2844-Pos

The Role of Soluble Guanylate Cyclase in Sepsis-Induced Cardiomyopathy in Mice

Ion A. Hobai[1,2], Eric Weiss[1], Antonis Armoundas[1], Emmanuel Buys[1], Peter Brouckaert[3], Wilson S. Colucci[2].
[1]Massachusetts General Hospital, Boston, MA, USA, [2]Boston University Medical Center, Boston, MA, USA, [3]Flanders Institute for Biotechnology (VIB), Ghent, Belgium.

Background. Soluble guanylate cyclase (sGC) plays multiple, conflicting roles during sepsis (*Am.J.Physiol.,2009;297:H654-663)*. Here we studied the impact of sGC deficiency on cardiac calcium (Ca^{2+}) handling in sepsis-induced cardiomyopathy (SIC).

Methods and Results. Cardiomyocyes were isolated from mice deficient in the α_1 subunit of sGC ($sGC\alpha_1^{-/-}$) and wild-type (WT), at baseline and 12h after administration of lipopolysacharide (LPS, 25 μg/g, ip). In $sGC\alpha_1^{-/-}$ cells (vs. WT), LPS induced a more marked decrease in externally paced Ca^{2+} transients (ΔCa_i, fura-2AM, Table), sarcoplasmic reticulum Ca^{2+} load (Ca_{SR}, using caffeine applications), fractional release (FR, ΔCa_i / Ca_{SR}) and trans-sarcolemmal Ca^{2+} entry (Ca_E, from the first ΔCa_i after caffeine removal). Ca^{2+} transient decay (τ_{Ca}) was slower in $sGC\alpha_1^{-/-}$ vs. WT after LPS, while Ca^{2+} decay during caffeine (τ_{Caff}, measuring Na^+/Ca^{2+} exchange) and L-type Ca^{2+} currents ($I_{Ca,L}$) were similar (Table; for all, n> 25 cells from >4 mice).

Conclusions. LPS induces a decrease in $I_{Ca\ ,L}$, Ca_E, FR, Ca_{SR} and ΔCa_i that does not require $sGC\alpha_1$. Moreover, sGC partially protects against Ca^{2+} handling alteration in SIC, through yet unidentified mechanisms.

2845-Pos

Effect of Training Induces Changes in Heart, CaMKII Dependent or Not?

Guri Kaurstad[1], Marcia N. Alves[2], Natale Rolim[1], Trine Skoglund[1], Helene Wisløff[3], Morten A. Høydal[1], Tomas O. Stølen[1], Ulrik Wisløff[1].
[1]NTNU, Department of Circulation and Medical Imaging, Trondheim, Norway, [2]Federal University of Minas Gerias, Department of Physiology and Biophysics, Belo Horizonte, Brazil, [3]VETHS, Oslo, Norway.

Background

Activation of the multifunctional Ca^{2+}/Calmodulin-dependant protein kinase II (CaMKII) is a decisive step in the development of heart failure. Interestingly, increased CaMKII activation was associated with improved cardiomyocyte function after exercise training in healthy mice. Here we determined whether CaMKII inhibition (KN-93) reduce the exercise training response on healthy hearts.

Methods

18 mice were included; KN-93 exercise, KN-93 sedentary, sham exercise and, sham sedentary. The exercise groups performed high intensity aerobic interval training 5 days a week for six weeks. KN-93 groups had daily intraperitoneal injections of KN-93 while sham were IP injected with DMSO. Ca^{2+} handling were measured in isolated cardiomyocytes, while *in vivo* myocardial function was assessed by echocardiography.

Results

VO_{2max} increased with 12 % in KN-93 exercise and 5 % in sham exercise, significant higher compared to sedentary groups ($P<0.05$). *In vivo* cardiac function was only improved in sham exercise ($P<0.02$). Fractional shortening from isolated cardiomyocytes improved in a similar magnitude in both exercise groups. KN-93 treated had reduced diastolic function, reflected by 25% slower re-lengthening than sham. Exercise training decreased time to 50% re-lengthening in KN-93 exercise ($P<0.05$) and sham exercise ($P<0.01$) vs. sedentary groups. This was reflected by Ca^{2+} decay, were both exercise groups reduced time to 50% Ca^{2+} decay, fastest in sham exercised. Cardiomyocyte hypertrophy occurred in both exercise groups with a significant higher response in the sham exercised compared to the KN-93 exercised (length; 13% vs. 8%, $P<0.05$, width; 30% vs. 14%, $P<0.05$, in sham exercised vs. KN-93 exercised, respectively).

Conclusion

CaMKII inhibition attenuated exercise training response on cardiomyocytes, but lead to higher levels of maximal oxygen uptake.

Cardiac Muscle II

2846-Pos

Increased Ca^{2+} Sensitivity and Length Dependent Activation in a Mouse Model with Increased Titin-Based Stiffness

Eun-Jeong Lee[1], Carlos Hidalgo[1], Michael Gotthardt[2], Henk Granzier[1].
[1]University of Arizona, Tucson, AZ, USA, [2]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany.

Length-dependent activation (LDA), the increase in Ca^{2+} sensitivity that occurs when sarcomere length (SL) is increased, is considered the cellular basis of the Frank-Starling law of the heart. Recent findings suggest that titin-based passive tension is a factor in LDA, and the aim of our study was to test this hypothesis in a mouse model in which the N2B spring element has been excised (N2B KO) and that therefore develops elevated passive tension. Fiber bundles of skinned papillary muscle from left ventricular of N2B KO and WT mice (eight per genotype) were isolated for mechanical tests. Active and passive tensions were measured and force-pCa curves were obtained at SL of 1.9, 2.1 and 2.3μm. The pCa_{50} was determined to characterize Ca^{2+} sensitivity and the difference in pCa_{50} at the different SLs (ΔpCa_{50}) was determined as a measure of LDA. We also studied the expression levels of thin and thick filament based regulatory protein, including their phosphorylation status, and found no significant differences between WT and KO mice. As expected from previous work (Radke *et al*. 2007), we found a significantly higher passive tension in N2B KO compared to WT mice at both SL of 2.1μm and 2.3μm ($p<.001$). The pCa_{50} increased proportionally to SL regardless of genotype, but the pCa_{50} was significantly greater in KO at SL of 2.1 and 2.3μm ($p<.05$ and $p<.001$, respectively). The increase in pCa_{50} from 1.9 to 2.3μm (ΔpCa_{50}), i.e. LDA, was significantly greater in KO compared to WT (0.19 and 0.15, respectively; $p<.001$). We also found that titin-based passive tension at SL 2.3μm was significantly correlated with ΔpCa_{50} ($p<.01$) in KO and WT mice. These results support that titin plays an important role in modulating LDA in cardiac muscle.

2847-Pos

Excision of Titin's Cardiac Pevk Spring Element Abolishes PKCα-Induced Increases in Myocardial Stiffness

Carlos G. Hidalgo[1], Bryan D. Hudson[1], Michael Gotthardt[2], Henk L. Granzier[1].
[1]The University of Arizona, Tucson, AZ, USA, [2]Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.
Carlos Hidalgo[1], Bryan D Hudson[1], Michael Gotthardt [2], Henk L Granzier[1].
[1]Department of Physiology, Molecular Cardiovascular Research Program, University of Arizona, Tucson AZ; [2]Neuromuscular and Cardiovascular Cell Biology, Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

Protein Kinase C-alpha (PKCα) was recently reported to increase myocardial stiffness, an effect that was proposed to be due to phosphorylation of two highly conserved sites (S11878 and S12022) within the proline-gluatamic acid-valine-lysine (PEVK) rich spring element of titin. To test this proposal we investigated the effect of PKCα on phosphorylation and passive stiffness in a mouse model lacking the titin exons that contain these two phosphorylation sites, the PEVK knockout (KO). We used skinned, gelsolin-extracted, left ventricular, myocardium from wildtype and PEVK KO mice. Consistent with previous work we found that PKCα increased passive stiffness in the WT myocardium by $27.4 \pm 6.2\%$. Importantly this effect was completely abolished in KO myocardium. In addition, increases in the elastic and viscous moduli (properties important in diastolic filling) following PKCα incubation was also ablated in the KO. Back phosphorylation assays showed that titin phosphorylation was significantly reduced by $36.1 \pm 12.3\%$ in skinned PEVK KO myocardial tissues following incubation with PKCα. The remaining phosphorylation in the KO suggests that PKCα sites exist in the titin molecule outside the PEVK region; these sites are not involved in increasing passive stiffness. Our results firmly support that the PEVK region of cardiac titin is phosphorylated by PKCα and that this increases passive tension. Thus, the PEVK spring element is the critical site of PKCα's involvement in myocardial stiffness.

2848-Pos

The N2B Element of Cardiac Titin Greatly Reduces Energy Loss During Loading Cycles of the Heart

Joshua Nedrud[1], Michael Gotthardt[2], Henk Granzier[1].
[1]University of Arziona, Tucson, AZ, USA, [2]Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

We recently published a N2B knockout (KO) mouse in which the exon that encodes the cardiac-specific N2B element (exon 49) has been deleted (Radke et al., 2007 PNAS, 104, 3444). The N2B element is one of the three titin spring elements found in the cardiac sarcomere; it provides a significant amount of extensibility within the physiological sarcomere length (SL) range of the heart. Earlier it had been proposed that the extensibility provided by the N2B element limits unfolding of titin's Ig domains that reside in series with the N2B element, thereby reducing energy loss during stretch and shortening (i.e., during diastole and systole) cycles of the beating heart. Here we tested this proposal by imposing loading cycles on skinned myocardium from wildtype (WT) and KO mice. Starting from the slack sarcomere length (SL~1.9 μm) we imposed triangular stretch/release protocols using a range of velocities (10, 100 and 1000 % /s) and three amplitudes (0.2, 0.3, and 0.4 μm/sarcomere); hysteresis was determined from the area between the stretch and release force-SL curves. In a separate set of experiments we imposed a sinusoidal small

amplitude oscillation (frequency: 1 −100Hz) and determined elastic and viscous moduli. Results showed that hysteresis was greatly increased in the KO over the WT (for example at a speed of 100 %/s and an amplitude of 0.3 μm/sarcomere, hysteresis was 1549 ± 379pJ/mm^2/sarcomere vs. 401 ± 94pJ/mm^2/sarcomere; $p<0.05$). It can be calculated that this differences in hysteresis is analogous to an energy difference in a 24 hour period of 600 BPM of ~16cal, or ~30% of the total energy consumed by the heart. We conclude that the N2B element greatly reduces energy loss during stretch/shortening cycles of the beating heart.

2849-Pos

Single Molecule Analysis of PKC Phosphorylation of Titin's PEVK Domain

Brian Anderson[1], Julius Bogolomovas[2], Siegfried Labeit[2], Henk Granzier[1].
[1]University of Arizona, Tucson, AZ, USA, [2]Universitätsmedizin Mannheim, Mannheim, Germany.

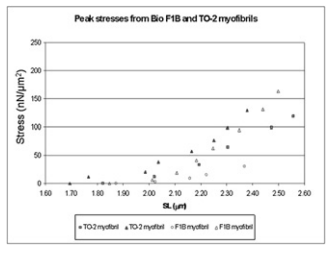
Titin's I-band region contains three spring-like domains that are primarily responsible for the development of passive tension in cardiac muscle. PKC phosphorylation targets one of these I-band domains_the PEVK region, which is rich in proline (P), glutamate (E), valine (V), and lysine (K). It has been shown that two serine residues within the PEVK are targeted by PKC phosphorylation, S26 and S170. We investigate the effects of PKC phosphorylation of these two residues on the single molecule level using force-extension curves generated by atomic force microscopy (AFM). We constructed four recombinant proteins: two PEVK single mutants (S26A and S170A), a PEVK double mutant (S26AS170A), and a wild-type PEVK segment. All constructs are flanked by immunoglobulin-like domains, Ig27 and Ig84, and the unfolding of these domains generates a single molecular ''fingerprint''. The force-extension curve leading up to the first unfolding peak describes the force-extension relationship of the PEVK. Preliminary data suggests that mutating either serine residue alters PEVK resistance to extension, which is quantified by the molecule's persistence length (PL). Wild-type PEVK underwent a large decrease in its PL after phosphorylation by PKC (by ~50%), and both single mutants have PLs similar to that of phosphorylated wild-type PEVK. Furthermore, phosphorylation of both single mutants resulted in a small PL decrease. Phosphorylation decreased PL for the S26A mutation by 16% (from 0.55 ± 0.02 to 0.46 ± 0.02 (mean ± SE)), and the serine-170 mutation PL by 11% (from 0.53 ± 0.04 to 0.47 ± 0.03). The double mutant was not affected by PKC (from 0.51 ± 0.04 to 0.51 ± 0.03). We conclude that both serines play a structural role in determining the relationship between longitudinal force and PEVK extension, and that this role is modulated through phosphorylation by PKC.

2850-Pos

Passive Stress in Myofibrils from Cardiomyopathic Hamsters

Audree McKenzie, Walter Herzog.
University of Calgary, Calgary, AB, Canada.

Dilated cardiomyopahty (DCM) is a frequent heart disease characterized by cardiac dilation and contractile dysfunction. The Bio TO-2 hamster is a genetic animal model of human DCM. The purpose of this research is to study the progression of DCM by comparing over time the passive mechanical properties of left ventricular wall myofibrils from TO-2 hamsters to those from F1B control hamsters. To date, we measured the passive stress-sarcomere length relations for two myofibrils each from experimental and control animals aged 36 weeks. Myofibrils were attached at one end to a glass needle controlled by a motor for stretching, and at the other end to a silicon-nitride nanolever of known stiffness for force measurements. Sarcomere lengths were measured from the myofibrillar striation patterns. Passive stresses in the experimental and control myofibrils were comparable. More passive mechanical experiments will be performed to confirm this result. In a single myofibril, titin is thought to be responsible for essentially all of the passive stress response to stretch (Linke et al., 1994; Bartoo et al., 1997). Titin depletion experiments and titin molecular weight determination will therefore also be performed to detect changes in titin isoform.



2851-Pos

Importance of Titin Based Viscosity in Cardiac Function: an Integrative Study on PEVK-Actin Interactions

Charles S. Chung[1], Methajit Methawasin[1], Joseph Popper[1], O. Lynne Nelson[2], Michael Gotthardt[3], Henk L. Granzier[1].
[1]Universityof Arizona, Tucson, AZ, USA, [2]Washington State University, Pullman, WA, USA, [3]Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

Viscosity has recently been hypothesized as an important regulator of diastolic relaxation during isovolumic relaxation and early rapid filling. A viscous interaction between the proline-glutamic acid-valine-lysine (PEVK) rich region of titin and the actin filaments has been shown at the protein level, but the physiologic relevance of such an interaction is unclear. A novel PEVK kockout (KO) mouse was utilized in order to investigate PEVK-actin based viscosity. KO and wild-type (WT) skinned cardiomyocytes were isolated subjected to ramp-hold protocols. Our data showed that the viscosity measured via stress relaxation was more than 2x greater in the WT vs KO cells and that WT cells showed a 2x faster relaxation to the steady state force at each of 4 stretch speeds, a hallmark of viscosity. Also using KO and WT mice, we examined the presence of viscosity in the intact ventricle. Using both ramp-hold and sinusoidal oscillations, we found that, in intact hearts, WT displayed greater viscosity than KO hearts. Ramp-hold analysis on isolated hearts again showed a 2x faster relaxation in WT (36ms) vs KO (53ms). Sinusoidal analysis provides KO viscosity nearly 30% lower than WT (Viscous modulus WT=0.97 vs KO=0.65 [mmHg/uL]). Because physiologic stretch speeds were probed in stretches on cells and isolated hearts, we analyzed in-vivo echocardiographic measurements utilizing kinematic models of stiffness and viscosity known as the Parameterized Diastolic Filling Formalism. As expected with a truncated titin, stiffness increased in the KO mouse (WT=10,700 vs KO=12,400 mass normalized stiffness [1/s2]). Importantly, a 30% reduction in viscous properties (WT=143 vs KO=99 mass normalized viscosity [1/s]). Titin based viscosity driven by PEVK-actin interactions are present in the ventricle and could play an important role in diastolic function and dysfunction.

2852-Pos

Titin Isoforms and Titin-Based Stiffness in Diastolic Heart Failure

Judith Krysiak[1], Lars S. Maier[2], Burkert M. Pieske[3], Margaret M. Redfield[4], **Wolfgang A. Linke**[1].
[1]Ruhr University, Bochum, Germany, [2]University of Göttingen, Göttingen, Germany, [3]Medical University Graz, Graz, Austria, [4]Mayo Clinic, Rochester, MN, USA.

Diastolic heart failure (DHF) is a common heart disease characterized, e.g., by delayed relaxation, impaired left ventricular (LV) filling and increased LV stiffness. Titin is an established contributor to LV stiffness, but little is known about the protein's contribution to altered diastolic function in DHF. We investigated LV tissue samples of several animal models of DHF, as well as interventricular septum samples of aortic stenosis (AS) patients, for titin-isoform composition by loose-gel electrophoresis and titin-based stiffness by skinned-fiber mechanics. Induction of diastolic dysfunction in a small animal model, the ''two kidney one clip'' (2K1C) rat, which develops LV hypertrophy due to chronic afterload increase, caused no significant changes in the titin-isoform expression pattern, both 6 weeks and 8 months following surgery (~6% N2BA in both 2K1C and SHAM-operated LV, the remainder being N2B isoform; N2B contains a stiffer, N2BA a more compliant titin spring). Similarly, in a volume-overload mouse model created by aortocaval fistula surgery, cardiac titin isoforms remained unaltered compared to SHAM-operated animals (18.5% vs. 19.8% mean N2BA). However, in an old dog model (8-12 years) made hypertensive by bilateral renal wrapping, the cardiac N2BA proportion was significantly lower ($41.6 \pm 4.9\%$; mean ± SD) than in normal old dog LV ($46.2 \pm 4.2\%$; $p<0.020$). Mechanical measurements revealed passive-stiffness modulations consistent with the magnitude of titin-isoform switching. In contrast, in human AS samples, the titin isoform composition showed $42.0 \pm 4.0\%$ N2BA, significantly higher than in location-matched normal donor hearts ($37.5 \pm 5.0\%$; $p<0.025$). We conclude that diastolic dysfunction is associated with changes in cardiac titin isoform composition in a large animal model and in humans. The direction and the magnitude of the isoform shift appear to be determined by multiple factors not excluding, but clearly not restricted to, hemodynamic overload.

2853-Pos

Characterization of a Mutant Rat Model with Altered Titin Isoform Expression

Wei Guo, Jonathan M. Pleitner, Kurt W. Saupe, Timothy A. Hacker, Marion L. Greaser.
University of Wisconsin-Madison, Madison, WI, USA.

Titin isoform expression is related to human cardiac disease. A mutant rat model with dramatically altered titin isoform expression has been described (Greaser et al. J Mol. Cell. Cardiol. 44:483, 2009), and the ultrastructural and physiological properties of mutant and wild type rats were compared in this study. Electron micrographs of homozygous mutant ventricles showed normal structure in most areas, but occasional regions of Z line streaming, myofibrillar disarray, lipofuchin granules, and myofibril degeneration were observed as found previously in human heart failure. Dobutamine administration caused an increased heart rate in wild type (Wt), heterozygotes (Ht) and homozygote mutants (Hm), but