526-Pos

Impaired Intracellular Calcium Handling in Atrial Cardiomyocytes from Rats Selected for Low Aerobic Capacity and Metabolic Syndrom

Anne Berit Johnsen, Marcia Alvez, Guri Kaurstad, Natale Rolim, Ulrik Wisløff, Morten A. Høydal.
Department of Circulation and Medical Imaging, University of Science and Technology, Trondheim, Norway.

Background

Atrial fibrillation (AF) is the most common arrhythmia and is often associated with altered Ca^{2+} handling. Evidence points to low aerobic capacity as predictor of cardiovascular disease and AF. The hypothesis of the present study was that rats, genetically selected on the basis of low running capacity has impaired Ca^{2+} handling compared to rats selected for high running capacity.

Method

Low capacity runners (LCR) (n=5) develop characteristics of the metabolic syndrome, and high capacity runners (n=5) develop a healthy and athletic phenotype. The exercise capacity was measured by VO_{2max}. Ca^{2+} handling were measured in isolated Fura-2/AM loaded atrial cardiomyocytes.

Results

VO_{2max} was 30% lower in LCR than HCR. Cardiomyocyte function was significantly depressed in LCR; fractional shortening was 52% lower and time to 50% relenghtening was 42% prolonged. We observed no differences in peak systolic and diastolic Ca^{2+}, as well as no significant difference in amplitude of Ca^{2+} transient between the two groups. This indicate reduced Ca^{2+}sensitivity in cardiomyocytes from LCR. SR Ca^{2+} load, assessed by caffeine-induced Ca^{2+} release, was not different between groups. Time to 50% Ca^{2+} transient decay was prolonged by 58% in LCR. We found no difference in Ca^{2+} decay between the two groups during caffeine induced Ca^{2+} transient, reflecting unaltered NCX function; neither did we find any changes in the plasma membrane Ca^{2+} exchanger (PMCA). The impaired diastolic Ca^{2+}removal is therefore mainly attributed to reduced SERCA function in LCR (39%). Diastolic SR Ca^{2+} leak was increased in LCR compared to HCR.

Conclusion

Our data demonstrate differences in atrial Ca^{2+}handling between rats selected for high and low aerobic capacity and we suggest that impaired Ca^{2+} handling in LCR may be an important factor in initiation of AF.

527-Pos

Role of the Putative NAADP Receptor, Two-Pore Channel 2, in Ventricular Myocyte Responses to Isoproterenol

Laura C. Elson, William D. Owen, Arie R. Gafson, John Parrington, Antony Galione, Derek A. Terrar, Stevan Rakovic.
University of Oxford, Oxford, United Kingdom.

NAADP is a potent endogenous Ca^{2+}-mobilizing molecule. It modulates signalling in several different cell types, including atrial[1] and ventricular[2] cardiomyocytes, by releasing Ca^{2+} from a lysosome-related acidic compartment distinct from the endo/sarcoplasmic reticulum. A recent study has suggested that the molecular target of NAADP is the two-pore (Ca^{2+}) channel, TPC2[3]. Here, we examine the role of TPC2 in ventricular myocyte responses to the β-adrenoceptor agonist isoproterenol, which has been demonstrated to utilize NAADP as a second messenger in these cells[2]. Ventricular myocytes isolated from transgenic mice with absence of TPC2 expression ($TPC2^{-/-}$) and corresponding wild-type (WT) control mice were electrically field-stimulated (3 ms pulses; 36°C). Cell contractions were measured using an edge detection system. Ca^{2+} transients were imaged using Nipkow spinning-disk confocal microscopy (fluo-5F-AM). There were no significant differences in contraction or Ca^{2+} transient amplitudes under control conditions. However, isoproterenol (3 nM) elicited significantly smaller increases in $TPC2^{-/-}$myocyte contraction amplitude when compared to WT myocytes (165 ± 57 % vs. 688 ± 114 % following 2 min application, n=6,4, $p<0.01$). The increases in Ca^{2+} transient amplitudes elicited by isoproterenol (3 nM) were also significantly smaller in $TPC2^{-/-}$ myocytes compared to those seen in WT myocytes (58 ± 17 % vs. 186 ± 37 %, n=6,5, $p<0.05$). There were no significant differences in contraction or Ca^{2+} transient amplitudes following washout of isoproterenol. We conclude that TPC2 is important in mediating the actions of isoproterenol in ventricular myocytes. These data also support the hypotheses that β-adrenoceptors utilize NAADP as an intracellular messenger, and that TPC2 is important in mediating NAADP actions in mammalian cells.

1. Collins *et al*., *Biophys. J.* 96(3):512a.
2. Macgregor *et al*., (2007) *J. Biol. Chem.*282(20):15302-15311.
3. Calcraft *et al*., (2009) *Nature*459(7246):596-60.

528-Pos

Biphasic Effect of Reactive Oxygen Species on Skeletal Muscle Sarcolemmal Ca^{2+} Influx

George G. Rodney, Luke Michaelson, Guoli Shi, Chris W. Ward.
Univerisity of Maryland School of Nursing, Baltimore, MD, USA.

In striated muscle, sarcolemmal Ca^{2+} influx (SCI) serves to maintain the store of Ca^{2+} within the sarcoplasmic reticulum during repetitive contractions. Furthermore, dysregulated SCI is suggested to underscore the altered Ca^{2+} homeostasis implicated in the pathogenic progression of muscular dystrophy. Despite its importance, we have little insight into the regulation of SCI in health and disease. In this study we investigated the role of oxidative stress on SCI. We report that in wild-type myofibers, oxidation has a biphasic effect on SCI as estimated via the Mn^{2+} quench technique. At low [H_2O_2] (<50μM), basal SCI was enhanced (+ 70%) while at higher [H_2O_2] (200μM) SCI was decreased (−48%). This biphasic effect of oxidation was also evident during long K^+ depolarization's in BTS paralyzed myofibers where low [H_2O_2] enhanced (+82%) while high [H_2O_2] decreased (−15%) SCI. Pre-incubation of myofibers with reduced glutathione diminished the oxidation induced alterations in SCI, allowing us to conclude that oxidation modulates SCI in wild-type myofibers. Myofibers from the *mdx* mouse exhibited an exuberant basal SCI compared to wild-type muscle (+873%). Pre-incubation of *mdx*fibers with reduced glutathione decreased SCI back toward wild-type, suggesting that the exuberant SCI in *mdx* myofibers may be a potential therapeutic target via. redox modulation. Taken together, these data support a hypothesis in which low levels of ROS stimulate SCI while higher levels are inhibitory. Ongoing studies are addressing the role of ROS production during strenuous exercise as a dynamic regulator of SCI in health and disease.

529-Pos

The Purinergic Agonist, ATP, Inhibits IP_3-Evoked Ca^{2+} Release in Smooth Muscle

Debbi MacMillan, Charles Kennedy, John G. McCarron.
University of Strathclyde, Glasgow, United Kingdom.

Adenosine 5-triphosphate (ATP) mediates a variety of biological functions following nerve-evoked release via activation of either G-protein coupled P2Y- or ligand-gated P2X- receptors. In various smooth muscle types, ATP, acting via P2Y receptors, acts as an inhibitory neurotransmitter. The underlying mechanism(s) remain unclear, but have been proposed to involve Ca^{2+} release from the internal store and subsequent activation of Ca^{2+}-activated potassium channels (K_{Ca}) to cause hyperpolarisation. Clearly, this proposal requires Ca^{2+} release from the internal store. In the present study ATP induced reproducible transient increases in the intracellular Ca^{2+}concentration in only approximately 10% of voltage-clamped single smooth muscle cells and failed to evoke Ca^{2+} release in the remainder. These results do not support activation of K_{Ca} as the major mechanism underlying inhibition of smooth muscle activity. Interestingly, ATP and its metabolite, adenosine 5-diphosphate, each inhibited inositol 1,4,5-trisphosphate (IP_3)-evoked Ca^{2+}release in cells that did not show a Ca^{2+} increase in response to purinergic stimulation. The inhibitory effect of ATP on IP_3-evoked Ca^{2+} release was blocked by the $P2Y_1$-selective receptor antagonist MRS2179. In conclusion, the present study demonstrates, for the first time, an inhibitory effect of purinergic activation via $P2Y_1$ receptors on IP_3-evoked Ca^{2+} release in smooth muscle.

Supported by the British Heart Foundation and Wellcome Trust.

530-Pos

AKAP150 is Required for NFATC3 Transcriptional Regulation of Kv2.1 and BK Channel Expression in Smooth Muscle

Madeline Nieves-Cintron, Manuel F. Navedo, John D. Scott, Luis F. Santana.
University of Washington, Seattle, WA, USA.

In smooth muscle, activation of the Ca^{2+}-sensitive phosphatase calcineurin dephosphorylates the transcription factor nuclear factor of activated T cells c3 (NFATc3). Upon de-phosphorylation, NFATc3 translocates into the nucleus of arterial smooth muscle cells where it modulates the expression of multiple genes including Kv2.1 and the α and β1 subunits of large conductance K^+ (BK) channels. Recent work by our group, suggested that L-type Ca^{2+} channels, the A-kinase anchoring protein 150 (AKAP150), calcineurin, and PKCα form a signaling triad that controls Ca^{2+} influx into these cells. Ca^{2+} influx via L-type Ca^{2+}channels is necessary for NFATc3 activation in smooth muscle cells during angiotensin II signaling. In this study, we tested the hypothesis that AKAP150 is required for the down regulation of Kv2.1 and BK channel α and

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