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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

Jaime DeSantiago, Dan J. Bare, Katherine A. Sheehan, Yunbo Ke, R. John Solaro, **Kathrin Banach**.
University of Illinois at Chicago, Chicago, IL, USA.

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

Francisco Altamirano[1], José R. López[2], Carlos Henriquez[1], Paul D. Allen[2], Enrique Jaimovich[1].
[1]Universidad de Chile, Santiago, Chile, [2]Brigham and Women's Hospital, Boston, MA, USA.

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

Sandra G. Velleman[1], Kelly R.B. Sporer[2], Catherine W. Ernst[2], Kent M. Reed[3], Katherine Stanchak[2], **Gale M. Strasburg**[2].
[1]Ohio State University, Wooster, OH, USA, [2]Michigan State University, East Lansing, MI, USA, [3]University of Minnesota, St. Paul, MN, USA.

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

Zuleika Calderin-Sollet, Oliver Hartley, **Alexandre Fuerstenberg**.
University of Geneva, Geneva, Switzerland.

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