Exponential growth of segments by this feed-forward mechanism is limited by the finite supply of tropomyosin in Position C, which can be controlled by troponin in Position B. A derivation and application to thin and thick filament regulation may be found on-line (www.westga.edu/STEMresearch). This work was supported by NSF grant MCB-0508203 (HGZ).

783-Pos

Determinants of Loaded Shortening in Cardiac Myocytes

Laurin M. Hanft, Kerry S. McDonald.
University of Missouri, Columbia, MO, USA.

Ventricular performance is dictated by stroke volume, which ultimately depends on the extent of myocyte shortening during loaded contractions. We propose that the extent of loaded shortening is determined by the balance between two processes: (i) Ca^{2+}-cross-bridge-induced cooperative activation of the thin filament and (ii) shortening-induced cooperative deactivation of the thin filament. Accordingly, any modulator that augments contractility (i.e., stroke volume) should favor process (i) and diminish process (ii). Since β-adrenergic stimulation is known to increase contractility, we tested whether PKA (the myofibrillar ligand of β-adrenergic signaling) would increase cooperative activation and diminish shortening-induced deactivation in rat permeabilized cardiac myocytes during submaximal Ca^{2+} activations. Regarding cooperative activation, PKA increased the slope of tension-pCa relationships ($n_H = 3.85 \pm 0.09$ before versus $n_H = 5.03 \pm 0.71$ after PKA). PKA also slowed rates of force redevelopment, increased the transient force overshoot after a slack-restretch maneuver, and increased the rate and amplitude of spontaneous oscillatory contractions (SPOCs); all of which are consistent with greater cooperative activation of the thin filament. Regarding cooperative deactivation, PKA increased the curvature of myocyte length traces during lightly loaded shortening ($k_{shortening} = 6.41 \pm 0.28$ before versus $k_{shortening} = 9.45 \pm 0.53$ after PKA) and steepened sarcomere length-tension relationships; both of which implicate enhanced (rather than diminished) shortening-induced cooperative deactivation. Taken together, PKA-induced myofibrillar phosphorylation appears to augment both Ca^{2+}-cross-bridge-induced cooperative activation of the thin filament and (ii) shortening-induced cooperative deactivation. Greater cooperative activation should lead to more cycling cross-bridges, which would speed loaded shortening against a given afterload. On the other hand, greater shortening-induced cooperative deactivation may be necessary to help accelerate relaxation and assist diastolic filling in the face of shorter systolic and diastolic times in the presence of higher heart rates induced by β-adrenergic stimulation.

784-Pos

The Role of Store-Operated Calcium Entry in Store Repletion During Repetitive High Frequency Tetanic Stimulation of Single Skeletal Muscle Fibers

Lan Wei, Alla D. Lyfenko, Robert T. Dirksen.
University of Rochester Medical Centre, Rochester, NY, USA.

Store-operated Ca^{2+} Entry (SOCE) involves a trans-sarcolemmal Ca^{2+} influx mechanism triggered by Ca^{2+} store depletion. Recently, we demonstrated that SOCE activation in skeletal myotubes involves a functional coupling between STIM1 Ca^{2+} sensor proteins in the sarcoplasmic reticulum (SR) and Ca^{2+}-permeable Orai1 channels in the sarcolemma. However, the physiological role of SOCE in muscle remains unknown. Here, we monitored myoplasmic Ca^{2+} transients in mag-fluo-4 loaded mouse flexor digitorum brevis fibres during repetitive high frequency tetanic stimulation (60 consecutive 500ms, 50Hz stimulation trains every 2.5s). In normal Ringer's solution, tetanic Ca^{2+} transient amplitude decays in three phases: an initial rapid phase (trains 1-10), a second phase of maintained amplitude (trains 10-40), and a final phase of decay (trains 40-60). The maintained phase corresponds to a slightly elevated tail transient integral during each interpulse interval, consistent with activation of Ca^{2+} influx between tetani. Addition of 0.5mM $CdCl_2$ plus 0.2mM $LaCl_3$ did not alter the initial or final phases of Ca^{2+} transient decay, but significantly ($p<0.01$) compromised both the maintained Ca^{2+} transient ($4 \pm 3\%$ reduction from trains 10 to 40 in normal Ringer versus $30 \pm 3\%$ reduction with Cd/La) and the increase in tail transient integral (which decreased $21 \pm 7\%$ with Cd/La) observed during the second phase. Similar results were obtained following addition of either BTP-2 or SKF96365, two known SOCE inhibitors, consistent with SOCE mediating store repletion during the secondary phase of maintained release. Together, these results suggest that repetitive high frequency tetanic stimulation activate a SOCE flux used to replenish SR Ca^{2+} stores required to maintain subsequent Ca^{2+} release. Current experiments are testing the validity of this assertion using molecular interventions (transient STIM1 knockdown and dnOrai1 expression) to more selectively inhibit SOCE.

785-Pos

Disruption of Circadian Gene Expression in Skeletal Muscle but not Liver in Pre-Hypertensive SHR Vs. WKY Rats

Mitsunori Miyazaki, Dawson F. Dean, Stephanie E. Edelmann, C. William Balke, Karyn A. Esser.
Center for Muscle Biology, Department of Physiology, College of Medicine, University of Kentucky, Lexington, KY, USA.

Recently, alterations of the molecular clock and circadian rhythms have been implicated as contributing factors to cardiovascular and skeletal muscle disease. Woon et al. (2007) determined that a polymorphism found in the congenic interval of the SHR rat is associated with hypertension and type II diabetes. Here, we examined the expression of circadian genes in striated muscle (cardiac and skeletal muscle) in young pre-hypertensive SHR (6 weeks old) and age-matched Wistar-Kyoto (WKY) male rats. The rats were entrained to a 12 hour light: 12 hour dark cycle for 2 weeks and then placed in constant darkness for 30 hours. Cardiac muscle (left ventricle), skeletal muscle (soleus) and non-muscle tissue (liver) were collected every 4 hours for 40 hrs, totally 10 time points. Expression of core clock genes (*Bmal1, Clock, Per2, Rorα, Rev-erb*) and the clock-controlled gene, *Dbp*, were analyzed using real-time quantitative PCR. Expression of *Bmal1* has a clear circadian pattern in muscle and liver tissue of rats. The pattern and amplitude of circadian expression of *Bmal1* were not altered between WKY and SHR strains in every tissue studied. In contrast, expression of the other clock genes, *Rorα, Dbp, Rev-erb, Clock and Per2*, were significantly dys-regulated in the soleus muscle from the SHR rat. In the left ventricle, circadian expression of *Per2* was dampened in the SHR but the other clock genes were unchanged. In liver, there were no differences in expression of any of the clock genes between the SHR and WKY rats. These data suggest that components of the molecular clock are disrupted in striated muscle prior to overt signs of hypertension. The contribution of this disruption in the clock to hypertension and type II diabetes are to be determined.

786-Pos

Effect of Cannabinoids on Choline Induced Contractures in Slow Skeletal Muscle Fibers of the Frog

Enrique Alejandro Sanchez-Pastor[1], Miguel Huerta-Viera[1], Adriana Valle-Chávez[2], Maria Felipa Andrade-Urzua[2], Xochitl Trujillo[1].
[1]University of Colima, Colima, Mexico, [2]University of Sonora, Sonora, Mexico.

Cannabinoids interact with membrane receptors causing, among others, psychoactive and motor effects. Recently it has been shown the presence of CB_1 receptors in amphibian skeletal muscle. The aim of our study was to approach the role of acetylcholine receptors (AchR) on the mechanism of cannabinoid action in skeletal muscle contraction. We isolated bundles of the slow portion of *cruralis* muscle of the frog and induced contractures by incubating with Ringer solution with choline 115 mM. Choline induces maintained contractures with a slow relaxation phase. As reported recently for other frog slow skeletal muscle, the choline-contracture in *cruralis* bundles depends on the interaction with AchR as the contracture is reduced almost completely (~95%, n=3) by blocking these receptors with tubocurarine 100 μM. To test the effect of cannabinoids we incubated with the CB_1 agonist ACPA (1 μM) and the choline-contracture was diminished by around 40% ($p<0.05$; n=4). This effect was blocked partially (~20%) by preincubating the bundles with the CB_1 antagonist AM281 (1 μM; n=3). Also, pretreating the bundles with *pertussis* toxin (2 μg/ml) causes a partial blockade of the ACPA effect (~20%). Both results strongly suggest that part of the effect is caused through a mechanism involving the activation of CB_1 receptors, being the rest a receptor-independent effect. On the other hand, blocking the Ryanodine receptor-α (RyR-α) with Dantrolene (150 μM) causes a reduction of the choline-contracture by approximately 45%. Once the RyR-α are blocked, ACPA did not cause further decrease, suggesting the involvement of the RyR-α in the effect caused by ACPA on the choline-contractures. Our results show that ACPA modulates choline-contractures and suggests that this effect involves the participation of CB_1, AchR and RyR-α.

Supported in part by CONACYT-MEX 83113 to MH.

787-Pos

The Alterations of Store-Operated Calcium Entry in TRPC1-Overexpressing C2C12 Myotubes

Tamás Oláh[1], János Fodor[1], Olga Ruzsnavszky[1], Celine Berbey[2], Bruno Allard[2], László Csernoch[1].
[1]University of Debrecen, Department of Physiology, Debrecen, Hungary, [2]Physiologie Intégrative Cellulaire et Moléculaire, Université Claude Bernard Lyon 1, Lyon, France.

When the endoplasmic reticulum (ER) calcium store is depleted, a Ca^{2+} influx is activated from the extracellular milieu to refill the intracellular stores. This well-regulated Ca^{2+} uptake mechanism, called store-operated Ca^{2+} entry

(SOCE), depends on the cooperation of several proteins as STIM1, Orai1 and TRPC1. The role of STIM1 as the calcium sensor of the ER and Orai1 as the Ca^{2+} influx channel is well-known from the recent publications, but the function of TRPC1 as a store-operated channel remains elusive.
Here TRPC1 was overexpressed by liposome-mediated transfection in C2C12 mouse skeletal muscle cell line. Overexpression was confirmed at mRNA level by RT-PCR and at protein level by immunostaining and Western-blot. The SOCE mechanism was studied by measuring the changes in $[Ca^{2+}]_i$ evoked by the re-addition of 1.8 mM $[Ca^{2+}]_e$ following the SERCA-inhibition by thapsigargin. As a result of TRPC1 overexpression, the amplitude and the maximum of the derivative of SOCE was significantly increased. When YM-58483, the antagonist of TRPC1 was used, these differences were eliminated, moreover in TRPC1-overexpressing myotubes the SOCE was slightly but not significantly lower, suggesting the downregulation of the STIM1-Orai1 system. This decrease in the expression level of STIM1 was confirmed by Western-blot together with the downregulation of SERCA. As a consequence a reduction in maximal Ca^{2+} uptake, and a higher resting $[Ca^{2+}]_i$ following the transients evoked by 120 mM KCl were detected. Morphological changes caused by the overexpression of TRPC1 were also observed. The differentiation of the myoblasts started later, and the myotubes were thinner in TRPC1-overexpressing cultures.
Our results suggest that enhancing the expression level of TRPC1 increases SOCE and has a negative feedback effect on the STIM1-Orai1 system, suggesting a cooperation between these proteins.

788-Pos

Leucine-Zipper Mediated Intermolecular Interaction between MG53 is Essential for Cellular Membrane Repair

Moonsun Hwang[1], Jae-Kyun Ko[1], Chuanxi Cai[1], Peihui Lin[1], Zui Pan[1], Noah Weisleder[1], Miyuki Nishi[2], Hiroshi Takeshima[2], Jianjie Ma[1].
[1]UMDNJ, Piscataway, NJ, USA, [2]Kyoto University, Kyoto, Japan.

We recently found that MG53, a muscle-specific TRIM family protein, functions as a sensor of oxidation to nucleate the assembly of cell membrane repair machinery (Cai et al, 2009 Nature Cell Biology). Our data showed that disulfide formation mediated by Cys242 is critical for MG53-mediated translocation of intracellular vesicles toward the injury sites. Here we test the hypothesis that leucine-zipper motifs in the coiled-coil domain of MG53 constitute an additional mechanism that can facilitate oligomeric formation of MG53 for assembly of the cell membrane repair machinery. Chemical cross-linking studies show that the coiled-coil domain of MG53, which contains the two putative leucine zipper motifs LZ1 (L176/L183/L190/V197) and LZ2 (L205/L212/L219/L226), is involved in formation of MG53 dimers. While mutation of LZ2 does not affect oligomeric interaction of MG53, replacement of 3 leucine residues to alanines in LZ1 leads to compromised oligomeric formation of MG53. Moreover, double mutation of LZ1 and LZ2 completely disrupts MG53 oligomeric formation, even under non-reducing conditions. Live cell imaging revealed that the movement of GFP-tagged MG53 mutants, GFP-LZ1 and GFP-LZ2, in response to mechanical damage of the cell membrane is significantly reduced relative to the wild type GFP-MG53 construct. Furthermore, the GFP-LZ12 double mutant is completely ineffective in translocation toward the injury sites, and cannot repair acute damage to cell membranes. Our data show that leucine-zipper mediated oligomer formation is essential for cell membrane repair. LZ1 likely constitutes a critical motif for disulfide cross-link between Cys242, during assembly of the membrane repair machinery.

789-Pos

Fatigue in Mouse Muscle Fibers: Role of Mitochondrial ATP-Sensitive Potassium Channels

Maria C. Garcia, Ascencion Hernández, Jorge A. Sanchez.
Cinvestav, I.P.N., Mexico City, Mexico.

Aim. The role of mitochondrial K_{ATP} (mitoK_{ATP}) channels on muscle fatigue was assessed in adult mouse skeletal muscle fibers.
Methods. Muscle fatigue was produced by eliciting short repetitive tetani. Isometric tension and the rate of production of reactive oxygen species (ROS) were measured at room temperature (20-22°C) using a force transducer and the fluorescent indicator CM-H_2DCFDA.
Results. We found that opening mitoK_{ATP} channels with diazoxide (100 ∈ μM) significantly reduced muscle fatigue. Fatigue tension was 34% higher in diazoxide-treated fibers relative to controls. This effect was blocked by the mito-K_{ATP} channel blocker 5-Hydroxydecanoate (5-HD), by the protein kinase C (PKC) inhibitor chelerythrine, and by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME). We found that the rate of ROS production in muscle fibers incubated with diazoxide under non-fatigue and fatigue conditions was similar to control experiments, but that the increase in the rate of ROS production during recovery from fatigue was greatly reduced.
Conclusions. A physiological role of mitoK_{ATP} channels on muscle fatigue is proposed.
Diazoxide, an opener of mitoK_{ATP} channels, reduces muscle fatigue possibly through a preservation of mitochondrial volume and function.
This work was supported in part by CONACYT grants 82667 (JS) and 60880 (MCG).

790-Pos

Inducible Activation of Akt Increases Skeletal Muscle Mass and Force Without Satellite Cell Activation

Bert Blaauw[1,2], Canato Marta[3], Lisa Agatea[1], Luana Toniolo[3], Cristina Mammucari[1], Eva Masiero[1], Reimar Abraham[1], Marco Sandri[1], Stefano Schiaffino[1], Carlo Reggiani[3].
[1]venetian institute of molecular medicine, padova, Italy, [2]department of Physiology, Padova, Italy, [3]department of Physiology, padova, Italy.

A better understanding of the signaling pathways that control muscle growth is required to identify appropriate countermeasures to prevent or reverse the loss of muscle mass and force induced by aging, disuse or neuromuscular diseases. However, two major issues in this field have not yet been fully addressed. The first concerns the pathways involved in leading to physiological changes in muscle size. Muscle hypertrophy based on perturbations of specific signaling pathways are either characterized by impaired force generation, e.g. myostatin knockout, or incompletely studied from the physiological point of view, e.g. IGF-1 over-expression. A second issue is whether satellite cell proliferation and incorporation into growing muscle fibers is required for a functional hypertrophy. To address these issues, we used an inducible transgenic model of muscle hypertrophy by short-term Akt activation in adult skeletal muscle. In this model, Akt activation for three weeks was followed by marked hypertrophy (approximately +50% of muscle mass) and by increased force generation, as determined in vivo by ankle plantar flexor stimulation, ex vivo in intact isolated diaphragm strips and in single skinned muscle fibers. No changes in fiber type distribution and resistance to fatigue were detectable. BrdU incorporation experiments showed that Akt-dependent muscle hypertrophy was accompanied by proliferation of interstitial cells but not by satellite cells activation and new myonuclei incorporation, pointing to an increase in myonuclear domain size. We can conclude that during a fast hypertrophic growth myonuclear domain can increase without compromising muscle performance.

791-Pos

Heat-Shock Treatment Induces Hypertrophy in C2C12 Muscle Cells

Todd Hall, Chad Touchberry, Robin Craig, Leticia Brotto, Michael Loghry, Michael Loghry, Jon Andresen, Michael Wacker, **Marco Brotto**.
University of Missori-Kansas City, Kansas City, MO, USA.

Heat shock proteins (Hsp's) are molecular chaperones that are critical for the maintenance of cellular homeostasis and have been shown to protect cells and tissues from a large variety of damaging insults. Conflicting reports have been published on the effects of HS on whole animal models of hypertrophy. Some reports suggest that HS inhibits skeletal muscle growth, while others show an increase in the size and proliferative potential of intact skeletal muscles. However recent studies have suggested that heat shock can induce hypertrophy in cardiac muscle cell lines. The cellular response of skeletal muscle cells exposed to heat shock (HS) is to our knowledge an untested treatment method for studying muscle hypertrophy. Therefore, our main goal was to examine the hypertrophic reactions to a mild HS treatment (43°C for 20 min) in C2C12 muscle cells. C2C12's are widely considered to be very representative of skeletal muscle cells in vivo, and can therefore serve as a useful model of physiological hypertrophy. We found that our mild HS treatment induced significant hypertrophy (> 30% of cell area growth) of differentiated C2C12 myotubes, indicating that HS is an effective treatment for induction of hypertrophy in C2C12 muscle cells. We are now investigating the mechanisms underlying HS-induced hypertrophy in C2C12 muscle cells. We believe that these studies will be useful in establishing the foundation for a better and in-depth understanding of HS-induced stress in skeletal muscles. *(Support:American Heart Association 0535355N, Missouri Life Sciences Research Board & NIH Opportunities Grant/Recovery Act (GO Grant) to MB*.