β1 subunits by calcineurin/NFATc3 signaling during chronic angiotensin II signaling.
To test this hypothesis, we used wild type and AKAP150 null (AKAP150$^{-/-}$) arteries. Experiments involved measurement of calcineurin activity in wild type (WT) and AKAP150 null (AKAP150$^{-/-}$) myocytes. We also used TIRF and confocal microscopy to image local Ca^{2+} signals by Ca^{2+} influx via L-type Ca^{2+} channels and nuclear NFATc3 translocation in WT and AKAP150$^{-/-}$ before and after application of angiotensin II. Finally, we examined the effects of chronic activation of angiotensin II and NFATc3 signaling on the expression of Kv2.1 as well as the α and β1 subunits of the BK channels in WT and AKAP150$^{-/-}$ arteries. We found that sustained activation of angiotensin II signaling down regulated these genes in arterial smooth muscle from WT but not AKAP150$^{-/-}$ arteries. These results suggest a model, in which AKAP150, calcineurin, and L-type Ca^{2+} channels form a signaling unit that regulates Ca^{2+} influx and gene expression in smooth muscle.

531-Pos
Cylic AMP Measured with ICUE3 in Vascular Smooth Muscle Cells
Li-ping He[1], Guiling Zhao[2], Withrow G. Wier[1], William J. Lederer[2].
[1]Unversity of Maryland, Baltimore, Baltimore, MD, USA, [2]Unversity of Maryland Biotechnology Institutie, Baltimore, MD, USA.
cAMP dependent protein kinase (PKA) activation represents a key signaling mechanism in the cardiovascular system. Here we used ICUE3, an Epac-based cAMP reporter based on Fluorescence Resonance Energy Transfer (FRET) to indicate cAMP activity in a smooth muscle cell line (a7r5). Cells were transfected with the ICUE3 vector and also loaded with fura-2 via exposure to fura-2/AM. Simultaneous imaging of ICUE and fura-2 fluorescence was by methods previously described. The β-adrenoceptor agonist, isoproterenol, potently increased cAMP, over the concentration range, 0.003 μM up to 0.1μM, with apparent EC_{50} of approximately 0.02 μM. Maximal increases in cAMP by isoproterenol were similar to those produced by exposure to high concentrations of forskolin (50 μM). The decline of cAMP transients was markedly slowed by exposure to the broad-spectrum phosphodiesterase inhibitor, IBMX (iso-butyl methylxanthine). We sought to determine whether cAMP might also be produced by Ca^{2+}-dependent isoforms of Adenylyl Cyclase. Elevation of [Ca^{2+}] by exposure to the SERCA pump inhibitor, CPA (cyclopiazonic acid, 50 uM) and elevated cAMP. However, when [Ca^{2+}] was elevated by exposure to the V1 receptor agonist, arginine vasopressin (AVP), cAMP did not increase. In conclusion, we demonstrated 1) receptor induced, 2) forskolin induced, and 3) Ca^{2+} induced increases in cAMP in a7r5 smooth muscle cells. The mechanism and or location of Ca^{2+} increase is important however, as release of Ca^{2+} from intracellular stores by SERCA pump inhibition increased cAMP, but receptor-induced Ca^{2+} release did not.

532-Pos
Aerobic Interval Training Prevents Cardiac Dysfunction and Mortality by Improving Calcium Handling in MI Diabetic Mice
Natale Rolim[1], Tomas Stølen[1], Charlotte B. Ingul[1], Anne Berit Johnsen[1], Guri Kaurstad[1], Harald E.M. Hansen[1], Marcia Alves[2], Kristine Skårdal[1], Marte Thuen[1], Morten Høydal[1], Ulrik Wisløff[1].
[1]NTNU, Trondheim, Norway, [2]Federal University of Minas Gerais, Belo Horizonte, Brazil.
Diabetic patients have greater risk of developing congestive heart failure (HF) after myocardium infarction (MI). Exercise training is an effective strategy for preventing the development of cardiomyopathies and the incidence of cardiovascular morbidity and mortality during diabetes.
Aim - To study the effects of aerobic interval training (AIT) on cardiac function and the role of calcium handling in a combined experimental model of MI-induced HF and diabetic cardiomyopathy.
Methods and Results - A cohort of male diabetic db/db and age-matched nondiabetic control mice was randomly assigned into untrained and trained sham and MI groups. MI was induced by coronary ligation. Exercise tolerance was evaluated by VO2 max. Standard echocardiography and tissue Doppler imaging were performed by high-resolution in-vivo imaging system, and diastolic sarcoplasmic reticulum (SR) calcium leak was measured in isolated cardiomyocytes using fluorescence microscope. MI diabetic mice displayed higher mortality rate compared to MI nondiabetic and sham mice (55% vs. 25% and 0%, respectively). In addition, exercise intolerance, reduced fractional shortening (FS), and cardiomyocyte dysfunction were observed in MI diabetic mice compared to other groups. AIT increased survival rate and exercise tolerance in MI diabetic to diabetic sham levels, paralleled by increased FS. AIT reestablished contractile function of MI diabetic to diabetic sham levels associated with improved SR calcium release synchronicity, T-tubule density and SR calcium leak.
Conclusion - These results provide evidence for improvement of calcium handling by AIT in MI-induced HF during diabetes. Therefore, AIT is a potential therapeutic tool for the management of HF associated with diabetes.

533-Pos
Cholesterol Elevation Impairs Glucose-Mediated Ca^{2+} Signalling in Mouse Pancreatic Beta Cells
Amy Tse, Valerie Yeung-Yam-Wah, Andy K. Lee, Frederick W. Tse.
University of Alberta, Edmonton, AB, Canada.
Elevation of cholesterol in pancreatic islets is associated with a reduction in glucose-mediated insulin secretion. We examined the effects of cholesterol elevation in β cells isolated from C57BL/6J mice by incubating the cells with 1 mg/ml of soluble cholesterol at 37 ^{o}C for 1 hour. In controls, ~80% of the β cells (identified by their Ca^{2+} response to the K_{ATP} channel blocker, tolbutamide) exhibited a $[Ca^{2+}]_i$ rise (monitored with fura-2 imaging) when exposed to glucose (20 mM). Cholesterol treatment reduced the fraction of glucose-responding β cells to ~19% but treatment with cholesterol plus excessive cholesterol chelator, methyl-β-cyclodextran (MβCD; 10 or 20 mM) did not affect the fraction of glucose-responding β cells. We found no significant difference in the resting potentials (perforated-patch recording) between the cholesterol-overload cells and controls. Nevertheless, glucose (20 mM) triggered only a small depolarization (~2 mV) in the cholesterol-overload cells (versus ~46 mV in controls). We examined whether the poor glucose response in the cholesterol-overload cells was related to an increase in the K_{ATP} or delayed rectifier current. The mean density of K_{ATP} current (normalized to cell capacitance) at −60 mV in the cholesterol-overload cells (perforated patch recording) was ~4-fold smaller than the controls. The current density of the delayed rectifier at +20 mV (whole-cell recording) in the cholesterol-overload cells was ~50% of the control values. Cholesterol-overload also reduced the density of the voltage-gated Ca^{2+} current (VGCC) to ~36% of the control values. Our results indicate that cholesterol elevation in β cells has inhibitory effect on the K_{ATP} channels, delayed rectifier and VGCC. A reduction in voltage-gated Ca^{2+} entry in conjunction with a decrease in the ability of glucose to evoke depolarization contribute to the impairment of the glucose-mediated Ca^{2+} signalling in cholesterol elevated β cells.

534-Pos
Role of Irbit in Regulation of IP_3-Induced Ca^{2+} Release in Superior Cervical Ganglion (SCG) Neurons
Oleg Zaika, Mark S. Shapiro.
UT Health Science Center, San Antonio, TX, USA.
Two modes of $G_{q/11}$-coupled receptor action have been described in SCG neurons. One mode, used by M_1 muscarinic receptors, depletes PIP_2 but does not generate IP_3-mediated $[Ca^{2+}]_i$ signals, whereas the other, used by bradykinin B_2 and purinergic P2Y receptors, does not deplete PIP_2 but generates IP_3-mediated $[Ca^{2+}]_i$ signals (Zaika et al., *J. Neurosci*. 27:8914-26). What accounts for the striking receptor specificity in $[Ca^{2+}]_i$ signals? There are two working hypotheses. The first involves co-localization of B_2 and P2Y, but not M_1, receptors with IP_3 receptors, allowing IP_3 produced to be in the right ''microdomain'' to trigger Ca^{2+} release (Delmas et al. *Neuron* 34:209-20). The second involves inhibition of IP_3 receptors by certain $G_{q/11}$-coupled receptors via some cytoplasmic messenger. We explored both hypotheses using fura-2 Ca^{2+} imaging. First, we over-expressed M_1 receptors in SCG neurons, which should disrupt any native ''micro-domain'' organization, but there were no effects. Thus, in cells transfected with EGFP only, application of the muscarinic agonist oxotremorine (oxo-M), bradykinin and the purinergic agonist UTP induced a $[Ca^{2+}]_i$ signal in 1/14, 13/14 and 12/14 neurons, and in neurons transfected with EGFP + M_1 receptors, oxo-M, bradykinin and UTP induced a $[Ca^{2+}]_i$ signal in 2/15, 14/15 and 13/15 neurons, respectively. We then tested the IP_3 receptor inhibitory protein, IRBIT (Ando et al. *Mol. Cell* 22:795-806). In SCG neurons over-expressed with wild-type IRBIT, application of oxo-M, bradykinin and UTP induced a $[Ca^{2+}]_i$ signal in 4/21, 12/21 and 14/21 neurons, respectively, whereas in neurons over-expressed with the dominant-negative IRBIT (S68A), application of oxo-M, BK and UTP induced a $[Ca^{2+}]_i$ signal in 9/14, 14/14 and 13/14 neurons, respectively. Our experiments suggest that IRBIT may play an important role in the regulation of IP_3-induced Ca^{2+} release induced by $G_{q/11}$-coupled receptors.

535-Pos
Kv Channel Suppression and Enhanced Cav Channel Activity Contribute to Increased Constriction of Parenchymal Arterioles from Subarachnoid Hemorrhage Model Rats
Matthew A. Nystoriak, Masayo Koide, Sheila R. Russell, George C. Wellman.
University of Vermont, College of Medicine, Burlington, VT, USA.
Subarachnoid hemorrhage (SAH) following cerebral aneurysm rupture is associated with substantial morbidity and mortality. Although extensive research has focused on the impact of subarachnoid blood on large diameter cerebral arteries, little is known regarding how SAH affects arterioles within the brain

parenchyma. To assess the impact of SAH on global cytosolic Ca^{2+} and arteriolar diameter, intact freshly-isolated parenchymal arterioles (PAs; 30-60 μm diameter) were loaded with the ratiometric Ca^{2+} indicator fura-2. At physiological intravascular pressure (40 mmHg), PAs from SAH animals exhibited significantly elevated cytosolic Ca^{2+} (349 ± 10 nM vs. 248 ± 14 nM) and developed significantly greater (~2-fold) myogenic tone compared with PAs from sham-operated animals. The L-type voltage-dependent Ca^{2+} channel (Ca_V) blocker nimodipine caused ~90% reduction in Ca^{2+} and tone in PAs from both groups suggesting elevated PA Ca^{2+} following SAH results from enhanced L-type Ca_V activity. Increased Ca_V activity may reflect Ca_V upregulation or membrane potential (V_M) depolarization of arteriolar smooth muscle. When V_M was clamped at K^+ equilibrium potential using elevated extracellular K^+ (60 mM; −22 mV), Ca^{2+} and tone were similar between groups, suggesting surface Ca_V density is unchanged by SAH. To examine whether suppression of voltage-dependent K^+ channel (K_V) activity contributes to V_M depolarization, outward K^+ currents were measured in isolated PA myocytes using conventional whole cell patch clamp electrophysiology. Currents sensitive to 4-aminopyridine, a K_V channel blocker, were reduced by ~70% (at +40 mV) in myocytes from SAH animals compared with controls. Taken together, our results suggest decreased K_V channel activity causes V_M depolarization, increased Ca_V activity, elevated cytosolic Ca^{2+} and enhanced constriction of PAs following SAH. Impaired regulation of PA diameter may contribute to local ischemia and neurological deterioration following SAH.

Calcium Fluxes, Sparks & Waves I

536-Pos

Nitric Oxide Can Mediate Beta-Adrenergic- and CaMKII-Dependent Spontaneous Ca^{2+} Waves in Cardiac Myocytes, Independent of PKA Activation

Jerry Curran[1], Donald M. Bers[2], Thomas R. Shannon[1].
[1]Rush University Medical, Chicago, IL, USA, [2]University of California Davis, Davis, CA, USA.

Increased diastolic SR Ca leak can initiate spontaneous Ca waves (SCaWs). SCaWs activate inward Na/Ca exchanger current causing an arrhythmogenic delayed afterdepolarization. Here we examine SCaWs in ventricular myocytes isolated from rabbit hearts. Myocytes did not exhibit SCaWs at baseline conditions, but 43% did when exposed to isoproterenol (ISO). This ISO-induced increase in activity was reversed by inhibition of CaMKII by KN93, but not with PKA inhibition by H89. At similar $[Ca]_{SRT}$ (121 μM) myocytes treated with ISO plus KN93 had significantly fewer SCaWs versus those treated with ISO or ISO plus H89 (0.2 ± 0.28 vs. 1.1 ± 0.28 & 1.29 ± 0.39 SCaWs $cell^{-1}$, respectively). We attribute this increase in activity to the previously characterized CaMKII-dependent increase in RyR-dependent leak. We also find that SR Ca leak is increased by the nitric oxide (NO) donor, SNAP; and this NO-dependent effect is also completely reversed by KN-93. We also show the increase in leak to be dependent on nitric oxide synthase 1 (NOS1) activity. At comparable SR Ca load (132 μM) ISO treated myocytes have significantly higher leak vs. control (8.4 vs. 3.8 μM). The ISO-induced leak (at constant SR Ca load) was attenuated by the NOS1 inhibitor, SMLT, but not the NOS3 inhibitor, L-NIO (3.5 vs. 6.8 μM). Moreover, ISO causes an upward trend in myocyte [NO] (sensed by the NO-dependent dye, DAF-2 A), and the NOS inhibitor, L-NAME significantly attenuated the development of SCaWs. Together this data suggests a novel pathway in which β1-adrenergic receptor activation stimulates NO production via NOS1, which in turn activates CaMKII to increase RyR gating, SR Ca leak, SCaWs and delayed afterdepolarizations.

537-Pos

On the "spark Frequency Vs. Leak Rate" Relationship in Ventricular Myocytes: A Study in the Rabbit

Demetrio J. Santiago, Eduardo Rios, Thomas R. Shannon.
Rush University, Chicago, IL, USA.

Recent studies have linked heart disease to mutated or hyperactive ryanodine receptors (RyRs) in ventricular myocytes, renewing the interest on how the sarcoplasmic reticulum (SR) Ca release process works during diastole. We addressed two questions of potential clinical relevance: (1) whether the SR Ca leak rate (J_{leak}) can be entirely explained by Ca sparks, and (2) whether the spark-dependent fraction of J_{leak} can be varied upon RyR phosphorylation. We used confocal microscopy to simultaneously measure J_{leak} and Ca sparks in Fluo 4-loaded rabbit ventricular myocytes. Control cells (C; n = 47) were paced at 0.5 Hz, while isoproterenol-treated cells (I; n = 14, [Isoproterenol] = 125 nM) were paced at 0.25 Hz to match the SR loads (C = 136.4 ± 5.6 μmoles/l cytosol; I = 126 ± 9.2 ; P = 0.47 in *t*-test with Welch correction). J_{leak} was quantified as in Shannon et al. (2002; *Circ Res* 91:594-600), but using a lower K_m for the forward rate of uptake in the I group. Although J_{leak} did not significantly differ among the groups (C = 10.87 ± 0.93 μM/s; I = 13.12 ± 2.53 ; P = 0.42), the spark frequency was more than doubled in the isoproterenol-treated cells (C = 1.21 ± 0.15 sparks * $(100\ \mu m)^{-1}$ * s^{-1}); I = 2.82 ± 0.51; P = 0.0082). These findings point to an increase in the spark-dependent fraction of J_{leak} upon RyR phosphorylation (for a given SR load), while suggesting an enhancement of Ca-induced RyR coupling relative to the influence of stabilizing RyR couplers.

538-Pos

Regulation of Sarcoplasmic Reticulum Calcium Leak by Cytosolic Calcium in Rabbit Ventricular Myocytes

Elisa Bovo[1], Lothar A. Blatter[1], Aleksey V. Zima[2].
[1]Rush University Medical Center, Chicago, IL, USA, [2]Loyola University Chicago, Maywood, IL, USA.

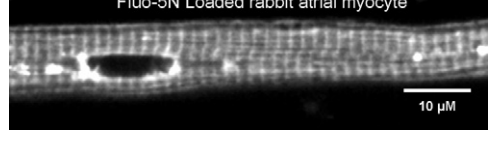
Diastolic sarcoplasmic reticulum (SR) Ca leak determines Ca content and, therefore, the amplitude of action potential-induced global Ca transients in ventricular myocytes. However, the pathways and properties of SR Ca leak have been poorly described. Here, we studied the effects of cytosolic [Ca] ($[Ca]_i$) on SR Ca leak in permeabilized rabbit ventricular myocytes. Using confocal microscopy we simultaneously measured intra-SR free Ca ($[Ca]_{SR}$) with fluo-5N and cytosolic Ca sparks with rhod-2, and monitored SR Ca leak as the change in $[Ca]_{SR}$ over time after complete SERCA inhibition with thapsigargin (10 μM). Increasing $[Ca]_i$ from 150 to 250 nM significantly increased SR Ca leak (by ~30%) over the entire range of $[Ca]_{SR}$. This increase in SR Ca leak associated with an increase in Ca spark frequency. Further increasing $[Ca]_i$ to 350 nM led to rapid $[Ca]_{SR}$ depletion due to the occurrence of spontaneous Ca waves. In contrast, lowering $[Ca]_i$ to 50 nM markedly decreased SR Ca leak rate (by ~60%) and nearly abolished Ca spark activity. When the ryanodine receptor (RyR) was completely inhibited with ruthenium red (50 μM), changes in $[Ca]_i$ between 50 and 350 nM did not produce any significant effect on SR Ca leak, showing that changes of $[Ca]_i$ over a physiological range alter SR Ca leak solely by regulating RyR activity. However, decreasing $[Ca]_i$ to a lower, non-physiologically level (5 nM) activated additional SR Ca leak pathway(s) that were insensitive to RyR or SR Ca ATPase inhibition. In summary, $[Ca]_i$ plays an important role in regulating SR Ca leak by activating RyR and preventing Ca leak through unspecified pathways.

539-Pos

Dynamic Changes of Calcium in Sarcoplasmic Reticulum of Rabbit Atrial Myocytes

Leyla Y. Teos, Julio Altamirano W.J. Lederer.
University of Maryland Biotechnology Institute, baltimore, MD, USA.

With each contraction in atrial muscle, Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptors and then reaccumulated by the activity of the SR Ca^{2+} ATPase (SERCA). Recent experiments by us and others have examined SR Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) using low affinity fluorescent Ca^{2+} sensors (e.g. fluo-5N) in ventricular myocytes (Brochet et al. PNAS 2005 102(8) 3099-104) however, similar characterization in atrial cells is missing. Here we examine $[Ca^{2+}]_{SR}$ dynamics in healthy adult rabbit atrial myocytes using fluo-5N. Fractional SR Ca^{2+}release at room temperature was 30% (n=12), where caffeine was used to determine 100% release. Restoration of $[Ca^{2+}]_{SR}$ by SERCA was consistent with the decline of $[Ca^{2+}]_i$ with each beat (1 Hz). The interconnectivity of the cell-wide SR and nuclear envelope and endoplasmic reticulum was characterized using fluorescence recovery after photobleaching (FRAP). Additional diverse modulations of atrial cell $[Ca^{2+}]_{SR}$ will be discussed.



540-Pos

Spatiotemporal Profiles of Sarcoplasmic Reticulum Ca^{2+} Release in Mouse Atrial Cardiomyoctes *in situ*

Asish Patel, Wen Tao, Hyder Khatri, Michael Rubart.
Indiana University, Indianapolis, IN, USA.

Previous studies in atrial cardiomyocytes isolated from various mammalian species have demonstrated poorly developed transverse(t)-tubules and inhomogeneities of sarcoplasmic reticulum (SR) Ca^{2+} release, but little is known of mice. Here, we examined the t-tubular organization and action potential-evoked Ca^{2+} release in *in situ* myocytes, using confocal microscopy of Langendorff-perfused hearts. Imaging of ANNINE-6plus-stained sarcolemmal membranes revealed paucity of t-tubules in atrial, but densely and regularly spaced t-tubules in ventricular myocytes. However, both myocyte types exhibited regular, striated appearance of type 2 ryanodine receptor distribution. Line-scans