Ca^{2+}/calmodulin dependent protein kinases, PKA and CaMKII respectively, has been linked to arrhythmogenic diastolic Ca^{2+} leak from intracellular Ca^{2+} stores (the sarcoplasmic reticulum, SR). Using confocal Ca^{2+} imaging, we have recently shown that β-adrenergic stimulation (1 μM isoproterenol, Iso) increases SR Ca^{2+} leak several fold in quiescent, whole-cell voltage-clamped guinea-pig ventricular myocytes without altering SR Ca^{2+} content (Ogrodnik & Niggli 2009, Biophys J 96:276a). Independent of extracellular Ca^{2+} and changes of diastolic intracellular Ca^{2+} concentration, this observation indicates a sensitization of the RyRs. Intriguingly, here we show that increasing cAMP production and PKA activity by direct stimulation of adenylate cyclase with forskolin (1 μM) does not significantly elevate SR Ca^{2+} leak under otherwise identical experimental conditions. As successful downstream activation of the cAMP/PKA pathway was confirmed by comparable stimulation of L-type Ca^{2+} current and SR Ca^{2+}-ATPase activity in both Iso and forskolin, these disparate results suggest a distinct signaling pathway by which β-adrenergic stimulation increases SR Ca^{2+} leak. Interestingly, we found that the increased SR Ca^{2+} leak observed in Iso was likely mediated by CaMKII, rather than PKA, as treatment with the CaMKII inhibitor KN-93 (5 μM) suppressed the increase without altering SR Ca^{2+} content, in contrast to inhibition of PKA with H-89 (5 μM). Taken together, we conclude that CaMKII activation during β-adrenergic stimulation may be rapid, may not require elevated cardiomyocyte Ca^{2+} cycling, and may increase SR Ca^{2+} leak independently of the cAMP/PKA signaling pathway, possibly via increased nitric oxide production (Curran et al. 2009, Biophys J 96:120-121a).

2836-Pos

Impaired Ca^{2+} Release Synchronization in RyR2-S2808a Mouse Cardiomyocytes During β-Adrenergic Stimulation

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Ca^{2+}-induced Ca^{2+} release via ryanodine receptors (RyR2) is crucial for cardiac contractile function. During periods of stress and exercise, the sympathetic nervous system stimulates cardiac contractility. β-Adrenergic receptor activation has been suggested to result in PKA-mediated phosphorylation of RyR2 at Ser2808. Hyperphosphorylation at Ser2808 has also been discussed as possible factor contributing to heart failure. However, the role of RyR2 phosphorylation in inotropic adaptations during β-adrenergic stimulation remains controversial. Previous reports on a mouse model with genetic ablation of this phosphorylation site (S2808A) did not confirm the putative involvement of RyR2 phosphorylation in EC-coupling changes during β-adrenergic stimulation. In the present study, we intensified the search for EC-coupling modifications in S2808A myocytes by challenging EC-coupling near threshold conditions. Single cardiomyocytes were patch-clamped in the whole-cell configuration to measure I_{CaL}, while Ca^{2+} transients were simultaneously recorded with confocal imaging of fluo-3. The EC-coupling gain, a measure for the effectiveness of I_{CaL} to trigger Ca^{2+} release from the SR, was determined from control and S2808A cardiomyocytes. Lowering the extracellular Ca^{2+} concentration, a maneuver often used to unmask latent EC-coupling problems, did not reveal significant differences in the EC-coupling gain in WT and S2808A myocytes before and during β-adrenergic stimulation with isoproterenol. However, comprehensive analysis of subcellular Ca^{2+} transient kinetics indicated subtle differences in coordination of RyR activation. Uncoupling of the EC-mechanism by reduced $[Ca^{2+}]_o$ resulted in a spatiotemporal de-synchronization of RyR openings. β-Adrenergic stimulation re-synchronized RyR openings under the same conditions less effectively in S2808A than in WT cardiomyocytes (time-to-peak of single Ca^{2+} release sites 181 ± 6 vs. 100 ± 3ms, respectively, $P<0.0001$). We conclude that although removal of the PKA phosphoepitope at Ser2808 does not critically derange EC-coupling, its ablation may interfere with synchronization of RyR2 activation during β-adrenergic stimulation.

2837-Pos

Ryanodine Receptors Outside of Couplons are Involved in Excitation-Contraction Coupling in Rabbit Ventricular Myocytes

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Current theories of excitation-contraction coupling (ECC) in ventricular myocytes assert that L-type Ca channels interact with clusters of ryanodine-sensitive Ca release channels (RyRs) within couplons. We hypothesized that RyR clusters exist also outside of couplons and contribute to ECC. We investigated this hypothesis by three-dimensional imaging of RyR clusters and sarcolemma of isolated myocytes lying flat (horizontal) and on end (vertical). We deconvolved the image stacks, created reconstructions of cell segments, and identified RyR cluster types. RyR clusters remote to sarcolemma were assumed to be outside of couplons. Similar studies were performed on intact ventricular tissue. Furthermore, we imaged evoked Ca transients and sarcolemma of horizontal cells labeled with fluo-4 and di-8-anepps. Image sequences were acquired using rapid two-dimensional scanning (Zeiss LSM5Live, rates up to 300HZ). The image sequences were corrected for bleaching and cross-talk. In horizontal and vertical isolated cells, RyR clusters appeared to be arranged in sheets in the vicinity of Z-disks. Some RyR clusters were associated with sarcolemma, in particular transverse tubules, and are presumably part of couplons. However, most RyR clusters were not. Examination of cells in intact tissue revealed a smaller number of RyR clusters not associated with sarcolemma than in isolated cells. The density of transverse tubules was higher than in isolated cells. This loss of transverse tubules might be caused by the isolation procedure. Analysis of the rapid image sequences indicated that both types of RyR clusters were activated during an action potential. However, the RyR clusters not associated with sarcolemma were activated with delays of up to 10ms. In conclusion, we demonstrated that RyR clusters outside of couplons are involved in ECC. We suggest that activation of RyR clusters outside of couplons occurs by a common pool mechanism.

2838-Pos

Remodelling of Calcium Handling, Ion Currents and Contraction in Rac1 Overexpressing Mouse Ventricle

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Increased production of oxygen radicals is involved in many cardiac deseases. In a cardiac-specific Rac1-overexpressing mouse line (RacET) NADPH oxidase activity is upregulated 6-7 fold. Here, we characterise ventricular remodelling processes with respect to calcium handling and ion currents in ventricular myocytes. We used 4-6 months old RacET and age-matched wt mice. In ventricular cells of RacET baseline calcium concentrations were significantly decreased. In post-rest behaviour the first amplitude was unchanged but the steady-state amplitude was down to almost 50% of the wt-value. Interestingly, RacET myocytes displayed significantly increased amplitudes of caffeine-induced calcium transients (up by 50%), while Na/Ca-exchange and SERCA-pump activity appeared unchanged. A similar behaviour was observed in cell-length experiments. Here, RacET myocytes displayed a significantly shorter resting cell length (down by 15%), in post-rest behaviour experiments the first twitch amplitude was unchanged while in steady-state their contraction was significantly reduced. When analysing calcium sparks we found that their amplitude was almost doubled in RacET cells while the recovery was speeded up 25%, their spatial spread was reduced by 25% when compared to wt. The membrane capacity of the RacET myocytes was significantly reduced (down by 40%) and action potentials were largely distorted, whereby both upstroke and repolarisation phase were altered. From these data we conclude that RacET overexpression and the accompanying increased oxygen radical load results in ventricular remodelling, even in the absence of hypertrophy.

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2839-Pos

Mesenchymal Stem Cell Conditioned Tyrode is a Potent Activator of Akt in Cardiomyocytes

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Transplantation of bone marrow-derived mesenchymal stem cells (MSCs) in clinical trails has been reported to decrease infarct size and benefit ventricular ejection fraction of the heart. Differentiation of the MSCs into cardiac myocytes has been postulated, but stronger evidence points toward a paracrine mechanism. We tested the hypothesis that MSC conditioned tyrode (conT) results in improved cardiomyocyte survival through activation of the anti-apoptotic Akt protein kinase pathway. HEPES/ Bicarbonate buffered tyrode (pH 7.4) was placed on MSCs for 16 hrs at 37°C for conditioning. Isolated mouse ventricular cardiomyocytes (VMs) were treated with conT. Immunoblotting of VM lysates was used to examine the activation Akt, a downstream effector of the receptor-mediated PI3-Kinase pathway in conjunction with confocal imaging of intracellular Ca2+ (FLUO 4-AM). Superfusion of VMs with conT resulted in a progressive decrease of the Ca2+ transient duration (31 ± 3.4 %) and an