muscle. This work was supported by the National Institutes of Health and the American Heart Association.

Platform Z: Cardiac Muscle I

1149-Plat
Ventricular Myocyte Morphology in Long Term Culture
Karin P. Hammer, Sandra Ruppenthal, Anne Vecerdea, Lars Kaestner, Peter Lipp.
Saarland University, Homburg/Saar, Germany.
Previously we have introduced a single cell system allowing long term culturing (1 week) of adult rat ventricular myocytes while maintaining their overall morphology, contractile behaviour and calcium-signalling.
Here, we characterize the subcellular morphology of the myocytes, including the Golgi apparatus, endo-/sarcoplasmic reticulum (ER/SR), plasma membrane and mitochondria. Cells were isolated from adult rats following a standard enzymatic procedure.
Organelles were labelled using targeted expression of fluorescent proteins, e.g. dsRed1 fused to the subunit VIII of human cytochrome C oxidase for mitochondria, YFP fused to a GPI-anchor for the plasma membrane, YFP fused to ts045G for the Golgi apparatus and dsRed2 fused to calreticulin for the ER/SR. Complementing this we also applied fluorescent dyes; di-8-ANEPPS for the plasma membrane and MitoTracker Green for the mitochondria. 3-dimensional stacks of individual cells were acquired with a nipkow-disc based confocal microscope.
Using both labelling approaches, the analysis of the plasma membrane illustrated a gradual loss of the t-tubules during culturing with cytosolic membrane fragments being present for extended time periods.
Mitochondria, which are very prominent and densely packed in cardiac myocytes, underwent an apparent fusion of originally isolated mitochondria, possibly reflecting the loss of t-tubules. While the structure of the ER/SR remained unaltered, the Golgi apparatus underwent a significant redistribution during the culturing time from a wide cytosolic distribution to a perinuclear accumulation.
We provide important evidence that cell morphology changes unavoidably occurring in adult cardiac myocytes during long term culture are highly reproducible and thus strongly support the application of such a single cell model in high-content screening applications.
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1150-Plat
Calcium Independent Positive Inotropy By Fast Cardiac Myosin Motor Gene Transfer In Slow Myosin Dominant Ventricular Myocytes
Todd J. Herron[1], Eric J. Devaney[1], Lakshmi Mundada[1], Sharlene Day[1], Guadalupe Guerrero-Serna[1], Immanuel Turner[1], Erik Arden[2], Margaret V. Westfall[1], Joseph Metzger[2].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of Minnesota, Minneapolis, MN, USA.
Current inotropic drug therapies used to boost cardiac muscle performance focus on elevating the amount of calcium that is mobilized for activation of the myofilaments. These calcium based therapies can provide short term benefits, but when administered long term can actually increase mortality due to calcium overload and the development of fatal arrhythmias. Calcium mobilization has also been the key target for current gene therapy strategies (i.e. SERCA2a over-expression, PLN knockdown, S100 protein expression) to treat the failing heart. Here we present a novel form of calcium independent positive inotropy by fast cardiac myosin motor gene transfer. We designed a recombinant adenovirus to express the full length human α-myosin heavy chain (α-MyHC, *MYH6*) gene in rabbit or human ventricular myocytes that endogenously express almost exclusively β-MyHC. Healthy or diseased adult cardiac myocytes were isolated by enzymatic digestion and maintained in primary culture for 48 hours. Highly efficient α-MyHC gene transfer was confirmed by fluorescent immunocytochemistry and Western blotting. In all cases contractility of single cardiac myocytes was measured 48 hours after gene transfer of α-MyHC by measuring sarcomere shortening and intracellular calcium transients. Sarcomere shortening was ~35% greater in cardiac myocytes transduced with the α-MyHC adenovirus when α-MyHC made up ~30% of the total myosin protein. Intracellular calcium transient amplitudes, however, were not affected by α-MyHC gene transfer. In permeabilized myocyte experiments we found that α-MyHC gene transfer did not affect myofilament calcium sensitivity, but did speed the kinetics of myosin cross bridge transitions from weakly-bound to strongly-bound states. We conclude that α-MyHC gene transfer offers a novel form of calcium independent positive inotropy for β-MyHC dominant adult ventricular myocytes.

1151-Plat
Role of Thin Filament Cooperative Activation in Length-dependent Activation in Skinned Porcine Ventricular Muscle
Takako Terui[1], Yuta Shimamoto[2], Munguntsetseg Sodnomtseren[1,2], Mitsuhiro Yamane[2], Iwao Ohtsuki[1], Shin'ichi Ishiwata[2], Satoshi Kurihara[1], Norio Fukuda[1].
[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Waseda University, Tokyo, Japan.
The basis of the Frank-Starling mechanism of the heart is the intrinsic ability of cardiac sarcomeres to produce greater active force in response to stretch (i.e., length-dependent activation). We have reported that troponin plays a key role in this phenomenon via on-off switching of the thin filament state, in concert with titin-based passive force (Terui et al., *J Gen. Physiol.* 131;275-283:2008). In the present study, we systematically investigated the role of thin filament cooperative activation in length-dependent activation using skinned porcine ventricular muscle at sarcomere lengths of 1.9 and 2.3 μm. MgADP (3 mM) increased Ca^{2+} sensitivity of force and enhanced the speed of contraction, indicating enhanced cooperative activation. MgADP was found to attenuate length-dependent activation, with and without quasi-complete reconstitution of thin filaments with fast skeletal troponin (sTn; from rabbits). Conversely, inorganic phosphate (Pi, 20 mM) decreased Ca^{2+} sensitivity of force and the speed of contraction, indicating reduced cooperative activation. Pi enhanced length-dependent activation, with and without sTn reconstitution. Qualitatively similar results were obtained with MgADP or Pi in rabbit fast skeletal muscle, with higher Ca^{2+} sensitivity of force than in cardiac muscle. Linear regression analysis revealed that the speed of contraction, Ca^{2+} sensitivity of force and length-dependent activation were strongly correlated in both cardiac and skeletal muscle. These results suggest that length-dependent activation is regulated via thin filament cooperative activation, such that the length-dependent increase in the fraction of cross-bridges is less in high cooperative activation states, coupled with a loss of recruitable cross-bridges.

1152-Plat
Length-Dependent Active Tension Development In Single Intact Cardiomyocytes, Isolated From Different Regions Of Guinea Pig Heart
Christian Bollensdorff[1], Oleg Lookin[2], Michiel Helmes[1], Peter Kohl[1].
[1]Oxford University, Oxford, United Kingdom, [2]Institute of Immunology and Physiology, Russian Academy of Sciences, Ekaterinburg, Russian Federation.
Introduction: Information on the force-length relation of intact myocytes from different regions of the heart is scarce. We therefore studied myocytes, isolated from apical and basal areas of guinea pig left and right ventricles (cell numbers: LV_A 22, LV_B 29, RV_A 11, RV_B 12). **Methods:** Force-length relations were measured by attaching carbon fibers to myocytes, allowing application of diastolic stretch while measuring passive and active force.[1] Cells were kept at $36 \pm 1°C$ and paced at 2 Hz. Recorded forces were normalized to cell cross-sectional area and used to construct end-diastolic, end-systolic and active tension-length relations (EDTL, ESTL and ATL=ESTL-EDTL; respectively). In addition, the ratio of the slopes of ESTL and EDTL was used as a cross-section independent factor to characterise the Frank-Starling Gain (FSG) in individual cells. **Results:** For all tissue regions ESTL, EDTL and, hence, ATL, are linear over the range of end-diastolic sarcomere lengths studied (1.88-2.15 μm). Plotting the slope values of ATL vs. EDTL for all cells shows a positive correlation (slope 1.29, R^2=0.24, 74 cells). In addition, FSG is larger than one for all cells studied: RV_B 3.91 ± 0.54), RV_A (2.84 ± 0.30), LV_B (2.77 ± 0.17), and LV_A (3.20 ± 0.24). **Conclusions:** Using the carbon fiber technique, it is possible to probe length-dependence of passive and active tension at the single cell level, without the interference of extracellular structures. The Frank-Starling Gain varies between the different regions of the heart and the positive correlation between ATL and EDTL confirms that in intact cells passive force bearing structures (such as titin) are likely to play a role in modulating length dependent activation.
[1] Iribe et al, Force-length relations in isolated intact cardiomyocytes subjected to dynamic changes in mechanical load. AJP 2007/292:1487-1497.

1153-Plat
Sarcomere Length Dependence Of The Force-pCa Relationship In Cardiac Muscle Is Influenced By Properties Of Thin Filament Regulatory Units
Frederick S. Korte, Dan Wang, Michael Regnier.
University of Washington, Seattle, WA, USA.
Myocardial performance is tightly regulated by sarcomere length (SL), which is thought to be due at least in part to concurrent changes in myofilament lattice spacing and the relative proximity (and binding probability) of myosin heads to actin. Furthermore, crossbridge binding enhances Ca^{2+} binding to troponin (Tn) in cardiac muscle, a unique form of cooperative thin filament activation