10 days after EC. Resting intracellular Ca^{2+} concentration ($[Ca^{2+}]_{rest}$) was measured *in vivo* using Ca^{2+} selective microelectrodes on days 0, 2, 4, 6, 8, and 10 after EC. In ***group A*** the average $[Ca^{2+}]_{rest}$ was 1113.8 nM on day 0 and did not change on subsequent days. On days 0, 2, 4, 6, 8 and 10 $[Ca^{2+}]_{rest}$ (in nM) in polarized-exercised muscle fibers in ***group B*** was 395 ± 38, 381 ± 35, 334 ± 23, 295 ± 39, 268 ± 27, and 223 ± 27**.** In ***group C*** (EC+pGz) $[Ca^{2+}]_{rest}$ was 402 ± 46, 304 ± 26, 260 ± 35, 145 ± 16, 121 ± 10 nM and 113 ± 6, showing a significant difference from group B by day 2 and reached control by day 10. In ***group D*** (EC+pGz+LNAME) the beneficial effect of pGz on $[Ca^{2+}]_{rest}$ was abolished. We conclude *that* EC induced a chronic elevation of $[Ca^{2+}]_{rest}$ in skeletal muscle that lasted up to 10 days and that pGz was able to accelerate the return to normal $[Ca^{2+}]$ homeostasis following EC induced muscle injury. The salutary effect of pGz on $[Ca^{2+}]_{rest}$ appears to be mediated by an increase in NO generation, since the NOS blocker, L-NAME, eliminated the effect of pGz on $[Ca^{2+}]_{rest}$ after EC.

2605-Pos Board B375

Basal PKC Activity Regulates Spontaneous Firing of Cardiac Pacemaker Cells

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Spontaneous beating of sinoatrial node cells (SANC) is linked to rhythmic, submembrane local Ca^{2+} releases (LCR) from the sarcoplasmic reticulum (SR). LCRs activate an inward Na^+/Ca^{2+} exchange current, imparting an exponential increase to the later diastolic depolarization, to bring membrane potential to the threshold and fire an action potential (AP). Previously we have demonstrated that basal PLC activity plays a key role in spontaneous firing of the cardiac pacemaker via regulation of SR Ca^{2+} cycling, but not IP_3 receptor Ca^{2+} release. The specific mechanisms that underlay PLC-dependent modulation of spontaneous SANC firing are still unclear. Here we show that SANC pacemaker activity is critically dependent on downstream target of PLC, i.e. protein kinase C (PKC). In freshly isolated rabbit SANC inhibition of PKC activity by GF109203X (10 μmol/L) markedly suppressed both spontaneous SANC beating by ~70% and LCR's parameters (confocal microscopy, Fluo-3 as Ca^{2+} indicator). Specifically, GF109203X decreased the LCR size (from 5.8 ± 0.3 to 2.8 ± 0.3 μm) and number per each spontaneous cycle (from 1.4 ± 0.2 to 0.7 ± 0.1); increased the LCR period, i.e. the time from the prior AP-induced Ca^{2+} transient to the subsequent LCR. The increase in LCR period during PKC inhibition predicted an increase in the spontaneous cycle length. All effects of GF109203X were reversed upon washout. Since Ca^{2+} cycling in SANC is critically dependent on L-type Ca^{2+} current ($I_{Ca,L}$), which contributes to the AP upstroke and modulates the SR Ca^{2+} content, we studied effects of GF109203X on $I_{Ca,L}$. PKC inhibition markedly suppressed $I_{Ca,L}$ amplitude (from 8.1 ± 1.0 to 4.6 ± 1.0 pA/pF), strongly suggesting that $I_{Ca,L}$ could be a major target of basal PKC activity in SANC. Thus, basal PLC-dependent regulation of spontaneous SANC firing is fulfilled through basal PKC-dependent modulation of Ca^{2+} cycling, specifically LCR's parameters and $I_{Ca,L}$ amplitude.

2606-Pos Board B376

How Ca^{2+}-Activated, cAMP/PKA-Dependent Phosphorylation Signaling Mediates Pacemaker Cell Activity: Experimental and *In Silico* Biochemical and Biophysics Perspectives

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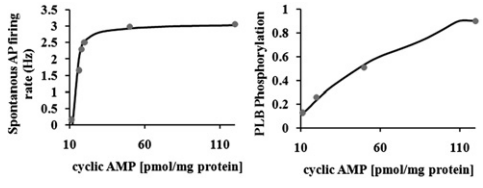
Ca^{2+}-activated, adenylate cyclase (AC)-cAMP/PKA-dependent phosphorylation of both surface membrane electrogenic proteins of (''Membrane-clock''), and of intracellular proteins that generate rhythmic Ca^{2+} oscillations (''Ca^{2+}-clock''), regulate the periodicity of each clock, and couple the two-clocks to regulate sinoatrial node cells (SANCs) normal automaticity.

We developed a novel numerical model to simulate the coupling of SANC **Ca^{2+}-AC-cAMP/PKA** signaling to functions of surface membrane and Ca^{2+} cycling molecules. The model incorporates experimentally measured a term for Ca^{2+}-dependent AC-activity, and when Ca^{2+} changes predicts resulting changes in downstream cAMP/PKA phosphorylation-dependent signaling that produce changes in ion channels conductance and intracellular Ca^{2+} kinetics that ultimately change the spontaneous action potential (AP) firing rate.



Model predictions of Ca^{2+}-dependent changes in the cAMP/PKA phosphorylation cascade, of spontaneous AP firing rate and of the stoichiometry-relationships between cAMP, phospholamban phosphorylation and AP firing rate (line in the figure) faithfully reproduced the experimentally measured variables and their stoichiometry (points in the figure).

The simulations of this novel integrative model of biochemical and biophysical signaling within the coupled-clock model further support the importance of high throughput signaling via Ca^{2+}-AC-cAMP-PKA phosphorylation cascade in normal SANC automaticity.

2607-Pos Board B377

Crosstalk Between Calcium and cAMP in Pancreatic Alpha Cells

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Glucagon secretion from pancreatic alpha cells can be controlled by a variety of factors including hypoglycemia-induced increase in calcium or by hormones that stimulate cAMP production. The mechanisms by which calcium and cAMP signals influence each other and glucagon secretion have yet to be resolved. Using fura2 imaging, we found that acute hypoglycemia-triggered calcium-induced calcium release (CICR) in clonal mouse alpha cells was sensitive to the calcium channel blockers, nifedipine and w-conotoxin-GVIA. CICR with similar channel pharmacology was triggered by forskolin-induced elevation of [cAMP] revealing cAMP-stimulation of $[Ca^{2+}]_i$. This CICR likely involved multiple cAMP targets as both PKA-selective and EPAC-selective cAMP analogs each triggered CICR. In addition, a role for calcium regulation of [cAMP] was revealed by hypoglycemia-triggered increase in [cAMP] as reported by imaging of an EPAC-based cAMP FRET indicator. This was presumably due to calcium regulation of adenylyl cyclase or cAMP phosphodiesterase (PDE). Multiple isoforms of adenylyl cyclase and PDEs including Ca-sensitive AC3 and PDE1 were detected by RT-PCR in alpha cells. PDE3- and PDE4-selective inhibitors had distinct effects on calcium and cAMP dynamics and whole cell calcium currents. These data suggest multiple points of cAMP and calcium crosstalk in regulating glucagon secretion.

2608-Pos Board B378

Subcellular Modeling of PKA Activation and cAMP Diffusion in Localized Microdomains of Adult Cardiomyocytes

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The beta adrenergic pathway in cardiomyocytes activates protein kinase A (PKA) to phosphoregulate several Ca^{2+} handling proteins, including the L-type Ca channel, ryanodine receptor, and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (via phospholamban), resulting in inotropic, lusitropic and chronotropic responses. However, based on PKA kinetics measured *in vitro*, almost all PKA should be activated by basal concentrations of cAMP. Several recent studies have postulated a role for local degradation of cAMP by phosphodiesterases (PDE) in maintaining microdomains with lower cAMP concentrations. However, the degradation rate of cAMP is slow compared with the rate of cytosolic diffusion, suggesting that local clustering of PDE is insufficient to maintain the cAMP microdomains alone. In this study, Virtual Cell, a finite volume solver, was used to create 3D diffusional models that were then validated with *in vitro* FRET experiments to probe other potential mechanisms of PKA's compartmentalized response. We examined the effects of structural obstruction, cAMP buffering by exchange protein directly activated by cAMP (EPAC), PKA isoform localization, ion gradients, and physical coupling between PKA and PDE, mediated by A-kinase anchoring protein (AKAP). Our results suggest that structural obstructions, obtained from cryo-TEM images, are insufficient to decrease the rate of diffusion of cAMP. However, PKA isoform localization around the SERCA pumps is essential in maintaining phosphoregulation. Furthermore, AKAP complexes and basal ion concentrations also contribute to localized control of PKA. These *in vitro* and *in silico* experiments help us understand how microdomains in adult cardiomyocytes are maintained.

2609-Pos Board B379

EPAC Activation of Cardiac Muscle

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Exchange protein-directly activated by cAMP (EPAC) has been shown to be involved in the classical cAMP-activated protein kinase A (PKA) pathway in many different cell types. However, in the cardiac muscle the effects of EPAC have yielded contradictory results with reports of increased (Oestrich, 2007) and decreased (Pereira, 2007 & Cazorla, 2009) Ca^{2+} transient amplitude in mice and rats, respectively. The aim of this study was to examine the role of EPAC activation in excitation-contraction (EC) coupling in isolated rat ventricular muscle preparations. To activate the EPAC pathway, we used

8-(4-chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate (cpTOME), an analogue of cAMP. Simultaneous measurements of intracellular calcium ($[Ca^{2+}]_i$) with Fura-2 and isometric force were made in RV trabeculae before and during cpTOME application. At 1.5 mM $[Ca^{2+}]_o$, 10 μM cpTOME had no effect on the amplitude of $[Ca^{2+}]_i$ transients (0.7 ± 0.1 to 0.7 ± 0.1, $P = 0.35$) or on twitch force (24.1 ± 5.2 mN mm^{-2} to 22.80 ± 4.62 mN mm^{-2}, $P = 0.20$) (n = 7). However, at 0.5 mM $[Ca^{2+}]_o$, cpTOME increased peak stress from 10.5 ± 2.8 mN mm^{-2} to 15.0 ± 2.7 mN mm^{-2}, $P = 0.01$ (n = 6), but without any change in $[Ca^{2+}]_i$ transients ($P = 0.16$). The force-$[Ca^{2+}]_i$ relationship of intact trabeculae exhibited increased myofilament Ca^{2+} sensitivity with cpTOME at low $[Ca^{2+}]_o$ but not at 1.5 mM $[Ca^{2+}]_o$. In isolated cells, cpTOME increased Ca^{2+} spark frequency (Fluo-4) from 6.6 per 100 μm^3 s (n = 3) to 32.3 per 100 μm^3 s (n = 9), $P = 0.05$, with a reduction in the peak amplitude of the sparks. The latter result recapitulates the idea that changes in RyR sensitivity do not alter the amplitude of Ca^{2+} transients (Eisner, 2009).

2610-Pos Board B380

P21-Activated Kinase (Pak1) is a Direct Modulator of Cardiac Excitation-Contraction Coupling (ECC) Gain

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Ras related proteins regulate the activity of the serine-threonine protein kinase Pak1, which has been implicated in the regulation of cytoskeletal dynamics and motility. Recent evidence points to its role in cardiac ECC by attenuation of the β-adrenergic stimulation of ICa,L and IKr through activation of the phosphatase PP2A. To further determine the role of Pak1 in cardiac ECC we analyzed the Ca handling properties of isolated ventricular myocytes (VM) from Pak1-/- mice by laser scanning confocal microscopy. Isolated VMs from Pak1-/- mice exhibited a reduced Ca transient amplitude (Δ F/F0 WT: 1.78 ± 0.22 n=10; Pak1-/-: 1.26 ± 0.18 n=7, $p<0.05$) that was not based on a decrease in the load of the sarcoplasmic reticulum (WT: 5.58 ± 0.32; Pak1-/-: 4.82 ± 0.31) or a decrease in the current density of ICa,L (@-10mV: WT: 4.1 ± 0.5 n=11 pA/pF; Pak1-/-: 4.02 ± 1.6 n=9). However, the rise time of the Ca transient in Pak1-/- myocytes was significantly delayed (WT: 79 ± 5 ms; Pak1-/-: 119 ± 7 ms, $p<0.05$). The reduced amplitude could be based on a modified gain between Ca influx and Ca induced Ca release through the ryanodine receptor. β-adrenergic stimulation with isoproterenol (100 nM) not only rescued the Ca transient rise time in Pak1-/- myocytes but induced an exaggerated increase in Ca-transient amplitude (F/F0: WT: 4.45 ± 0.25; Pak1-/-: 5.22 ± 0.26; $p<0.05$) and decrease in tau (WT: 159.8 ± 8ms; Pak1-/-: 121 ± 3ms; $p<0.05$). The direct involvement of Pak1 in this process is suggested by the reversal of all parameters to control conditions by adenoviral overexpression of Pak1 in Pak1-/- VMs. Our results further support the role of Pak1 as a modulator of β-adrenergic stimulation in VMs and indicate a novel role in the maintenance of cardiac ECC gain.

2611-Pos Board B381

Increased Resting Calcium Modulates NF-κB Activity and iNOS Expression in mdx Myotubes

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Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by severe muscle wasting. Dystrophic muscles exhibit activated immune cell infiltrates with up-regulated inflammatory gene expression and increased NF-κB activity, but the contribution of the skeletal muscle cell to this process has been unclear. The aim of this work was to study the resting calcium [Ca2+] rest deregulation and its possible link with NF-κB up-regulation and iNOS expression in mdx myotubes.

[Ca2+]rest was measured with Ca2+-selective microelectrodes and NF-κB transcriptional activity was studied using luciferase reporter and immunofluorescence in wt and mdx myotubes. Gene expression was studied by real time PCR.

[Ca2+]rest was higher in mdx than in wt myotubes (308 ± 6 vs 113 ± 2 nM, $p<0.001$). Both the inhibition of Ca2+ entry (Gd3+ and low Ca2+ solutions) and blockade of ryanodine (Ry) receptors or IP3 receptors (XeB), reduced [Ca2+]rest in mdx myotubes. Basal activity of NF-κB was significantly up-regulated in mdx myotubes. This was shown by an increased p65 nuclear localization and increased transcriptional activity, which could be reversed by inhibitors that reduced [Ca2+]rest. Levels of mRNA for TNFα, IL-1β and IL-6 were similar in wt and mdx myotubes, whereas iNOS expression was increased 5-fold in mdx myotubes. Moreover, both NF-κB inhibition and [Ca2+] rest inhibitors reduced iNOS gene expression.

We propose that NF-κB is constitutively active in mdx myotubes, modulated by increased [Ca2+]rest and this condition can account for iNOS overexpression in dystrophic myotubes. We hypothesize that the differences in NF-κB activity may help to understand the mechanisms of muscle damage in DMD.

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2612-Pos Board B382

Versican, Matrix-Gla Protein, and Death-Associated Protein Expression Affect Muscle Satellite Cell Proliferation and Differentiation

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Skeletal muscle growth and development from embryo to adult consists of a series of carefully regulated changes in gene expression. In our previous transcriptional profiling study [Sporer et al., BMC Genomics 12:143 (2011)], our experiments using a turkey skeletal-muscle-specific oligonucleotide microarray revealed that more than 3,000 genes were differentially expressed as a function of three critical stages of muscle development: hyperplasia (18 d embryo), hypertrophy (1 d post-hatch), and mature muscle (16 wk). The genes versican, matrix Gla protein (MGP), and death-associated protein 1 (DAP1) were selected for further study for their potential effects on modulation of muscle satellite cell proliferation and differentiation. Moreover, these genes exhibited large fold-changes in expression as a function of muscle development in the turkey. Small interfering RNA was used to knock down expression of these genes during proliferation and differentiation of cultured turkey muscle satellite cells; DNA content and creatine kinase activity were quantified as markers of proliferation and differentiation, respectively. Knockdown of each of the genes was associated with altered rates of proliferation and differentiation. Versican and MGP predominantly affected proliferation, but later stages of differentiation were also affected by the knockdown of versican and MGP. The knockdown of DAP1 dramatically inhibited satellite cell differentiation to form myotubes, with reduction in creatine kinase activity of up to 90% compared to the control. Microarray and pathway analysis of the proliferating and differentiating DAP1 knockdown cells indicated that several genes associated with calcium signaling were differentially expressed. This is the first report that these genes, with no previously documented functions in regulation of muscle development, may play critical roles in muscle cell proliferation and differentiation.

Membrane Receptors & Signal Transduction I

2613-Pos Board B383

Effect of Ligand Binding on the Diffusion and Distribution of the G Protein-Coupled Receptor CCR5 in the Cell Membrane

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The human immunodeficiency virus (HIV) almost entirely depends on CCR5, a host-encoded chemokine receptor member of the G protein-coupled receptor (GPCR) superfamily, for infection of target cells and hence for transmission from person to person. Inhibiting CCR5 is a viable strategy to prevent viral infection since individuals expressing a truncated version of this protein are perfectly healthy. While native chemokines can display some weak anti-HIV activity, it has been shown that N-terminally-modified analogues of the native CCR5 ligand RANTES/CCL5 are much more potent inhibitors: analogues such as PSC-RANTES, 6P4-RANTES, 5P12-RANTES, and 5P14-RANTES, have potencies in the picomolar range.

PSC-RANTES owes its anti-HIV potency to its capacity to induce long-term sequestration of CCR5 inside target cells. This molecule is a strong agonist of CCR5 and activation of the receptor was believed to be required for internalization. While some recombinant chemokines like 6P4-RANTES exhibit a comparable pharmacological profile to PSC-RANTES, others such as 5P12-RANTES and 5P14-RANTES do not, which led us to conclude that anti-HIV potency can be achieved by different mechanisms: 5P12-RANTES is capable of efficiently blocking CCR5 without removing the receptor from the cell surface and without activating it; 5P14-RANTES displays comparable anti-HIV potency while causing only partial internalization in the absence of detectable receptor activation.

Because CCR5 needs to be mobile in the cell membrane for HIV infection, we investigated the effect of RANTES analogues on the diffusion dynamics and spatial distribution of CCR5 in the cell membrane, using single-particle tracking and blink microscopy techniques in combination with quantum-dot or