2844-Pos

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

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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?

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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

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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

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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

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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