+]. The enhanced Pi sensitivity of the actin-myosin interaction suggests that the Pi release step of the actin-myosin cross-bridge cycle is modified during end-stage human heart failure. This might be of functional importance when Pi accumulates (e.g. during cardiac ischaemia). Moreover, this alteration can influence cardiac energetics and the clinical efficacy of sarcomere targeted agents in human heart failure.

6/ Kalász J, Pásztor ET, Fagyas M, Balogh Á, Tóth A, Csató V, Édes I, Papp Z, Borbély A.: Myeloperoxidase impairs the contractile function in isolated human cardiomyocytes, Free Radical Biology and Medicine 84: 116–127, 2015

Original article

We set out to characterize the mechanical effects of myeloperoxidase(MPO) in isolated left-ventricular human cardiomyocytes. Oxidative myofilament protein modifications (sulfhydryl(SH)-group oxidation and carbonylation) induced by the peroxidase and chlorinating activities of MPO were additionally identified. The specificity of the MPO-voked functional alterations was tested with an MPO inhibitor (MPO-I) and the antioxidant amino acid Met. The combined application of MPO and its substrate, hydrogenperoxide(H2O2), largely reduced the active force (Factive),

increased the passive force (Fpassive), and decreased the Ca2+ sensitivity of force production (pCa50) in permeabilized cardiomyocytes. H2O2 alone had significantly smaller effects on Factive and Fpassive and did not alter pCa50. The MPO-I blocked both the peroxidase and the chlorinating activities, whereas Met selectively inhibited the chlorinating activity of MPO. All of the MPO-induced functional effects could be prevented by the MPO-I and Met. Both H2O2 alone and MPO H2O2 reduced the SH content of actin and increased the carbonylation of actin and myosin-binding protein C to the same extent. Neither the SH oxidation nor the carbonylation of the giant sarcomeric protein titin was affected by these treatments. MPO activation induces a cardiomyocyte dysfunction by affecting Ca2+-regulated active and Ca2+-independent passive force production and myofilament Ca2+ sensitivity, independent of protein SH oxidation and carbonylation. The MPO-induced deleterious functional alterations can be prevented by the MPO-I and Met. Inhibition of MPO may be a promising therapeutic target to limit myocardial contractile dysfunction during inflammation.

7/ Csató V, Pető A, Fülöp GA, Rutkai I, Pásztor TE, Fagyas M, Kalász J, Édes I, Tóth A, Papp Z.: Myeloperoxidase evokes substantial vasomotor responses in isolated skeletal muscle arterioles of the rat, Acta Physiol. (Oxf.) 214: 109–123, 2015

Original article

The consequences of Myeloperoxidase (MPO) catalyzed oxidative protein changes on vascular functions were also studied. To this end the following exposure to MPO (1.92 mU mL-1) in the presence of increasing concentrations of hydrogen peroxide (H2O2), changes in arteriolar diameter of isolated gracilis skeletal muscle arterioles (SMAs) and coronary arterioles (CAs) and in the isometric force in basilar arteries (BAs) of the rat were monitored. Myeloperoxidase increased vascular tone to different degrees in CAs, SMAs and BAs. The mechanism of increased vasoconstriction was studied in detail in SMAs. MPO-evoked vasoconstrictions were prevented by the MPO inhibitor 4-aminobenzhydrazide (50 lM), by endothelium removal in the SMAs. Surprisingly, the HOCl scavenger L-methionine (100 uM), the thromboxane A2 (TXA2) antagonist SQ-29548 (1 uM) or the non-specific cyclooxygenase (COX) antagonist indomethacin (1 uM) converted the MPO-evoked vasoconstrictions to pronounced vasodilations in SMAs, not seen in the presence of H2O2. In contrast to noradrenalineinduced vasoconstrictions, the MPO-evoked vasoconstrictions were not accompanied by significant increases in arteriolar [Ca2+] levels in SMAs. Conclusion: These data showed that H2O2-derived HOCl to be a potent vasoconstrictor upon MPO application. HOCl activated the COX pathway, causing the synthesis and release of a TXA2-like substance to increase the Ca2+ sensitivity of the contractile apparatus in vascular smooth muscle cells and thereby to augment H2O2-evoked vasoconstrictions. Nevertheless, inhibition of the HOCl–COX–TXA2 pathway unmasked the effects of additional MPO-derived radicals with a marked vasodilatory potential in SMAs.

8/ Nagy L, Kovács Á, Bódi B, Pásztor ET, Fülöp GÁ, Tóth A, Édes I, Papp Z.: The novel cardiac myosin activator omecamtiv mecarbil increases the calcium sensitivity of force production in isolated cardiomyocytes and skeletal muscle fibres oftherat, Br. J. Pharmacol. 172 (18): 4506–4518., 2015

Original article

Increased Ca2+-sensitivity of force production can contribute to diastolic dysfunction upon the administration of postive inotropes. Omecamtiv mecarbil (OM) is a novel cardiac myosin activator drug for inotropic support in systolic heart failure. Here we have assessed the concentration-dependent mechanical effects of OM in permeabilized cardiomyocyte-sized preparations and single skeletal muscle fibres of Wistar-Kyoto rats under isometric conditions. Ca2+-dependent active force production (Factive), its Ca2+ sensitivity (pCa50), the kinetic characteristics of Ca2+-regulated activation and relaxation, and Ca2+-independent passive force (Fpassive) were monitored in Triton X-100-skinned preparations with and without OM (3nM-10 μM). In permeabilized cardiomyocytes, OM increased the Ca2+ sensitivity of force production (ΔpCa50: 0.11 or 0.34 at 0.1 or 1 μM respectively). The concentration–response relationship of the Ca2+ sensitization was bell-shaped, with maximal effects at 0.3–1 μM OM (EC50: 0.08 ± 0.01 μM). The kinetics of force development and relaxation slowed progressively with increasing OM concentration. Moreover, OM increased Fpassive in the cardiomyocytes with an apparent EC50 value of 0.26 ± 0.11 μM. OM-evoked effects in the diaphragm muscle fibres with intrinsically slow kinetics were largely similar to those in cardiomyocytes, while they were less apparent in muscle fibres with fast kinetics. OM acted as a Ca2+-sensitizing agent with a downstream mechanism of action in both cardiomyocytes and diaphragm muscle fibres. The mechanism of action of OM is connected to slowed activation–relaxation kinetics and at higher OM concentrations increased Fpassive production.

2016:

9*/ Alvarado G, Jeney V, Tóth A, Csősz É, Kalló G, Huynh AT, Hajnal Cs, Kalász J, Pásztor ET, Édes I, Grame M, Akerström B, Smithf, Eaton JW, Balla Gy, Papp Z, Balla J: Heme-induced contractile dysfunction in Human cardiomyocytes caused by oxidant damage to thick filament proteins, Free Radical Biology and Medicine 89, 248–262, 2015*

Original article

We set out to characterize the mechanical effects of free heme in isolated left-ventricular human cardiomyocytes. Intracellular free heme predisposes to oxidant-mediated tissue damage. We hypothesized that free heme causes alterations in myocardial contractility via disturbed structure and/or regulation of the contractile proteins. Isometric force production and its Ca2+-sensitivity (pCa50) were monitored in permeabilized human ventricular cardiomyocytes. Heme exposure altered cardiomyocyte morphology and evoked robust decreases in Ca2+-activated maximal

active force (Fo) while increasing Ca2+-independent passive force (Fpassive). Heme treatments, either alone or in combination with H2O2, did not affect pCa50. The increase in Fpassive started at 3 mM heme exposure and could be partially reversed by the antioxidant dithiothreitol. Protein sulfhydryl (SH) groups of thick myofilament content decreased and sulfenic acid formation increased after treatment with heme. Partial restoration in the SH group content was observed in a protein running at 140 kDa after treatment with dithiothreitol, but not in other proteins, such as filamin C, myosin heavy chain, cardiac myosin binding protein C, and α-actinin. Importantly, binding of heme to hemopexin or alpha-1-microglobulin prevented its effects on cardiomyocyte contractility, suggesting anallosteric effect. In line with this, free heme directly bound to myosin light chain 1 in human cardiomyocytes. Our observations suggest that free heme modifies cardiac contractile proteins via posttranslational protein modifications and via binding to myosin light chain 1, leading to severe contractile dysfunction. This may contribute to systolic and diastolic cardiac dysfunctions in haemolytic diseases, heart failure, and myocardial ischemia–reperfusion injury.

10/ Kovács Á, Fülöp GÁ, Kovács A, Csípő T, Bódi B, Priksz D, Juhász B, Beke L, Hendrik Z, Méhes G, Granzier HL, Édes I, Fagyas M, Papp Z, Barta J, Tóth A.: Renin overexpression leads to increased titin-based stiffness contributing to diastolic dysfunction in hypertensive mRen2 rats, Am J Physiol Heart Circ Physiol 310: H1671–H1682., 2016

Original article

In this study we investigated the influence of hypertension (HTN) on cardiac contraction and relaxation in transgenic renin overexpressing rats (carrying mouse Ren-2 renin gene, mRen2, n = 6). Blood pressure (BP) was measured. Cardiac contractility was characterized by echocardiography, cellular force measurements, and biochemical assays were applied to reveal molecular mechanisms. Sprague-Dawley (SD) rats (n=6) were used as controls. Transgenic rats had higher circulating renin activity and lower cardiac angiotensin-converting enzyme two levels. Systolic BP was elevated in mRen2 rats (235.11 ± 5.32 vs. 127.03 ± 7.56 mmHg in SD, P = 0.05), resulting in increased left ventricular (LV) weight/body weight ratio (4.05 ± 0.09 vs. 2.77 ± 0.08 mg/g in SD, P = 0.05). Transgenic renin expression had no effect on the systolic parameters, such as LV ejection fraction, cardiomyocyte Ca2+-activated force, and Ca2+ sensitivity of force production. In contrast, diastolic dysfunction was observed in mRen2 compared with SD rats: early and late LV diastolic filling ratio (E/A) was lower (1.14 ± 0.04 vs. 1.87 ± 0.08, P = 0.05), LV isovolumetric relaxation time was longer (43.85 ± 0.89 vs. 28.55 ± 1.33 ms, P = 0.05), cardiomyocyte passive tension was higher (1.74 ± 0.06 vs. 1.28 ± 0.18 kN/m2, P = 0.05), and lung weight/body weight ratio was increased (6.47±0.24 vs. 5.78±0.19 mg/g, P=0.05), as was left atrial weight/body weight ratio (0.21 ± 0.03 vs. 0.14 ± 0.03 mg/g, P = 0.05). Hyperphosphorylation of titin at Ser-12742 within the PEVK domain and a twofold overexpression of protein kinase Cα in mRen2 rats were detected. Our data suggest

a link between the activation of renin-angiotensin-aldosterone system and increased titin-based stiffness through phosphorylation of titin's PEVK element, contributing to diastolic dysfunction.

11/ Nagy L, Pollesello P, Haikala H, Végh Á, Sorsa T, Levijoki J, Szilágyi Sz, Édes I, Tóth A, Papp Z, Papp JGY: ORM-3819 promotes cardiac contractility through Ca2+ sensitization in combination with selective PDEIII inhibition, a novel approach to inotropy, European Journal of Pharmacology 775, 120–129, 2016

Original article

This study is the first pharmacological characterization of the novel chemical entity, ORM-3819(L-6-{4-[N'-(4-Hydroxi-3-methoxy-2-nitro-benzylidene)-hydrazino]-phenyl}-5-methyl-4,5-dihydro-2H-pyr-idazin-3-one), focusing primarily on its cardiotonic effects. ORM-3819 binding to cardiac troponinC (cTnC) was confirmed by nuclear magnetic resonance spectroscopy, and a selective inhibition of the phosphodiesteraseIII (PDEIII) isozyme (IC50= 3.88±0.3 nM) was revealed during in vitro enzyme assays. The Ca2+-sensitizing effect of ORM-3819 was demonstrated in vitro in permeabilized myocyte-sized preparations from left ventricles (LV) of guinea pig hearts (ΔpCa50 = 0.12±0.01; EC50 = 2.88±0.14 mM). ORM-3819 increased the maximal rate of LV pressure development (+dP/dtmax) (EC50=8.9±1.7nM) and LV systolic pressure (EC50=7.63±1.74nM) in Langendorff-perfused guinea pig hearts. Intravenous administration of ORM-3819 increased LV +dP/dtmax (EC50 = 0.13±0.05 mM/kg) and improved the rate of LV pressure decrease (-dP/dtmax); (EC50 = 0.03 ± 0.02 mM/kg) in healthy guinea pigs. In an in vivo dog model of myocardial stunning, ORM-3819 restored the depressed LV +dP/dtmax and improved %seg-mental shortening (%SS) in the ischemic area (to 18.8±3), which was reduced after the ischaemia-reperfusion insult (from 24.1±2.1 to 11.0±2.4). Our data demonstrate ORM-3819 as a potent positive inotropic agent exerting its cardiotonic effect by a cTnC-dependent Ca2+-sensitizing mechanism in combination with the selective inhibition of the PDEIII isozyme. This dual mechanism of action results in the concentration-dependent augmentation of the contractile performance under control conditions and in the postischemic failing myocardium.

12/ Úri K, Fagyas M, Kertész A, Borbély A, Jenei C, Bene O, Csanádi Z, Paulus WJ, Édes I, Papp Z, Tóth A, Lizanecz E. Circulating ACE2 activity correlates with cardiovascular disease development. J Renin Angiotensin Aldosterone Syst. 2016 Dec 12;17(4). pii: 1470320316668435.

Original article

It was shown recently that angiotensin-converting enzyme activity is limited by endogenous inhibition in vivo, highlighting the importance of angiotensin II (ACE2) elimination. The potential contribution of the ACE2 to cardiovascular disease progression was addressed. Serum ACE2 activities were measured in different

clinical states (healthy, n=45; hypertensive, n=239; heart failure (HF) with reduced ejection fraction (HFrEF) n=141 and HF with preserved ejection fraction (HFpEF) n=47). ACE2 activity was significantly higher in hypertensive patients (24.8±0.8 U/ml) than that in healthy volunteers (16.2±0.8 U/ml, p=0.01). ACE2 activity further increased in HFrEF patients (43.9±2.1 U/ml, p=0.001) but not in HFpEF patients (24.6±1.9 U/ml) when compared with hypertensive patients. Serum ACE2 activity negatively correlated with left ventricular systolic function in HFrEF, but not in hypertensive, HFpEF or healthy populations. Serum ACE2 activity had a fair diagnostic value to differentiate HFpEF from HFrEF patients in this study. Serum ACE2 activity correlates with cardiovascular disease development: it increases when hypertension develops and further increases when the cardiovascular disease further progresses to systolic dysfunction, suggesting that ACE2 metabolism plays a role in these processes. In contrast, serum ACE2 activity does not change when hypertension progresses to HFpEF, suggesting a different pathomechanism for HFpEF, and proposing a biomarker-based identification of these HF forms.

2017:

13/ Bódi B, Pásztorné Tóth E, Nagy L, Tóth A, Mártha L, Kovács Á, Balla G, Kovács T, Papp Z. Titin isoforms are increasingly protected against oxidative modifications in developing rat cardiomyocytes Free Radic Biol Med. 2017 Sep 21. pii: S0891-5849(17)30760-8. doi: 10.1016/j.freeradbiomed.2017.09.015. [Epub ahead of print]

Original article

During the perinatal adaptation process N2BA titin isoforms are switched for N2B titin isoforms leading to an increase in cardiomyocyte passive tension (Fpassive). Here we attempted to reveal how titin isoform composition and oxidative insults (i.e. sulfhydryl (SH)-group oxidation or carbonylation) influence Fpassive of left ventricular cardiomyocytes during rat heart development. Moreover, we also examined a hypothetical protective role for titin associated small heat shock proteins (sHSPs), Hsp27 and αB-crystallin in the above processes. Single, permeabilized left ventricular (LV) cardiomyocytes of the rat (at various ages following birth) were exposed either to 2,2'-dithiodipyridine (DTDP) to provoke SH-oxidation or Fenton reaction reagents (iron(II), hydrogen peroxide (H2O2), ascorbic acid) to induce protein carbonylation of cardiomyocytes in vitro. Thereafter, cardiomyocyte force measurements for Fpassive determinations and Western immunoblot assays were carried out for the semiquantitative determination of oxidized SH-groups or carbonyl groups of titin isoforms and to monitor sHSPs' expressions. DTDP or Fenton reagents increased Fpassive in 0- and 7-day-old rats to relatively higher extents than in 21-day-old and adult animals. The degrees of SH-group oxidation or carbonylation declined with cardiomyocyte age to smiliar extents for both titin isoforms. Moreover, the above characteristics were mirrored by increasing levels of HSP27 and αB-crystallin expressions during cardiomyocyte development. Our data implicate a gradual build-

up of a protective mechanism against titin oxidation through the upregulation of HSP27 and αB-crystallin expressions during postnatal cardiomyocyte development.

14/ Kovács Á, Kalász J, Pásztor ET, Tóth A, Papp Z, Dhalla NS, Barta J. Myosin heavy chain and cardiac troponin T damage is associated with impaired myofibrillar ATPase activity contributing to sarcomeric dysfunction in Ca2+-paradox rat hearts. Mol Cell Biochem. 2017 Jun;430(1-2):57-68. doi: 10.1007/s11010-017-2954-8.

Original article

This study aimed to explore the potential contribution of myofibrils to contractile dysfunction in Ca^{2+}-paradox hearts. Isolated rat hearts were perfused with Krebs-Henseleit solution (Control), followed by Ca^{2+}-depletion, and then Ca^{2+}-repletion after Ca^{2+}-depletion (Ca^{2+}-paradox) by Langendorff method. During heart perfusion left ventricular developed pressure (LVDP), end-diastolic pressure (LVEDP), rate of pressure development (+ dP/dt), and pressure decay (-dP/dt) were registered. Control LVDP (127.4 ± 6.1 mmHg) was reduced during Ca^{2+}-depletion (9.8 ± 1.3 mmHg) and Ca^{2+}-paradox (12.9 ± 1.3 mmHg) with similar decline in +dP/dt and -dP/dt. LVEDP was increased in both Ca^{2+}-depletion and Ca^{2+}-paradox. Compared to Control, myofibrillar Ca^{2+}-stimulated ATPase activity was decreased in the Ca^{2+}-depletion group (12.08 ± 0.57 vs. 8.13 ± 0.19 μmol P_i/mg protein/h), besides unvarying Mg^{2+} ATPase activity, while upon Ca^{2+}-paradox myofibrillar Ca^{2+}-stimulated ATPase activity was decreased (12.08 ± 0.57 vs. 8.40 ± 0.22 μmol P_i/mg protein/h), but Mg^{2+} ATPase activity was increased (3.20 ± 0.25 vs. 7.21 ± 0.36 μmol P_i/mg protein/h). In force measurements of isolated cardiomyocytes at saturating [Ca^{2+}], Ca^{2+}-depleted cells had lower rate constant of force redevelopment ($k_{tr,max}$, 3.85 ± 0.21) and unchanged active tension, while those in Ca^{2+}-paradox produced lower active tension (12.12 ± 3.19 kN/m^2) and $k_{tr,max}$ (3.21 ± 23) than cells of Control group (25.07 ± 3.51 and 4.61 ± 22 kN/m^2, respectively). In biochemical assays, α-myosin heavy chain and cardiac troponin T presented progressive degradation during Ca^{2+}-depletion and Ca^{2+}-paradox. Our results suggest that contractile impairment in Ca^{2+}-paradox partially resides in deranged sarcomeric function and compromised myofibrillar ATPase activity as a result of myofilament protein degradation, such as α-myosin heavy chain and cardiac troponin T. Impaired relaxation seen in Ca^{2+}-paradoxical hearts is apparently not related to titin, rather explained by the altered myofibrillar ATPase activity.

15/ Mátyás C, Németh BT, Oláh A, Török M, Ruppert M, Kellermayer D, Barta BA, Szabó G, Kökény G, Horváth EM, Bódi B, Papp Z, Merkely B, Radovits T. Prevention of the development of heart failure with preserved ejection fraction by the phosphodiesterase-5A inhibitor vardenafil in rats with type 2 diabetes. Eur J Heart Fail. 2017 Mar;19(3):326-336. doi: 10.1002/ejhf.711.

Original article

Heart failure with preserved ejection fraction (HFpEF) has a great epidemiological burden. The pathophysiological role of cyclic guanosine monophosphate (cGMP) signalling has been intensively investigated in HFpEF. Elevated levels of cGMP have been shown to exert cardioprotective effects in various cardiovascular diseases, including diabetic cardiomyopathy. We investigated the effect of long-term preventive application of the phosphodiesterase-5A (PDE5A) inhibitor vardenafil in diabetic cardiomyopathy-associated HFpEF. Zucker diabetic fatty (ZDF) rats were used as a model of HFpEF and ZDF lean rats served as controls. Animals received vehicle or 10 mg/kg body weight vardenafil per os from weeks 7 to 32 of age. Cardiac function, morphology was assessed by left ventricular (LV) pressure-volume analysis and echocardiography at week 32. Cardiomyocyte force measurements were performed. The key markers of cGMP signalling, nitro-oxidative stress, apoptosis, myocardial hypertrophy and fibrosis were examined. The ZDF animals showed diastolic dysfunction (increased LV/cardiomyocyte stiffness, prolonged LV relaxation time), preserved systolic performance, decreased myocardial cGMP level coupled with impaired protein kinase G (PKG) activity, increased nitro-oxidative stress, enhanced cardiomyocyte apoptosis, and hypertrophic and fibrotic remodelling of the myocardium. Vardenafil effectively prevented the development of HFpEF by maintaining diastolic function (decreased LV/cardiomyocyte stiffness and LV relaxation time), by restoring cGMP levels and PKG activation, by lowering apoptosis and by alleviating nitro-oxidative stress, myocardial hypertrophy and fibrotic remodelling. We report that vardenafil successfully prevented the development of diabetes mellitus-associated HFpEF. Thus, PDE5A inhibition as a preventive approach might be a promising option in the management of HFpEF patients with diabetes mellitus.

16/ Nagy L, Gödény I, Nánási P, Leskó Á, Balogh L, Bánhegyi V, Bódi B, Csípő T, Csongrádi A, Fülöp GÁ, Kovács Á, Lódi M, <u>Papp Z.</u> A szív pozitív inotróp támogatása a miozin-aktivátor hatású omecamtiv mecarbil segítségével / Inotropic therapy with the myosin activator omecamtiv mecarbil. Cardiol. Hung. 2017; 47: 145–152.

Review article in Hungarian

The novel cardiotonic drugs, called cardiac myosin activators have not been commercialized for clinical administrations yet. Omecamtiv mecarbil, the only known representative of the above class of drugs, appeared to be effective in preclinical investigations. Moreover, promising results have been also reported for phase I and phase II clinical trials as well. Nevertheless, our understanding on the exact mechanism of action and safety of omecamtiv mecarbil is still limited. The aim of the present overview is to summarize the currently available preclinical and clinical data on omecamtiv mecarbil, and thereby to estimate its potential clinical benefit.

Debrecen, 2017. szeptember 26. Dr. Papp Zoltán