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
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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
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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
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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
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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
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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