Cardiac Muscle III

3894-Pos Board B622

Analysis of the Inner and Outer Environment of Sarcolemma in Cardiac Muscle Cells

Zuzana Nichtová[1,2], Marta Novotová[1], Ivan Zahradník[1].
[1]Institute of Molecular Physiology and Genetics, Slovak Academy of Science, Bratislava, Slovakia, [2]Faculty of Pharmacy, Comenius University, Bratislava, Slovakia.

The peripheral envelope of cardiac myocytes interfaces their inner and outer environment to perform its specific functions. We used stereological analysis of electron micrographs to estimate the association of plasmalemmal structures with their neighbors. Left ventricular papillary muscles of adult rats were dissected and processed for electron microscopy. Uniform random images of the sarcolemma were taken from longitudinal sections at 50,000× magnification and analyzed using a cycloid grid. Intersections of cycloids with structures occurring in the 100 nm wide layer adjacent to the surface sarcolemma of myocytes were counted and the relative occurrence of individual structures was calculated. The cytoplasm dominated at caveolae and nexuses. Myofibrils reached the highest relative occurrence at desmosomes and nexuses, while mitochondria and sarcoplasmic reticulum at the plain plasmalemma (PPL) and at the mouths of t-tubules (MTT). Other small cytosolic structures occurred rarely and only at the PPL. The cavity of caveolae was almost completely covered by the basal lamina (BL). The desmosomes were equally covered with BL and by neighbor myocytes. The PPL was covered by BL 10x more than by myocytes. The MTTs were covered 10x more by BL than by external fluid. The nexuses, by definition, connected neighbor myocytes. Endothelium or collagen approached the surface of myocytes very rarely. The stereologic analysis of the peripheral envelope of cardiac myocytes revealed how specifically is the sarcolemma structured within its inner and outer environment and provided its quantitative characterization.
Supported by APVV-0721-10, VEGA 2/0116/12, VEGA 2/0203/11, by FaF UK/29/2013.

3895-Pos Board B623

Bridging Integrator 1 (BIN1)-Induced T-Tubule Formation in Cardiomyocytes

David B. Lipsett[1,2], Neha Singh[3], Michael Frisk[1,2], Jan Magnus Aronsen[1,2], Ole M. Sejersted[1,2], Ivar Sjaastad[1,2], J. Andrew Wasserstrom[3], Geir Christensen[1,2], William E. Louch[1,2].
[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway, [2]KG Jebsen Cardiac Research Center and Center for Heart Failure Research, University of Oslo, Oslo, Norway, [3]Feinberg Cardiovascular Research Institute, Northwestern University School of Medicine, Chicago, IL, USA.

The T-tubule system is a branching network of membrane invaginations essential for efficient Ca^{2+}-induced Ca^{2+} release in myocytes. Significant remodeling of this system has been observed during heart failure (HF), resulting in delayed Ca^{2+} release at sites lacking T-tubules. Despite its considerable role in HF aetiology, the signaling mechanisms behind T-tubule remodeling remain largely unknown. Bridging integrator 1 (BIN1) has recently drawn considerable interest due to its altered expression during HF and ability to tubulate membranes. In rats examined 6 weeks following myocardial infarction, BIN1 transcription was significantly elevated in animals with compensated or end-stage HF, compared to SHAM operated controls. Although overall T-tubule density was unchanged, confocal imaging of isolated cells stained with di-8-ANEPPS confirmed that the transverse pattern of T-tubules was lost in failing cardiomyocytes, as indicated by reduced peak power in fast Fourier transforms. Interestingly, between-peak power was increased, consistent with the increased fraction of longitudinal tubules visible in failing cardiomyocytes. The role of BIN1 in T-tubule growth was investigated using HL-1 cells, a differentiated murine cardiac cell line lacking both intrinsic BIN1 and T-tubules. When transfected with hBIN1, HL-1 cells developed BIN1-positive invaginations as early as 12 hours following transfection. Whereas BIN1 transcript and protein levels rapidly rose to a peak 24 hours following transfection, BIN1-generated tubules gradually increased in density up until 48 hours after transfection. Additionally, early Ca^{2+} release at sites containing BIN1-positive tubules indicated that these structures improve Ca^{2+} release synchrony across the cell. We propose that BIN1 is a crucial regulator of T-tubule development in both healthy and failing cardiomyocytes. In the setting of heart failure, increased BIN1 expression may promote growth of longitudinal tubules that compensate for loss of transverse elements, thereby improving calcium homeostasis in this disease.

3896-Pos Board B624

Bin-1 Expression in Normal Rat Cardiac Myocytes and in Myocytes with Reduced T-Tubule Density Due to Cell Culture or Heart Failure

Hannah M. Kirton, Matthew Hardy, Edward White, Derek Steele.
School of Biomedical Sciences, University of Leeds - Faculty of Biological Sciences, LEEDS, United Kingdom.

Bridging Integrator-1 (BIN-1), member of the BAR domain protein superfamily, has been implicated in the formation and maintenance of membrane invaginations in skeletal and cardiac muscle cells. However, it is not clear whether BIN-1 expression and t-tubule density are correlated in all circumstances. The aim of the present study was to investigate the relationship between t-tubule density and BIN-1 expression in (i) fresh atrial and ventricular myocytes (ii) ventricular myocytes cultured for 24-72 hours and (iii) ventricular myocytes from animals with monocrotaline-induced right heart failure (HF). Confocal microscopy was used to assess t-tubule density and BIN1 expression in rat myocytes using di-8-ANEPPS fluorescence or immunofluorescence respectively. In fresh ventricular myocytes, a regular t-tubule network was apparent and BIN-1 exhibited a corresponding striated pattern (n=10). In atrial myocytes t-tubules were typically absent and BIN-1 immunofluorescence was only detectable in the peripheral sarcolemma (n=20). In ventricular myocytes after 24, 48 or 72 hrs cell-culture, t-tubule density was reduced to 61 $\pm$ 1.4%, 26 $\pm$ 0.6% and 11 $\pm$ 0.5% respectively (n=10, 8 and 6, $P<0.05$), while mean cell area did not change significantly. Correspondingly, BIN1 expression was significantly reduced in ventricular myocytes at 48 (to 17 $\pm$ 0.14%, n=7, $P<0.05$) and 72 hrs (to 16 $\pm$ 0.09%; n=5, $P<0.05$) compared with control. In right ventricular myocytes, from rats with right HF and reduced t-tubule density, BIN1 expression decreased significantly (69 $\pm$ 1.3%; n=7, $P<0.05$) compared with saline-injected rats. These results suggest that where t-tubules are absent (e.g. atrial cells) or in circumstances where t-tubule density is decreased (cell culture, HF) BIN-1 expression is correspondingly reduced. This supports the hypothesis that BIN-1 has an important role in formation and maintenance of t-tubules.

3897-Pos Board B625

Involvement of TRPC in the Slow Force Response Observed in Mouse Ventricular Myocytes

Yohei Yamaguchi, Toshiyuki Kaneko, Keiji Naruse, Gentaro Iribe.
Okayama university, Okayama, Japan.

When cardiac muscle is stretched persistently, the contractile force slowly increases over several minutes. This phenomenon is termed by a slow force response (SFR). The SFR is due to a stretch-induced increase in Ca^{2+} influx, however, underlying mechanism remains unclear. The transient receptor potential, canonical (TRPC) channels are mechano-sensitive, non-selective cation channels. We hypothesized that activation of TRPC channels is involved in stretch-induced increase in Ca^{2+} influx in SFR. To test this hypothesis, mouse cardiac myocytes were isolated with collagenase, and a pair of computer-controlled piezo-positioned carbon fibers was attached to each cell-end. Cells were paced at 1 Hz and superfused in normal Tyrode solution. Passive and active forces were calculated from carbon fiber bending. Stretch was applied by moving carbon fiber positions to achieve 3-10 % increase in sarcomere length and maintained for 200 to 300 seconds to obtain SFR. At the plateu of SFR, the contractile force increased to 115.7 $\pm$ 3.7% (n = 16) of the value immediately after the stretch. Inhibition of TRPC channel with BTP-2 (10 μM) significantly reduced the magnitude of SFR (103.5 $\pm$ 1.0%, n = 8). It has been reported that activation of angiotensin II type 1 (AT1) receptor induces Ca^{2+} influx via TRPC channels. To reveal whether TRPC channels are activated secondarily by AT1 receptor activated by strech, we tested the effect of Olmesartan, an AT1 receptor inhibitor, in SFR. Olmesartan (10 μM) also reduced the magnitude of SFR (101.1 $\pm$ 2.4%, n = 8). These results suggest that stretch-induced activation of AT1 receptors leads to increase in Ca^{2+} influx via TRPC channels in SFR.

3898-Pos Board B626

Hypercholesterolemia Protects Against Ischemia-Induced Ventricular Tachycardia and Ventricular Fibrillation

Antonius Baartscheer, Cees A. Schumacher, Marieke W. Veldkamp, Arie O. Verkerk, Ruben Coronel.
Academic Medical Center, Amsterdam, Netherlands.

Background:
Membrane cholesterol regulates ion channels. Hypercholesterolemia protect against ventricular fibrillation in patients with myocardial infarction. Hypothesis: Hypercholesterolemia increases action potential duration due to altered ion channels function and protects against ischemia induced re-entrant arrhythmias.

Methods:
QTc intervals were measured in 10 week old LDL-receptor (LDLr-/-) and APO1 (APO1-/-) knockout mice, and wild type mice (WT). Action potentials, calcium handling and ion currents were recorded in ventricular myocytes. In perfused hearts regional ischemia was induced by ligating the left anterior descending artery . Arrhythmias inducibility was tested every 30 s by applying premature stimuli followed by a 500 ms pause. The area at risk (AAR) was determined by perfusion with Evans Blue.
Results:
Serum LDL cholesterol was higher in LDLr-/- and serum HDL cholesterol was lower in APO1-/- mice than in WT. Resulting in an increased cholesterol content in ventricular myocytes. The L-type calcium current was increased in LDLr-/- and APO1-/- (12.1 ± 0.7 and 12.8 ± 0.8) compared to WT (9.4 ± 1.1 pA/pF) resulting in altered calcium handling in LDLr-/- and APO1-/- vs WT mice (increased calcium transient and fractional SR calcium release) and prolongation of AP and QTc duration (APD90 102 ± 4 and 106 ± 3 vs 84.4 ± 3.1 and QTc 50.9 ± 1.3 and 52.3 vs 43.8 ± 1.18 ms, respectively.In LDLr-/- and APO1-/- hearts 1.7 and 1.3% of the attempts to induce arrhythmias resulted in VT/VF compared to 6.9% in WT. There was no significant difference in the induction of premature beats although (12.8% in WT vs 15.5 and 15.8 in LDL-/- and APO-/-). The AAR was not significantly different in LDLr -/-, APO1-/- and WT mice.
Conclusion:
Hypercholesterolemia protects against the occurrence of re-entrant arrhythmias during myocardial ischemia due to AP prolongation caused by an increase of the L-type calcium current.

3899-Pos Board B627
Apico-Basal Gradient of Repolarization Over the Left Ventricle Determines Arrhythmia Susceptibility in Mice

Morten B. Thomsen, Tobias Speerschneider, Søren Grubb, Søren-Peter Olesen, Kirstine Calloe.
University of Copenhagen, Copenhagen, Denmark.

Combined prolongation and spatial heterogeneity of cardiac action potential (AP) duration is proarrhythmic in larger mammals; however the short murine AP suggests a different mechanism underlying arrhythmia induction. Expression of KChIP2 is critical for the stabilization of Kv4.2 in mice, and KChIP2$^{-/-}$ abolishes the transient outward K^+ current, $I_{to,f}$. We have previously shown that KChIP2$^{-/-}$ mice have lower susceptibility to pacing-induced ventricular tachyarrhythmia than normal wild-type mice. Moreover, aorta constriction causing heart failure also lowered proarrhythmia susceptibility in wild-type mice.
In vivo recording of QRS duration from surface ECG suggested comparable conduction velocities in KChIP2$^{-/-}$ and wild-type mice and QRS prolongation associated with heart failure. Next, we used floating microelectrodes to record APs from different regions of the explanted, perfused mouse heart to test the hypothesis that short and spatially heterogeneous AP morphologies contributes to arrhythmia susceptibility in mice. Left ventricular (LV) APs were prolonged in KChIP2$^{-/-}$ (APD$_{90}$:50 ± 3ms, n=6) compared to wild-type hearts (39 ± 3ms, n=6; $P<0.05$). Right ventricular APs were similar in KChIP2$^{-/-}$ and wild-type hearts, producing a larger left-to-right AP dispersion in KChIP2$^{-/-}$ (17 ± 5 versus 7 ± 3ms, respectively). Importantly, LV apico-basal dispersion of AP duration was smaller in KChIP2$^{-/-}$ hearts than in wild-type hearts (2 ± 1 versus 13 ± 4ms, respectively; $P>0.05$).
Despite prolonged APs in KChIP2$^{-/-}$ and heart failure, arrhythmia susceptibility was low. A large apico-basal AP gradient was found in the proarrhythmic wild-type mice, strongly suggesting that this is an important determinant for arrhythmia vulnerability. Contrary to larger mammals, left-to-right ventricular dispersion of AP duration was not associated with vulnerability to pacing-induced arrhythmia.

3900-Pos Board B628
Effect of Different Currents and Extracellular Potassium Ion Concentration on Anodal Excitation of Cardiac Tissue

Sunil M. Kandel, Bradley J. Roth.
Physics, Oakland University, Rochester, MI, USA.

Background:
In order to understand defibrillation completely and thereby to design better defibrillators, we need to understand fibrillation and the effect of electrical stimulation on cardiac tissue. We address this issue by studying anodal excitation applied to the refractory tissue and by measuring the refractoriness of the cardiac tissue through the strength-Interval (SI) curve. The anodal SI curve contains a "dip" in which the S2 threshold increases as the interval increases. Our goal is to find the mechanism of the dip and to determine how calcium currents, sodium calcium exchange (NCX) current and elevated extracellular potassium ion concentration, [K]e, influences the dip in the anodal strength-interval curve.
Methods and Results:
Computer simulations of unipolar stimulation were performed using the bidomain model, with membrane kinetics governed by the Luo-Rudy model. The SI curve is determined by applying a threshold stimulus at different time intervals after a previous action potential. The dip disappears with no NCX current, but is present with 50% and 75% reduction of normal NCX current. The calcium induced calcium release (CICR) current and/or calcium uptake current are not responsible for the dip in the anodal SI curve. High [K]e results in the disappearance of the dip in the anodal SI curve because the tissue remained refractory after the transmembrane potential returned to its resting state.
Conclusions:
Neither NCX nor calcium current is responsible for the dip in the anodal SI curve. It is due to the electrotonic interaction between regions of depolarization and hyperpolarization following the S2 stimulus. The dominance of the electrotonic mechanism emphasizes the importance of the spatial distribution of virtual electrodes during excitation, which ultimately clarifies tissue-shock interactions and optimizes advanced defibrillation protocols.

3901-Pos Board B629
Examination of the Heat-Stress Relationship of Rat Cardiac Trabeculae using an Improved Muscle Calorimeter

Callum M. Johnston[1], June-Chiew Han[1], Bryan P. Ruddy[1,2], Poul M.F. Nielsen[1,2], Andrew J. Taberner[1,2].
[1]Auckland Bioengineering Institute, Auckland, New Zealand, [2]Department of Engineering Science, University of Auckland, Auckland, New Zealand.

The energetic output of cardiac muscle is closely correlated with its mechanical outputs. Using isolated cardiac preparations (papillary muscles and trabeculae) most investigators have found a linear relation between heat production and developed force, where the heat production at zero force indexes the metabolic cost associated with Ca^{2+} cycling, predominantly by the sarcoplasmic reticulum Ca^{2+}-ATPase. To our knowledge, the heat-stress relationship of cardiac muscle at physiological temperature has not yet been reported. Thus, it is of paramount important to extend previous studies to determine the heat-stress relation at 37°C.
Our flow-through micro-mechano-calorimeter is capable of simultaneously measuring the heat output, length change, and force production of isolated rat cardiac trabeculae, but was restricted to room temperature measurements (22°C). Two modifications to the instrument have been made to allow measurements at physiological temperature. We substituted thermoelectric heat pumps for our thin-film thermopile temperature sensors, thereby reducing light sensitivity and lowering the electrical noise floor. We also added other thermoelectric heat pumps for controlling the temperature of the instrument to a set-point of up to 40°C, with 100 µK resolution. Finite element modelling was used to inform and optimise the choice and position of the heat pumps within the device. The heat-rate resolution of our instrument has thereby been improved ten-fold to 5.8 nW over a 5 Hz bandwidth, corresponding to a temperature change of approximately 0.9 µK.
Using the improved calorimeter, we have determined, for the first time, the heat-stress relation of isolated cardiac trabeculae at 37°C. The value of the heat-intercept is at the vicinity of 1.5 kJ m^{-3} per twitch, which amounts to approximately 25 Ca^{2+} ions sequestered by the sarcoplasmic reticulum Ca^{2+}-ATPase within a sarcomere with each twitch.

3902-Pos Board B630
Sarcomere Length Nanometry in Cardiomyocytes Expressed with α-Actinin-AcGFP in Z-Discs

Seine A. Shintani[1], Kotaro Oyama[1], Takashi Ohki[1], Shin'ichi Ishiwata[1,2], Norio Fukuda[3].
[1]Department of Physics, Waseda Univ., Tokyo, Japan, [2]Waseda Bioscience Research Institute in Singapore, Singapore, Singapore, [3]Department of Cell Physiology, Jikei University School of Medicine, Tokyo, Japan.

Nanometry is widely used in today's biological sciences to analyze the movement of molecules or molecular assemblies in cells and in vivo. In cardiac muscle, a change in sarcomere length (SL) by a mere ~100 nm causes a dramatic change in contractility (i.e., the Frank-Starling mechanism), indicating the need for the simultaneous measurement of SL and intracellular Ca^{2+} concentration ([Ca^{2+}]i) in cardiomyocytes at high spatial and temporal resolution. To accurately analyze the motion of individual sarcomeres with nanometer precision during excitation-contraction coupling, we in the present study applied nanometry techniques to primary-cultured rat neonatal cardiomyocytes. First, we developed an experimental system for simultaneous nanoscale analysis of single sarcomere dynamics and [Ca^{2+}]i changes via the expression of AcGFP in Z-discs. We found that the averaging of the lengths