Ca^{2+} release. Exposure to the hypoosmotic (254mOsm) extracellular solution elevated $[Ca^{2+}]_i$ by only 7.3 ± 0.04% (n=8; P<0.001) of the response to sucrose. Assessment of t-tubule integrity revealed that exposure to hyperosmotic-sucrose, but not hyperosmotic-Ca^{2+} or hypoosmotic solution, generated visible t-tubule disruption. The mechanism underlying the marked difference in response to sucrose or Ca^{2+} is not presently known.

1522-Pos MG53 Is An Essential Component Of The Acute Membrane Repair Machinery

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An elemental process in cell biology involves dynamic membrane remodeling and repair, which mediates efficient cellular signaling and maintains cell integrity. Membrane repair defects have been linked to numerous disease states including muscular dystrophy, heart failure and neurodegeneration. Repair of the plasma membrane damage requires intracellular vesicular trafficking to injury sites, a process that is thought to involve entry of extracellular Ca through the damaged plasma membrane. While there is significant interest in establishing the mechanisms underlying plasma membrane repair, the molecular machinery that facilitates vesicle translocation during acute membrane repair has not been fully resolved. Here we show that mitsugumin 53 (MG53), a novel muscle-specific tri-partite motif (TRIM) family protein, is a critical component of the acute cell membrane-repair machinery. MG53 interacts with phosphatidylserine to associate with intracellular vesicles, which display dynamic trafficking to and fusion with sarcolemmal membranes. Mice null for MG53 exhibit progressive muscular dystrophy with defective membrane-repair capacity. Acute injury of the sarcolemmal membrane leads to exposure of the cell interior to an external oxidized environment that produces oligomerization of MG53, followed by recruitment of MG53-containing vesicles to patch the injury site. Mutation of a critical cysteine residue in MG53 that disrupts oligomerization of MG53 leads to impairment of repairsome formation at the sites of membrane damage. Together, our dada demonstrate that muscle membrane repair involves two physiological steps that require oxidation-mediated nucleation of MG53 at the injury site followed by vesicle accumulation leading to repairsome formation.

1523-Pos Blebbistatin: Use As A Myocardial Excitation-Contraction Uncoupling Agent

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Blebbistatin (BLEB) is a recently discovered compound that specifically inhibits myosin II ATPase activity. If specific, this agent could be used to uncouple myofilament contractility from the electrical events that lead to cytosolic Ca^{2+} release in the cardiac myocyte. We investigated the mechanical and structural actions of BLEB inhibition in intact and skinned rat cardiac trabeculae, single rabbit psoas myofibrils and in intact isolated rat myocytes. Application of BLEB (10 Î¼M) reduced twitch force in intact trabeculae without altering the orientation or position of the myosin heads under diastolic conditions. BLEB reduced contractile force in skinned trabeculae without affecting tension dependent myofilament ATPase activity (index of cross-bridge cycling kinetics). Application of BLEB to skinned trabeculae (overnight) or intact trabeculae (>15 min/dose) in the dark reduced Ca^{2+} activated force (EC_{50}=0.38+/−0.03 ÃŽÂ¼M skinned fibers; EC_{50}=3.92+/−0.73 ÃŽÂ¼M intact fibers). While the rapid (<5ms) application of BLEB to single Ca^{2+} activated rabbit myofibrils immediately reduced force this rate of force decline depended on the [BLEB]. Two-photon line scan microscopy in isolated myocytes (0.5 μM BLEB, >1hr) with Indo1-AM ratiometric 2-photon analysis revealed no significant impact of BLEB on the Ca^{2+} transient (ΔCa^{2+} 0.033+/−0.002 vs. 0.034+/−0.003; μM, τCa^{2+} 263+/−3 ms vs. 278+/−3 ms; control vs. BLEB resp.; n=31–39). We conclude that BLEB specifically uncouples cardiac myofilament activation from Ca^{2+} activation without affecting the cross-bridge cycling kinetics, EC-coupling, or structural parameters. However, the compound is very sensitive to light, a property that severely limits its application to mechanistic physiological studies.

Excitation-Contraction Coupling - II

1524-Pos Efficient Excitation-Contraction Coupling (ECC) in Adult Rabbit Ventricular Cardiomyocytes Requires Brain-type Na Channels, Na/Ca Exchanger (NCX) and L-type Ca Channels

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We investigated the effect of the Na gradient on transmembrane and Ca release fluxes evoked by action potentials recorded with a patch electrode in ventricular myocytes loaded with fluo-4. In the absence of a Na gradient, both Ca release and transarcolemmal Ca fluxes were reduced. Ca spikes recorded in the absence of a Na gradient occurred at significantly lower probability. We estimated the ECC gain by taking the ratio of release flux to transarcolemmal flux (the latter provides an estimate of the trigger flux). In the presence of a Na gradient the gain was 7.95±1.71 (mean±SEM, n=7 cells). In the absence of the Na gradient the gain was reduced to 3.51±0.6 ($P<0.05$). Since brain-type Na channels may be involved in ECC, we performed additional experiments in which we investigated the effects of TTX 200 nM on Ca fluxes in the presence of a Na gradient. 200 nM TTX selectively blocks brain-type Na channel isoforms Nav 1.1, 1.3 and 1.6 found in the t-tubules of cardiac myocytes. As with removal of the Na gradient, TTX reduced the magnitude and rate of rise of Ca transients and transarcolemmal Ca fluxes. Gain dropped from 7.24±1.27 to 3.31±0.66 (n=7 cells, $p<0.05$), also similar to the decline in the gain obtained by removing the Na gradient. We propose that Na entry via brain Na channels produces an early rise of Na in the dyadic cleft. This stimulates reverse NCX, which primes the dyadic cleft with Ca. In the presence of a dyadic cleft primed with Ca, the Ca current then becomes a more effective sarcoplasmic reticulum Ca release trigger.

1525-Pos $Ca_v3.1$ T-type Calcium Channels are Inefficient in Cardiac Excitation Contraction Coupling and Localize to the Surface Sarcolemma

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Localized primarily in the transverse tubules of ventricular myocytes (VMs), the L-type calcium channels (LTCCs) initiate excitation-contraction coupling (ECC). The function of T-type calcium channels (TTCCs) in VMs, however, remains largely undetermined. Transgenic (TG) mice with cardiac-specific $Ca_v3.1$ expression were generated to explore TTCC function in VMs.

Methods and Results: Isolated TG VMs showed robust $I_{Ca,T}$ (−32.8±3.1 pA/pF, n=15), whereas control VMs showed no $I_{Ca,T}$ (n=11). Despite large $I_{Ca,T}$, ejection fraction of TG hearts (63.1±5.0%, N=11) evaluated by echocardiography was not different from control hearts (61.8±2.5%, N=8). Similarly, contraction of isolated TG VMs (6.4±0.4%, n=13) was not different from control VMs (6.0±0.8%, n=9). However, calcium transients from TG VMs (3.5±0.3, n=13) were larger than control VMs (2.7±0.2, n=9; $p<0.01$). No difference in SR calcium load after caffeine application was seen between TG (4.2±0.4, n=17) and control VMs (4.2±0.8, n=8). TG VMs, however, showed decreased $I_{Ca,L}$ (−9.6±0.7 pA/pF, n=15) compared to control VMs (−12.0±0.4 pA/pF, n=11; $p<0.01$). The voltage dependence of LTCC was shifted to positive potentials with larger half-activation potentials in TG (−5.5±0.8 mV, n=15) than control VMs (−8.6±1.2 mV, n=11; $p<0.05$). Despite a three-fold larger amplitude of $I_{Ca,T}$ than $I_{Ca,L}$, peak systolic calcium and contraction induced by $I_{Ca,T}$ were only 30.2±4.3% (n=5) and 19.9±6.1% (n=5) of those induced by $I_{Ca,L}$, respectively. Immunostaining with $Ca_v3.1$ and $Ca_v1.2$-specific antibodies revealed TTCC localization to the surface sarcolemma and LTCC localization to the T-tubule system. Detubulation of VMs via formamide-induced osmotic shock resulted in 31.9% decrease in $I_{Ca,T}$ (−22.3±1.9 pA/pF, n=5) and 65.5% decrease in $I_{Ca,L}$ (−3.3±0.6 pA/pF, n=5) suggesting differential membrane localization of TTCCs and LTCCs.

Conclusion: Calcium entry via $I_{Ca,T}$ is distant from the T-tubule system and thus ineffective in triggering ECC.

1526-Pos The Differences Between Physiological And Pathological Cardiac Hypertrophy In The Aspect Of Signaling Efficiencies

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The molecular mechanisms deciphering physiological and pathological cardiac hypertrophy have remained unclear. For investigating the mechanisms of the two types of hypertrophy, we attempted a manual curation to collect biochemical information concerning the cardiac hypertrophy from available scientific publications, and constructed a comprehensive hypertrophy signaling pathway map. For efficient analysis, the map was dissected into 14 signaling pathways (SP), and the frequency of differentially expressed gene (DEG) in individual SP was analyzed using Fischer's exact hypergeometric test. By the analysis, we have found three crucial SP that have different patterns of signaling efficiency between the two types of hypertrophy. The quantitative information for the efficiency of individual SP could be used in formulating a mathematical model for the hypertrophy signaling pathways.

1527-Pos Spark Generating Stochastic Dynamics Of Release Unit In A Cardiac Cell

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We apply a simple biophysically-reasonable electron-conformational (EC) model [Moskvin ea, PBMB, 2006] for the ryanodine receptor channel (RyR) to describe the stochastic RyR's cluster gating in calcium release unit (RU) in cardiomyocytes under steady-state conditions. Single RyR channels are characterized by a fast electronic and slow classical conformational degrees of freedom. The RyR gating implies Ca^{2+}-induced Franck-Condon electronic transitions between two branches of a conformational potential, a conformational Langevin dynamics, thermoactivated transitions and quantum tunneling. The sarcoplasmic (SR) load is incorporated into the model through the effective conformational strain. Computer simulation of a single RyR gating shows the electron-conformational model reproduces all the features observed in lipid bilayer experiments. The cooperativity in the RyR lattice is assumed to be determined by the inter-channel conformational coupling. Model simulations for the 11×11 RyR cluster in a calcium RU have revealed different gating regimes with a single RyR channel openings generating a Ca^{2+} synapse (quark) and a cooperative cluster mode, due to a nucleation process with a step-by-step domino-like opening of a fraction of coupled RyR channels, providing for a sufficient release to generate Ca^{2+} sparks. Nucleation and typical features of the spark-generating openings are specified mainly by the strength of the RyR-RyR coupling, SR calcium load, and lumenal Ca^{2+} refilling rate. The SR overload leads to instabilities with strong spontaneous low-frequency modulations of calcium release and clear tendency to auto-oscillation regime with spontaneous RyR channel opening and closure. Overall we show that the model can both successfully reproduce essential features of the steady-state RyR gating in cardiac muscle and uncover the role played by different physiological factors.

Supported by The Wellcome Trust, RFBR Grants 05-04-48352, 07-04-96126, Russian President Leading Scientific School Grant #4923.2006.

1528-Pos Characterization of Calcium Sparks in Transgenic Calsequestrin 2 Knockout Mice

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To further understand the role of calsequestrin 2 (CSQ2) on cardiac function, we studied calcium sparks in isolated ventricular myocytes from transgenic mice partially or totally lacking this protein. Fluo 4 AM-loaded cells were paced at 1 Hz and constantly perfused with physiological saline at room temperature. Once a steady state was reached, the electrical stimulator was turned off and calcium sparks recorded using confocal line-scanning. Eleven frames were routinely collected from each cell, at different locations. Data analysis started once a relatively constant cytosolic [Ca] was reached (from frames 2 to 11) and the detection criteria remained unchanged regardless of the mouse genotype analyzed. Spark frequency, relative amplitude, full width at half maximum, full duration at half maximum, duration, rise time, time to peak and signal mass were compared among samples. Statistical significance was addressed using the unpaired two-tailed *t*-test with Welch's correction for unequal variances, $p < 0.05$. Both rise time and full duration at half maximum were significantly shortened in $CSQ2^{+/-}$ (average ± SEM, respectively, 6.13 ± 0.95 ms and 4.15 ± 0.65 ms, n=24) and $CSQ^{-/-}$ (6.58 ± 0.91 ms and 4.56 ± 0.51 ms, n=43) when compared to the wild-type (13.32 ± 2.09 ms and 9.9 ± 1.13 ms, n=13). The other parameters, including frequency, were not significantly different among the three groups. Our results are in agreement with the previous literature indicating that CSQ2 depletion alters the SR Ca release process through regulation of the sarcoplasmic reticulum Ca channels (Chopra et al, *Circ Res. 101* (6):617–26, 2007).

Supported by R01-HL88635 to BCK

1529-Pos Excitation-Contraction Coupling in Human Embryonic Stem Cell Derived Cardiomyocytes

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Human embryonic stem cell derived cardiomyocytes (hESC-CMs) have tremendous potential both in regenerative medicine and as a model system of early cardiac development. Work to date has generated conflicting results with regard to the mechanisms of excitation-contraction coupling in hESC-CMs as well as their state of maturation. These uncertainties arise in part from the availability of preparations of only low cardiac purity. In the present study, we investigated the calcium handling and contractile properties of near-homogenous preparations of cardiomyocytes, generated from the H7 hESC line using a recently reported, directed differentiation procedure (Laflamme et al. *Nat Biotechnol*. 2007; *25*:1015–1024). After three weeks of in vitro differentiation, hESC-CMs were loaded with the calcium indicator fluo-4/AM, and robust spontaneous and evoked (field stimulation at 1 Hz) $[Ca^{2+}]_i$ transients were observed by confocal microscopy. Transients were completely eliminated by the removal of extracellular calcium (n=13 cells) or the addition of the L-type Ca^{2+}channel blocker diltiazem (10 μM, n= 9 cells), indicating a requirement for trans-sarcolemmal Ca^{2+} entry. On the other hand, the addition of the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase inhibitor thapsigargin (100 nM) also reduced the amplitude of $[Ca^{2+}]_i$ transients by 78±8% (n= 4 cells), indicating a substantial contribution by SR Ca^{2+} cycling. In spontaneously contracting myocytes (24 ± 7 BPM, n = 11), the amplitude of shortening was a robust 10 ± 3 % of muscle length (ML) with a shortening rate of

1.12 ± 0.34 ML/s and a relaxation rate of 0.43 ± 0.13 ML/s. We conclude that the calcium handling and contractile properties of hESC-CMs are surprisingly similar to those of adult human cardiomyocytes.

1530-Pos Regulation of $[InsP_3]_i$ in Embryonic Stem Cell-Derived Cardiomyocytes

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The spontaneous activity in embryonic stem cell-derived cardiomyoyctes (ESdCs) depends on $InsP_3$ mediated Ca^{2+}-release. It has been proposed that the nuclear envelope, where predominant IP_3R staining is described, is the source of this diastolic $InsP_3$-mediated Ca^{2+}-release. In this study we aimed to determine by which mechanism basal $InsP_3$ production is maintained in ESdCs and where the relevant region of $InsP_3$ signaling is located.

We monitored the intracellular $InsP_3$ concentration ($[InsP_3]_i$) with a FRET-based $InsP_3$-biosensors FIRE (Fluorescent $InsP_3$ Responsive Element) where the $InsP_3$-binding domain of $InsP_3$ receptor-1 is fused between CFP and YFP. FIRE-1 exhibits an increase in the fluorescence ratio (F_{530}/F_{480}) upon increasing concentrations of $InsP_3$. Stimulation of ESdCs with Endothelin-1 (100 nM) or phenyephrine (10 μM) resulted in a significant 3.77 % and 1.7 % increase of $[InsP_3]_i$ respectively, concurrent with a 3.04 and 1.51 fold increase in the ESdCs beating frequency. Under control conditions, the phospholipase C (PLC) inhibitor U73122 (1 μM) resulted in a decrease of basal $[InsP_3]_i$ (3.2 %) and suppressed ESdCs spontaneous activity. This data indicates a basal activity of PLC. The basal level of $[InsP_3]_i$ remained unchanged during ESdCs superfusion with 2-APB (2 μM), zero $[Ca^{2+}]_o$, or BAPTA/AM (1 μM) although all interventions significantly attenuate ESdCs spontaneous activity and reduce basal $[Ca^{2+}]_i$ by 18.1, 27.05, and 18.6 % respectively. Attenuation of localized $InsP_3$ signaling by expression of a membrane targeted form of Inositol polyphosphate-5-phosphatase resulted in a 54 % decrease in ESdCs beating frequency and abolished the cells positive chronotropic response to ET-1. In conclusion our data support that

(i) ESdCs spontaneous activity depends on an increased basal PLC activity,
(ii) basal PLC activity is not maintained by $[Ca^{2+}]_i$ and
(iii) ESdCs spontaneous activity and positive chronotropic response depend on $InsP_3$ production at the plasma membrane.

1531-Pos Mechanism of Action of A Novel Positive Inotropic Agent

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N'1-(3,3,6,8-tetramethyl-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yliden)-2-cyanoethanohydrazide (TMOTHNYCE) has been found to increase intracellular Ca^{2+} level in non-myocardial cells in preliminary screening assays. The purpose of the present study was to examine if TMOTHNYCE exerts positive inotropic effects on acutely isolated rabbit left ventricular myocytes, and if so to further define the potential mechanism of action. Sarcomere length of contracting rabbit ventricular myocytes (electronically paced at 1 Hz and 37 °C) was continuously recorded using Ion Optix data acquisition system. Changes in fractional shortening of sarcomere length (calculated from the diastolic and systolic sarcomere length) were used to evaluate changes in myocyte contractility. L-type Ca^{2+} current of rabbit ventricular myocytes was also measured in separate experiments using whole-cell voltage-clamp techniques. TMOTHNYCE increased the fractional shortening of sarcomere length in a concentration-dependent manner and the peak L-type Ca^{2+} current in a voltage-dependent way (for example, current amplitudes were increased by 4.0-fold at −10 mV and 1.5-fold at +10 mV). Voltage-dependence of steady-state activation of L-type Ca^{2+} current was shifted by 15 mV in the negative direction with the slope factor being unchanged. Inactivation time course of the L-type Ca^{2+} currents at voltages of −10 to 20 mV was significantly slowed by 0.3 uM TMOTHNYCE (at 20 mV: fast time constant (τ) = 34 ± 4 ms for control, 54 ± 3 ms for the drug, n = 6, $P < 0.01$; slow τ = 115 ± 16 vs 415 ± 46 ms, n = 6, $P < 0.01$). In conclusion, this novel positive inotropic agent increases ventricular myocyte contractility by enhancing Ca^{2+} influx through L-type Ca^{2+} channel. Both negative shifting of the voltage-dependence of the L-type Ca^{2+} channel activation and the reduced inactivation are two mechanisms responsible for the enhanced L-type Ca^{2+} current.

1532-Pos Excitation-Contraction Coupling in a Mice Model of CPVT

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare disease that occurs in subjects without obvious structural heart disease and characterized by adrenergically mediated bidirectional or polymorphic ventricular tachycardia leading to syncope and/or sudden cardiac death. Point mutations in the Ca^{2+} release channel (RyR-2) were found in CPVT patients, suggesting that alterations in Ca^{2+} handling underlie this type of arrhythmia. Here we analyzed

excitation-contraction (EC) coupling using a knock-in mouse-model carrier of the $RyR^{R4496C+/-}$ mutation, equivalent to the human mutation R4497C identified in CPVT families. Littermates $RyR^{R4496C-/-}$ were used as controls. We analyzed Ca^{2+} release in whole hearts by two-photon microscopy using Rhod-2 as a dye. EC coupling was investigated by simultaneously recording L-type Ca^{2+} currents (I_{Ca}) by patch-clamp and Ca^{2+} release by confocal microscopy (MetaZeiss LSM510). The patch pipette contained 50 μmol/l Fluo-3 pentapotassium salt. Sarcoplasmic reticulum Ca^{2+} load was estimated by rapid 10 mmol/L caffeine application to isolated cells. The associated Na^+/Ca^{2+} exchanger current (I_{NCX}) was recorded by patch clamp and integrated to estimate the amount of Ca^{2+} released by the sarcoplasmic reticulum. The amplitude of the I_{NCX} current was also analyzed. We observed no difference in basal conditions in I_{Ca} in control *vs* R4496+/− cells at all voltages tested (in pA/pF, at 0 mV: −6.98±0.39 in 13 control cells, *vs* −5.5±0.7 pA/pF in 5 R4496+/− cells; p=0.06). $[Ca^{2+}]_i$ transient elicited by I_{Ca} was also not significantly different between control and R4496+/− cells at all voltages tested (in peak F/F_0 at 0 mV: 2.42±0.20 F/F_0 in 13 control cells *vs* 2.44 ± 0.21 in 5 R4496+/− cells; p>0.05). Our results are in consonance with the normal cardiac function of $RyR^{R4496C+/-}$ mice showed under basal conditions.

1533-Pos Phospholamban Phosphorylation Increases the Rate of Calcium Leak From the SR

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Phospholamban (PLB) is a 52 amino acid integral membrane protein of the sarcoplasmic reticulum (SR) that exists in both monomeric and pentameric forms. In its unphosphorylated state, PLB inhibits the SR Ca^{2+}-pump (SERCA). This inhibition is relieved when PLB is phosphorylated by protein kinase A (PKA) as a result of beta-adrenergic stimulation to the heart. Consistent with predictions from molecular models, pentameric PLB has also been shown to form Ca^{2+}-selective channels in artificial liposomes. Although regulation of SERCA by PLB is well established, it is not known if PLB also functions as a channel *in situ* nor is it known if phosphorylation affects the conductance of the PLB channel. Using the calcium indicator dye Fura2, we have observed that phosphorylation of PLB by PKA leads to an increase in the rate of calcium leak from the SR. This enhanced rate of calcium leak from the SR is also observed when a PLB specific antibody (A1) which mimics phosphoryaltion of PLB is used. Presence of 20 μM ruthenium red did not affect the increased rate of calcium leak from the SR after phosphorylation, arguing against a possible role of ryanodine receptors in mediating the leak. Further evidence for an *in situ* PLB channel was obtained from HEK cells co-expressing SERCA2a with either WT- PLB or I40A-PLB (which does not form pentameres). Phosphorylation of WT-PLB with PKA or presence of A1 antibody increased the rate of calcium leak from ER membranes of the HEK cells. This increased rate of calcium leak was not observed when I40A mutant was co-expressed with SERCA2a. These results suggest that PLB as a pentamer forms a calcium permeable channel in the SR and that the permeability of the PLB channel is enhanced when PLB is phosphorylated.

1534-Pos Reduced Ca Buffering And SR Ca Content In The Phospholamban Knockout Mouse During Beta-Adrenergic Stimulation

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Ca buffering is important in determining the size and shape of the systolic Ca transient. The greater the buffering power then the smaller the amplitude of free Ca will be for a given change in total Ca. In cardiac cells, Ca can be bound to various buffers including troponin (Robertson et al., 1982, J. Biol. Chem.) and potentially the SR-Ca-ATPase (SERCA; Higgins et al., 2006, Biophys. J.). Phosphorylation of troponin following beta-adrenergic stimulation increases the Kd for Ca resulting in a decrease in the buffering power. Conversely, SERCA phosphorylation via an effect on phospholamban increases Ca affinity, thereby decreasing the Kd and resulting in increased Ca buffering. We were interested in examining the effects of these two buffers and thus performed studies in the phospholamban knockout ($PLN^{-/-}$) mouse in which we hypothesised that a decrease in buffering would be apparent following beta-adrenergic stimulation. Buffering experiments were performed with isolated ventricular myocytes using the whole cell patch clamp technique with Cs^+-based pipette solutions containing the Ca indicator Fluo-5F. Caffeine was used to empty the SR and buffering measured as previously described (Trafford et al., 1999, Plfügers Archiv.). No differences in Ca buffering were observed in the wild-type mouse (n=7) following beta-adrenergic stimulation with isoproterenol (100 nM). However, as predicted isoproterenol decreased the Ca buffer slope in the $PLN^{-/-}$ mouse ($p<0.01$, n=4). Furthermore, SR content was decreased in the $PLN^{-/-}$ mouse after beta-adrenergic stimulation but was increased in the wild-type mouse. We conclude that phosphorylation of phospholamban increases Ca buffering by SERCA but that in control mice this effect is opposed by a decrease of buffering by troponin.

1535-Pos Calcium Overload Induces Ectopic Electrical Activity And Abnormalities Of Left Ventricular Function In An Isolated Working Heart

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Calcium overload in cardiomyocytes results in spontaneous sarcoplasmic reticulum mediated Ca^{2+} release with the potential for triggering lethal arrhythmias. While the effects of calcium overload have been extensively studied within isolated cell and papillary muscle preparations, the effects on the whole heart remain unclear. To investigate changes in cardiac function during Ca^{2+} overload, a rabbit working heart preparation perfused with a modified Tyrodes's solution (2.5mM [Ca^{2+}]) was established. Simultaneous measurement of intraventricular pressure and volume with an intraventricular catheter enabled characterisation of left ventricular function. Preload and afterload were set at 10cmH_20 and 75cmH_20 respectively. Aortic pressure, flow rates and electrical activity were measured with an additional pressure catheter, in-line flow meters and a bi-polar electrogram. Ca^{2+} overload was induced by increasing extracellular [Ca^{2+}] to 4.5mM and then addition of 150nM isoproterenol (ISO). While perfusion with a higher [Ca^{2+}] did not alter heart rate (HR) (206.9±10.1 vs. 205.1±10.5bpm; 2.5mM vs. 4.5mM [Ca^{2+}]; n= 7), developed pressure (DP) (80.4±2.0 vs. 84.1±2.2mmHg) and +dp/dt (1669±109 vs. 1944±109mmHg.s^{-1}) increased significantly. Addition of ISO significantly increased HR (206.9±10.1 vs. 251.7±24.9; 2.5mM vs. 4.5mM [Ca^{2+}] + ISO after 50s), DP (80.4±2.0 vs. 97.4±3.8) and +dp/dt (1669±109 vs. 3530±183 mmHg.s^{-1}) with no change in coronary flow. After 50s, ectopic events were observed. These events increased in frequency from 0.08±0.03.s^{-1} (50s after ISO) to 0.61±0.14.s^{-1} (200s after ISO) while HR, DP and +dp/dt continued to decrease (230±18bpm, 85.2±18.0mmHg, and 2885±217mmHg.s^{-1}; 200s after ISO). Aortic flow ceased approximately 600s after ISO. Ventricular fibrillation did not occur in any of the hearts. These observations suggest that Ca^{2+} overload not only induces ectopic activity but also compromises ventricular mechanical function.

1536-Pos Spatiotemporal Dynamics of Subcellular Ca^{2+} Alternans

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SR Ca^{2+} release\reuptake in Langendorff-perfused intact normal rat heart measured by confocal fluorescence (fluo-4) microscopy during rapid pacing exhibits considerable heterogeneity at the sarcomere level, which can and typically does proceed to the development of subcellular Ca^{2+} alternans–i.e., Ca^{2+} alternans comprised of multiple sarcomeric-level regions of beat-to-beat out-of-phase alternations in SR Ca^{2+} release\re-uptake. We have recently begun to explore whether a model that emphasizes bi-directional coupling between Ca^{2+} and voltage in alternans development can be utilized to elucidate mechanisms underlying these complex patterns of subcellular Ca^{2+} cycling. The dynamic Ca^{2+} cycling in the empirical development and stability of subcellular Ca^{2+} alternans was found to be consistent with positive Ca^{2+}-voltage coupling–i.e., spatially dyssynchronous sarcomeric Ca^{2+} release\re-uptake manifest in subcellular Ca^{2+} alternans eventually synchronizes as whole-cell Ca^{2+} alternans. Yet, in many instances there was redevelopment of subcellular Ca^{2+} alternans later in the rapid pacing epoch after sarcomeric Ca^{2+} release\re-uptake synchronized, which positive Ca^{2+}-voltage coupling dynamics alone cannot explain. Careful examination of the empirical records revealed two important features of such behavior. First, the degree of sarcomeric Ca^{2+} transient alternans varied substantially across the cell, even when spatially synchronized. Second, when subcellular Ca^{2+} alternans emerge from an initially synchronized (but heterogeneous) pattern, the timing of synchronized activations also alternated–i.e., cycle length (CL) alternans were observed that were out-of-phase with the synchronized sarcomeric Ca^{2+} alternans (large/small Ca^{2+} transient corresponded to an early/late activation). Upon incorporating these features into the model, subcellular Ca^{2+} alternans that emerged from initially synchronized Ca^{2+} alternans could be simulated. These results represent a novel mechanism for the formation of subcellular Ca^{2+} alternans in which intrinsic differences in sarcomeric properties leading to varying degrees of Ca^{2+} alternans magnitude, and small CL alternations out-of-phase with the initial synchronized Ca^{2+} alternans are essential elements.

1537-Pos Trimetazidine Rescues Calcium Transient and Mechanical Alternans in Cardiac Myocytes from the Failing Heart

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Background: Heart failure (HF) and ischemia associate with high plasma levels of free fatty acids (FFA) that lead to energy depletion and decreased contractility. Clinical parameters of ischemia are improved by trimetazidine (TMZ), an agent that inhibits FFA oxidation. However, the potential of TMZ for HF treatment remains to be established. The aim of this study was to evaluate the effects of TMZ on altered Ca^{2+} signaling and contractility observed during HF.

Methods: Experiments were performed on ventricular myocytes isolated from an arrhythmogenic rabbit model of nonischemic HF (combined aortic insufficiency and constriction). Fluorescence microscopy with indo-1, fluo-4, CM-H_2DCF or calcein was used to measure intracellular calcium ($[Ca^{2+}]_i$), ROS production or mitochondrial permeability transition pore (PTP) activity, respectively. Cell shortening was recorded simultaneously with $[Ca^{2+}]_i$.

Results: Field stimulation (1 Hz) of single HF myocytes elicited $[Ca^{2+}]_i$ transient and mechanical alternans which were rescued by treatment with 1 μM TMZ. The alternans ratio (AR), an indicator of the severity of arrhythmogenic alternans, decreased (from 0.50±0.08 to 0.06±0.04, n=6) after 15 min of TMZ application. Moreover, addition of the β-adrenergic agonist isoproterenol (1 μM) to HF cells increased the severity of $[Ca^{2+}]_i$ transient alternans (AR=0.69±0.04, n=4) and led to cell death; both were prevented by TMZ (AR=0.05±0.01, n=4). The critical event leading to cell death is the opening of mitochondrial PTP. Therefore, we examined PTP activity in HF cells, and found that TMZ prevented Ca^{2+}-induced

opening of the mitochondrial PTP. This preventative effect was directly related to the ability of TMZ to decrease the amount of ROS generated during fast pacing or mitochondrial Ca^{2+} overload.

Conclusion: TMZ rescues $[Ca^{2+}]_i$ transient and mechanical alternans by inhibiting the mitochondrial PTP opening induced by elevated ROS generation in the failing heart.

1538-Pos I_{K1} Remodeling In Ageing; A Protective Role Against Arrhythmias

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Board B514

In a population of increasing longevity, ageing is a major risk factor for the development of heart failure and arrhythmias. The inward rectifier potassium current, I_{K1}, is crucial for stabilising the resting membrane potential of cardiac myocytes. Reductions in I_{K1} contribute to an increased susceptibility to arrhythmias in heart failure (Pogwizd *et al*., *Circ Res* 88: 1159). We investigated the role of I_{K1} in age-associated arrhythmogenesis.

Currents were recorded from left ventricular myocytes from young (approx. 18 months) and old (> 8 years) sheep using the whole-cell voltage clamp technique. Protein expression was assessed using Western blotting.

I_{K1} currents were confirmed by sensitivity to Ba^{2+}. Peak and steady-state I_{K1} currents were increased in the old sheep ($P<0.05$ @ −130mV). This increase was not accompanied with a difference in steady-state I_{K1} conductance. I_{K1} activation kinetics were increased in the old sheep ($P<0.05$ @ −130mV), however, the fractional inactivation of I_{K1} was also increased in these cells ($P<0.05$ @ −130mV). We then used the Hodgkin Huxley model to assess whether or not the slower activation in the young myocytes masked inactivation and thus true peak current amplitude. Peak current amplitude remained greater in the old myocytes together with faster activation and inactivation time constants ($P<0.05$). We investigated if subunit isoform switches could explain these kinetic changes using Western blotting. We found no change in K_{ir} 2.1 expression but a down-regulation of K_{ir} 2.3 in the old myocytes. Using action potential modeling, the increase in I_{K1} was sufficient to prevent the generation of EADs that occurred when simulating the age-associated increase in $I_{Ca\text{-}L}$ (Dibb *et al.*, *J. Mol Cell Cardiol* 37: 1171). We conclude that age-associated changes in I_{K1} may confer a protective effect resulting in an anti-arrhythmogenic mechanism.

1539-Pos Vdac2 Interacts With Ryr2 In Mouse Cardiomyocytes

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Ryanodine receptor type 2 (RyR2) is the major homotetrameric intracellular Ca^{2+} release channel located in sarcoplasmic reticulum of cardiac cells. The N-terminal side of RyR2 serves as a scaffold protein which holds proteins to regulate Ca^{2+} release from SR. To identify putative RyR2 binding proteins, we performed a bacterial two-hybrid screening using a cytoplasmic region of mouse RyR2 and found VDAC2 as a candidate protein for the RyR2 binding. The interaction of VDAC2 with RyR2 was further confirmed by GST pull down and co-immunoprecipitation assays. To investigate the roles of VDAC2 in cardiomyocytes, we attempted to use adenovirus-based siRNA system to knock down VDAC2 gene expression. Viral infection of HL-1 cells with VDAC2-siRNAs effectively reduced the expression of VDAC2 protein to 25%. The basal level of cytosolic Ca^{2+} increased significantly in VDAC2 knockdown cells, as compared with control ($p < 0.001$). The underlying mechanism for the increased basal Ca^{2+} level by knockdown of VDAC2 is currently under investigation using various molecular approaches.

1540-Pos Knock-down (kd) Of Histidine-rich Calcium Binding Protein (hrc) Elevates Ca^{2+} Cycling Functions In Cardiac Sarcoplasmic Reticulum (sr)

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Board B516

The histidine-rich Ca^{2+} binding protein (HRC) is a SR luminal protein that binds to triadin and SERCA and could affect Ca^{2+} cycling in the SR through the interaction with RyR and SERCA (Arvanitis *et al. Am. J. Physiol. Heart. Circ. Physiol,*. 2007). In the present study, we tested the hypothesis that HRC regulates the Ca^{2+} cycling activity through the interactions with RyR2 and SERCA2 in cardiac cells using a siRNA approach to KD HRC. The results showed that the expression level of HRC was effectively decreased by 70% without any noticeable changes in the expression levels of other major E-C coupling proteins. HRC-KD led to 106% higher maximal velocity of Ca^{2+} release and 135% higher maximal velocity to reach the baseline. HRC-KD also increased the peak height by 76%, but decreased the time to 50% decay by 20%. There was no significant change in the SR Ca^{2+} load. Taken together, these results suggest that HRC play an important role in the regulation of SR Ca^{2+} cycling, through the interactions with RyR2 and SERCA2.

1541-Pos Overexpression Of Calumenin Impairs Sarcoplasmic Reticulum(sr) Ca2+ Homeostasis In Rat Neonatal Heart Cells

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Calumenin is a 6 EF-hand calcium binding protein localized in the lumen of SR. It contains N-terminal signal peptide and C-terminal ER retention signal, HDEF. The previous report showed that calumenin was up-regulated in human idiopathic dilated cardiomyopathy (Grzeskowiak et.al. *Cardiovasc. Res.* 2003). To gain insight into the physiological function of calumenin in heart, calumenin was overexpressed (Cal-Over) in rat neonatal cardiomyocytes with adenovirus-mediated overexpression system. The results showed no change in the expression levels of major EC-coupling proteins such as SERCA2, RyR, PLN and calsequestrin in Cal-Over cells. However, field stimulation-induced Ca^{2+} transients in fura-2 loaded Cal-Over cells showed increased time to peak Ca^{2+} transient (0.09±0.002 vs. 0.076±0.001 s, n=28 and 22, $P<0.001$) and time to 50% base line (0.270±0.008 vs. 0.216±0.007 s, n=28 and 22, $P<0.001$). SR Ca^{2+} uptake rate, however, decreased in Cal-Over cells (5.89±0.465 vs. 8.38±0.53 nmol/min/mg protein, n =3, $P<0.05$). We found evidence that calumenin is directly associated with SERCA2. Taken together these results suggest that calumenin is involved in the regulation of SR Ca^{2+} homeostasis perhaps through the direct interaction with SERCA2.

1542-Pos Three Separate Microfluidic Compartments For Fast Solution Change Around Regions Of The Adult Ventricular Myocytes

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Previously intracellular gradients in cardiomyocytes have been experimentally established through the exposure of two separate extracellular regions of the cell to different concentrations of a permeable agent, e.g. caffeine. Here we describe the microfluidic integration of the perfusion of three separate extracellular compartments, a central pool in addition to the two endpools. An adult ventricular myocyte was laid across three open microfluidic channels, the two outer ones for the cell ends and the middle one for the cell centre. This arrangement generated three extracellular compartments sealed against each other through paraffin oil, which in addition formed the lid of the open channels. The leakage across the seal was compensated for through the continuous perfusion of the three microfluidic channels. For the fast solution change in the end pools a micropipette mounted on a piezo translator was stepped from the overlayer of paraffin oil through the oil/water interface into the selected channel. The performance of the experimental setup was tested using the chemical stimulation of Ca2+ transients with caffeine in electrically paced cardiomyocytes. The uniform rise in [Ca2+]i upon electrical stimulation was replaced through regional Ca2+ transients in the cell ends stimulated with caffeine (1 mM). The automated separate caffeine application to the two endpools enabled the generation of trains of regional Ca2+ transients in response to repetitive caffeine puffs (0.5 Hz), where the transients were out of phase in the separate cell ends if the puffs were out of phase. This arrangement was used to examine the refilling of the intracellular Ca2+ store (sarcoplasmic reticulum, SR) in one cell end through Ca2+ uptake and transport from the other cell end.

1543-Pos Effects Of Hydrogen Peroxide On Calcium Handling In Rat Cardiac Myocytes

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Board B519

Reactive oxygen species (ROS) are implicated in the pathogenesis of myocardial depression in disease states such as sepsis. Hydrogen peroxide (H_2O_2) can be used as a free radical generating system to mimic intracellular enzymatic ROS production. The effects of mM concentrations of H_2O_2 on cardiac myocyte calcium handling at room temperature were assessed by Goldhaber *et al* (1994: J. Physiol. *477*, 135) who found a decreased L-type Ca^{2+} current and sarcoplasmic reticulum (SR) content. However, since H_2O_2 cytotoxicity is temperature dependent we have reinvestigated the effects at 37°C. Rat ventricular myocytes were loaded with Fluo-3AM for cytoplasmic calcium ($[Ca^{2+}{}_i$) measurement. Membrane currents were measured via perforated patch under voltage clamp conditions. 200μM H_2O_2 caused a 44% decrease in $[Ca^{2+}]_i$ transient amplitude (n=10, Control:272(±29), H_2O_2:153(±17)(nmol/l), $p<0.0001$) and a 30% increase in diastolic $[Ca^{2+}]_i$ (n=10, Control:93(±5), H_2O_2:121(±7)(nmol/l), $p<0.0001$). Associated with this was a 61% decrease in systolic cell shortening (n=7, Control:2.24 (±0.43), H_2O_2:0.87(±0.14)%, $p<0.05$), a 27% decrease in SR Ca^{2+} content (n=6, Control:95(±11), H_2O_2:69(±7)(μmol/l), $p<0.05$), a 2.4% decrease in diastolic cell length (n=7, Control:123(±8), H_2O_2:120(±8)(μm), $p<0.005$) and a 42% increase in cell relaxation time (n=7, Control:526(±57), H_2O_2:746(±118)(ms), $p<0.05$). We found no significant effect on the L-type Ca^{2+} current amplitude and therefore, this cannot account for the decreased $[Ca^{2+}]_i$ transient amplitude. The rate of decay of the $[Ca^{2+}]_i$ transient was decreased by 18% (n=10, Control:4.63(±0.49), H_2O_2:3.79(±0.36)(RC/s), $p<0.005$) consistent with a decrease of SERCA activity which may also account for the decreased SR Ca^{2+} content. The decreased $[Ca^{2+}]_i$ transient amplitude and contractility can be explained by the decrease in SR Ca^{2+} content. In summary, under the conditions of our experiments, the major factor accounting for the depressed contractility is a decrease of SR Ca^{2+} content independent of the L-type Ca^{2+} current.

1544-Pos Impact of Hydroxyl Radicals on Calcium Handling and Myofilament Sensitivity in Isolated Myocardium

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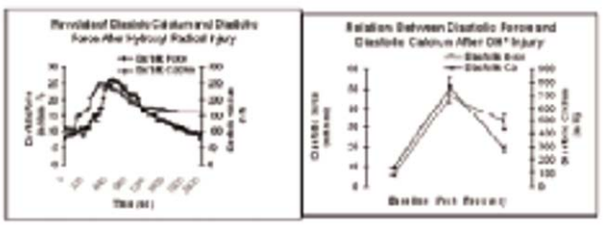
Hydroxyl radicals (OH*) are involved in the pathogenesis of reperfusion injury, which is observed in many clinical situations

including acute heart failure, stroke and myocardial infarction. Two different sub-cellular defects are involved in the phenotypical OH*-injury, deranged calcium handling and alterations of myofilament responsiveness. In this study we temporarily delineate the impact of these mechanisms at different time points on OH* injury. In order to elucidate the injury response, we measured the amplitude and kinetics of calibrated intracellular Ca^{2+} transients and twitch contractions simultaneously using ionophoretically loaded bis fura-2. This figure shows the relationship between intracellular calcium and diastolic force after the exposure of OH* in rabbit trabeculae (n=8). Initially there is a marked increase in resting tension (6.27± 0.71 to 46.4 ± 3.49 mN/mm^2) in parallel with an increase in diastolic calcium (136.6 ± 7.65 to 779.4 ± 64.63 nM). After the peak injury response, diastolic calcium returned to near-normal levels, whereas diastolic force development remained significantly elevated. Our results indicate that calcium overload is mainly responsible for acute diastolic dysfuntion after OH* injury, while sustained myocardial dysfunction is mainly due to the alterations in myofilament responsiveness.



1545-Pos Multi-Scale Modeling of Ventricular Cardiac Myocytes: Image Analysis, Geometric Processing, and Numerical Simulation

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The geometry of sub-cellular structures in ventricular cardiac myocytes plays a critical role in regulating the cells' functions. There have been a number of mathematical models that aim to simulate calcium release and propagation both locally and globally in ventricular cells. However, most of the existing models are still proof-of-concepts, based on certain simple geometries. Building realistic geometric models from imaging data still poses a big challenge in mathematical simulation of ventricular cardiac myocytes.

We present a chain of image and geometric processing approaches for constructing multi-scale and realistic 3D models of ventricular cardiac myocytes. Two types of imaging data are considered: one is the confocal light microscopy images of whole cells and the other is the electron microscopy images of individual calcium release units (CRUs). Images are pre-processed by anisotropic filtering and contrast enhancement techniques. Sub-cellular structures (T-tubules, junctional SRs, etc.) are extracted by automatic image analysis techniques including 3D image segmentation and skeletonization. High quality surface and volumetric meshes are generated for finite element simulation of excitation-contraction (E-C) coupling in ventricular cardiac myocytes. Stochastic models are combined with continuum methods to better describe calcium spark generation and wave propagation based on the realistic geometries extracted from imaging data.

We have developed novel image analysis techniques, high-quality mesh generation toolkits, and fast numerical solvers based on finite element methods. The software packages will be made available under the standard license terms in the Finite Element ToolKit (FETK) website (http://www.fetk.org) and the National Biomedical Computation Resource (NBCR) website (http://nbcr.sdsc.edu/).

1546-Pos An Integrative Model for the Mechanisms of Excitation-Contraction Coupling in Rat Ventricular Myocytes

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Board B522

An integrative biophysical detailed model of cardiac excitation-contraction (EC) coupling for the rat ventricular myocytes was developed to undertand its mechanism. Due to the rapid rate of Ca2+ cycling in the rat (resulting from the high heart rate) there are fundamental differences from the guinea pig myocyte data which Jafri and co-workers modeled previously (Jafri et al., 1998). This requires the reformulation of Ca2+-handling by developing a modified description of L-type Ca2+ current and Na+-Ca2+ exchanger. The rat has a shorter action potential with different morphology than the guinea pig. This is thought to be due to the properties of the potassium channels. We reformulate three potassium currents (Ito, Iss, IK1), which give strong characteristics of the cardiac AP in rat ventricular myocytes (no plateau phase and shorter APD) and the Na+ current, based on the experimental literature. This new formulation simulates the negative force-frequency relation observed in experiments on rat ventricular myocytes. In the models this is due to the interplay between the rate of recovery of the ryanodine receptor (RyR) and loading of the sarcoplasmic reticulum (SR). The peak RyR open probability decreases with increased pacing frequency as there is less time for RyR recovery from inactivation (restitution). The SR Ca2+ load decreases with increased frequency in contrast to the guinea pig which does the opposite and has a dome shaped force-frequency relation. The new rat model also simulated mechanical restitution and low levels post-extrasystolic potentiation levels seen in experiments with rat.

Electron Transfer Systems

1547-Pos Effect of Q_o site Inhibitor JG144 on Electron Transfer Reactions of Cytochrome bc_1

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Long range movement of the iron-sulfur protein (ISP) between the cytochrome b (cyt b) and cyt c_1 redox centers plays a key role in electron transfer within the cyt bc_1 complex. JG144 is a new cytochrome bc_1 Q_o site inhibitor that immobilizes the ISP in the b conformation. Electron transfer in the cyt bc_1 complex was studied using the ruthenium dimer, Ru_2D, to rapidly photooxidize cyt c_1 and initiate the reaction. Flash photolysis of a solution containing reduced wild-type *Rhodobacter sphaeroides* cyt bc_1 and Ru_2D results in photooxidation of cyt c_1 within 1 μs, followed by electron transfer from the iron-sulfur center [2Fe2S] center to cyt c_1 with a rate constant of $k_1 = 60{,}000\ s^{-1}$. JG144 binding to the Q_o site decreases k_1 to $10{,}000\ s^{-1}$ indicating that a conformational change on the surface of cyt b decreases the rate of release of the ISP from cyt b. The effects of JG144 binding on electron transfer were also studied in a series of mutants in the cyt b *ef* loop of *Rhodobacter sphaeroides* cyt bc_1 in order to examine the role of this loop in controlling the capture and release of the ISP from cyt b.

Supported by NIH grants GM20488, GM30721, and RR15569.

1548-Pos Mutagenesis of Cytochrome f Y160 and R156: Effects on Redox Properties

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Protein functional groups interacting with heme propionates have often been suggested to affect the redox properties of the heme. Structures of cytochrome f show one of the heme propionates to interact with Y160 and R156. To examine whether such interactions influence the redox properties of the cytochrome f heme, we have performed site directed mutagenesis of these sites in the lumenal domain of cytochrome f from Chlamydomonas reinhardtii. A Y160L mutant was found to shift the redox potential of cytochrome f by −20–30 mV. Such a shift could be due to either the loss of a hydrogen bond between the Y160 phenolic group and the heme propionate or due to the loss of aromaticity at this position. To answer this question, we have prepared a Y160F mutant of cytochrome f and found its redox potential to be identical to that of the wild type protein. We conclude that the loss of aromaticity at this position and not the loss of a hydrogen bond to the heme propionate is responsible for the redox potential change in the Y160L mutant. The same heme propionate is also observed to interact electrostatically with R156. A R156L mutant has also been prepared. In contrast to the other mutants in which the reduced form is stable to air, the resulting R156L mutant is highly susceptible to air oxidation, suggesting that the loss of the electrostatic interaction between R156 and the heme propionate significantly alters of the protein's redox properties.

1549-Pos Cation-Pi Interactions in Cytochrome f: Relation to Electron Transfer Between Cytochrome f and Plastocyanin

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Board B525

Structures of cytochrome *f* show the presence of two cation-pi interactions which appear to be in locations of potential structural or functional importance. One is the K165/Y171 interaction which is located at the interface of the large and small domains of cytochrome f. The other interaction is the R13/Y149 interaction which appears to help position the heme binding loop relative to the rest of the large domain. At last year's meeting, results were presented indicating these interactions to be contributors to the stability and redox properties of cytochrome f. To assess whether these interactions might also influence the electron transfer properties of cytochrome *f*, we have performed of site directed mutagenesis of these sites in the water soluble lumenal domain of cytochrome *f* from Chlamydomonas reinhardtii. R13 and K165 were replaced with I, A, and G in an S106C mutant designed for covalent attachment with photoactive Ru-complexes. The mutants were labeled with Br-CH2-(bpy)Ru(bpy)2. Flash illumination results in rapid electron transfer from the excited state of the Ru-complex to the heme. When plastocyanin is included, the subsequent electron transfer from the cytochrome f to the plastocyanin copper can be observed. Only minor differences were observed among the mutants when compared to wild type suggesting that the K165/Y171 and R13/Y149 cation-pi interactions do not have a major impact on the interaction of plastocyanin with cytochrome f nor on electron transfer between the two proteins.

1550-Pos Stigmatellin Induces Reduction of Iron Sulfur Protein in the Oxidized Cytochrome bc_1 Complex

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Board B526

Stigmatellin, a Q_P site inhibitor, inhibits electron flow from iron-sulfur protein (ISP) to cytochrome c_1 in the cytochrome bc_1