Cardiac Muscle III

3894-Pos Board B622
Analysis of the Inner and Outer Environment of Sarcolemma in Cardiac Muscle Cells
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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.

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

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