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

Nina D. Ullrich, Ernst Niggli.
Dept. Physiology, University of Bern, Bern, Switzerland.

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

Natalia S. Torres[1], Eleonora Savio-Galimberti[1], Joshua I. Goldhaber[2], Christian Soeller[3], **John H.B. Bridge[1]**, Frank B. Sachse[1,4].
[1]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA, [2]Department of Medicine, Division of Cardiology, Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, [3]Department of Physiology, School of Medicine, University of Auckland, Auckland, New Zealand, [4]Bioengineering Department, University of Utah, Salt Lake City, UT, USA.

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

Peter Lipp[1], Martin Oberhofer[1], Qinghai Tian[1], Jue Wang[1], Karin Hammer[1,2], Jan Reil[3], Hans-Ruprecht Neuberger[3], Lars Kaestner[1].
[1]Molecular Cell Biology; Saarland University, Homburg/Saar, Germany, [2]Dept. Pharmacology; UC Davis, Davis, CA, USA, [3]Cardiology Department; University Hospital, Homburg/Saar, Germany.

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

Dan Bare, Iurii Semenov, Kathrin Banach.
University of Illinois at Chicago, Chicago, IL, USA.

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

increase in Ca2+ transient amplitude (84 ± 1.5 %; n=218) that reached steady-state approx. 3 hrs post-treatment. ConT increased the activation of Akt as indicated by phosphorylation of Akt (p-Akt) on Ser473 at 15 min and remained elevated relative to non-treated VMs at 3 hrs. A faster migrating p-Akt immunoreactive band was also identified. This putative Akt-cleavage product was not seen with insulin (10 μM) stimulation of p-Akt and not prevented with inhibitors of caspase activity (Z-DEVD-FMK (40 μM), Boc-D-FMK (50 μM)). This activation was not myocyte specific and conT treatment produced similar results in fibroblasts. The results demonstrate a paracrine mechanism of MSCs on improved cardiomyocyte survival through activation the PI3K/Akt pathway that also triggers remodeling of EC coupling. Stimulation of Akt in fibroblasts presents an additional indirect means by which cardiac repair could be modulated following injury.

2840-Pos

Rac1-Induced Remodelling is Different in the Left and Right Atrium of RacET Mice

Peter Lipp[1], Martin Oberhofer[1], Qinghai Tian[1], Jue Wang[1], Karin Hammer[1,2], Jan Reil[3], Hans-Ruprecht Neuberger[3], Lars Kaestner[1].
[1]Molecular Cell Biology; Saarland University, Homburg/Saar, Germany, [2]Department of Pharmacology; UC Davis, Davis, CA, USA, [3]Cardiology Department; University Hospital, Homburg/Saar, Germany.

In human atrial fibrillation (AF), increased levels of reactive oxygen species, increased activity of the NADPH oxidase and increased expression of the small GTPase Rac1 have been reported. In a RacET transgenic mouse line, spontaneous AF occurs at ages >12 weeks. Here, we characterise atrial remodelling processes that take place in calcium handling and ion current. We used 4-6 months old male mice and age-matched control mice. Echocardiography indicated mildly enlarged left atria while the right atria displayed several fold dilation. The analysis of whole cell calcium transients revealed that although both cell types displayed reduced calcium transient amplitudes, basal calcium concentration was significantly increased only in cells of the right atrium. Post-rest behaviour was unchanged. While in the left atrium (LA) caffeine responses were unchanged in amplitude but SERCA activity was down by almost 50%, cells from the right atrium (RA) showed significantly decreased caffeine signals (by 40%) and increased Na/Ca exchanger with an unchanged SERCA pump activity. Calcium sparks also displayed striking differences. While in RA their amplitude was reduced, recovery was slowed down and spatial spread was only slightly decreased (by 10%), cells from LA showed an increased amplitude (by 40%), faster recovery (up 20%) and a decreased spatial spread (down by >25 %). Action potentials recorded from LA and RA cells showed a slightly more negative resting membrane potentials in the myocytes from RacET with otherwise unchanged properties. From these data we conclude that Rac1 overexpression and the accompanying increased oxygene radical load results in atrial remodelling that is significantly different between the left and the right atrium.

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2841-Pos

Increased Susceptibility to Ventricular Arrhythmias Relates to Diastolic Ca^{2+} Leak in Rats with Metabolic Syndrome

Morten A. Høydal, Anne B. Johnsen, Marcia Alvez, Guri Kaurstad, Natale Rolim, Ulrik Wisløff.
Norwegian University of Science and Technology, Trondheim, Norway.

Introduction:
Rats genetically selected and bred for low aerobic capacity (LCR) develop characteristics resembling to the metabolic syndrome and animals with heart failure. Rats selected for high aerobic capacity (HCR) develop athletic characteristics. We hypothesized that LCR rats are more susceptible for ventricular arrhythmias and that this relates to increased diastolic SR Ca^{2+} leak.
Methods:
We included 10 LCR and 10 HCR rats. We assessed susceptibility to ventricular fibrillation by burst pacing in Langendorff perfused hearts. In isolated cardiomyocytes we measured Ca^{2+}-handling with FURA2/AM and action potential by di-8-ANEPPS on an inverted epi-fluoresence microscope.
Results:
LCR rats were more susceptible for ventricular fibrillation and monophasic action potential at the left ventricle was prolonged. Prolongation of the action potential was confirmed in single di-8-ANEPPS loaded cardiomyocytes. Cardiomyocyte function was significantly depressed in LCR rats compared to HCR; fractional shortening was 37% lower and time to 50% relenghtening was 53% longer. Ca^{2+}-handling was impaired by elevated diastolic Ca^{2+}, reduced Ca^{2+} amplitude and prolonged time to 50% Ca^{2+} decay. SR Ca^{2+} content was 21% lower and fractional Ca^{2+} release was 10% lower in LCR. During caffeine induced Ca^{2+} transient, we found no difference in Ca^{2+} decay between the two groups, reflecting unaltered NCX function; nor did we find any changes in the plasma membrane Ca^{2+}, assessed by caffeine induced transients in a $0Na^{+}$/$0Ca^{2+}$ solution. Diastolic Ca^{2+} removal is mainly attributed to reduced SERCA function by 15% in LCR rats. By measuring diastolic Ca^{2+} in quiescent myocytes with and without tetracaine we found 46% increased SR Ca^{2+} leak LCR rats.
Conclusion:
Impaired Ca^{2+} handling and increased diastolic SR Ca^{2+} leak together with prolonged action potential duration may explain increased susceptibility to ventricular arrhythmias in LCR rats with metabolic syndrome.

2842-Pos

Effects of Membrane Calcium Flux Localizations and Realistic T-Tubule Geometry on Cardiac Excitation-Contraction Coupling

Yuhui Cheng[1], Zeyun Yu[1,2], Masahiko Hoshijima[1], Michael Holst[1], Andrew McCulloch[1], Andrew McCammon[1], Anushka Michailova[1].
[1]University of California San Diego, La Jolla, CA, USA, [2]University of Wisconsin, Milwaukee, WI, USA.

The sub-cellular anatomy and ionic flux distributions are considered important in calcium (Ca) regulation and their variations over time may hold clues to the progression of heart diseases. To investigate how these coupled processes may affect the cardiac cell function we developed 3D continuum model of Ca-signaling, buffering and diffusion in rat ventricular myocytes. The model geometry was derived from high definition light and electron microscopy images in rodent cardiac cells through a process called tomographic reconstruction [1, 2]. The current model includes: the 3D geometry of a single t-tubule and its surrounding half-sarcomeres; spatially distributed Ca handling proteins along the t-tubule and surface membrane; stationary and mobile Ca buffers (ATP, calmodulin, fluo-3, troponin C). A finite element software package SMOL-SubCell was used to solve the PDE system on cluster of computers [3]. In agreement with experiment [4,5], model suggests that the rat t-tubule anatomy and the heterogeneous distribution of Ca fluxes along the cell membrane might be important mechanisms for maintaining uniform Ca concentration in presence of 100 microM fluo-3 and sarcoplasmic reticulum inhibited. In the absence of fluo-3, model predicts that the overall Ca distribution can not be maintained uniform when the membrane Ca fluxes were heterogeneously distributed.

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*Corresponding author: amihaylo@ucsd.edu

2843-Pos

The Generation of a Novel Animal Model of Inducible Hypertrophy: Overexpression of NCX1 in the Murine Heart Using the Doxycycline-Dependent Promoter

Yuki Katanosaka[1], Keiichiro Iwasaki[1], Satoshi Mohri[1,2], Keiji Naruse[1].
[1]Okayama Univ, Okayama, Japan, [2]Kawasaki Medical School, Kurashiki, Japan.

The Na^{+}/Ca^{2+} exchanger (NCX1) is essential regulator of Ca^{2+} homeostasis in cardiomyocytes. In normal cardiac muscle, the primary role of NCX1 is to extrude cytoplasmic Ca^{2+} during myocyte repolarization and diastole. In hypertrophic and failing hearts, sarcolemmal NCX1 expression has often been shown to be elevated, which could be compensatory for the reduced ability of the sarcoplasmic reticulum to maintain low diastolic $[Ca^{2+}]_i$ under these pathological conditions. However, whether increased NCX1 expression invariably leads to enhanced function under disease conditions is not clear. In this study, we have produced a line of the transgenic mice in which expression of the canine cardiac NCX1 transgene was induced using the doxycycline (DOX) dependent promoter. After the injection of 100 μL of DOX (2 mg/mL in normal saline solution) into the abdominal cavity per 1 day during 2 weeks, the expression of canine NCX1 protein in the hearts of the DOX-treated transgenic mice was 3-fold higher, but the protein levels involved in excitation-contraction coupling, sarcoplasmic reticulum Ca^{2+}-ATPase, phospholamban, ryanodine receptor, and L-type Ca^{2+} channel were unchanged compared with those of wild-type (WT) and the non-induced transgenic mice. The total heart-to-body weight ratio was increased by 45% in the DOX-treated transgenic mice compared with WT or non-induced mice. The DOX-treated transgenic mice developed cardiac hypertrophy accompanied by elevation of multiple hypertrophic markers, but not by the any signs of other typical heart failures. We suggest that the overexpression of NCX1 is sufficient to develop cardiac hypertrophy and these mice would be useful as the animal model to study on the pathological role of NCX1 in cardiac hypertrophy and progression of cardiac dysfunction on heart failure.