

type 1) in the sarcoplasmic reticulum (SR) causing Ca^{2+} release from the SR. Canonical-type transient receptor potential cation channel 3 (TRPC3), an extracellular Ca^{2+} entry channel in the t-tubule and plasma membrane, is required for full-gain of skeletal EC coupling. The present study examined additional role(s) for TRPC3 in skeletal muscle other than mediation of EC coupling. We created a stable myoblast line (MDG/TRPC3 KD myoblast) with reduced TRPC3 expression by knock-down of TRPC3 using retrovirus-delivered small interference RNAs in $\alpha 1\text{sDHPR}$ -null muscular dysgenic myoblasts to eliminate any DHPR-mediated EC coupling-related events. Unlike $\alpha 1\text{sDHPR}$ -null muscular dysgenic myoblasts, MDG/TRPC3 KD myoblasts exhibited dramatic changes in cellular morphology (e.g., unusual expansion of both cell volume and the plasma membrane, and multi-nuclei) and increased Ca^{2+} content in both the endoplasmic reticulum and cytoplasm of resting myoblasts. Moreover, these myoblasts failed to differentiate into myotubes. Therefore, TRPC3 in skeletal myoblasts is essential for maintenance of skeletal muscle.

1784-Pos

Effects of Cardiac Specific Inhibition of TRPC Channels on Cardiac Hypertrophy and Ca^{2+} Handling

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TRPC channels have been identified as components of Ca^{2+} signaling pathways that promote maladaptive growth of the myocardium. Transgenic mice over-expressing TRPC3 or 6 exhibited an increased propensity towards hypertrophic progression in response to pressure overload, in part through a Ca^{2+} -dependent calcineurin-NFAT signaling pathway. Moreover, different TRPC subunits are known to be up-regulated in heart failure and in hypertrophic hearts after pressure overload. In this study we inhibited TRPC channel function with dominant negative (dn) TRPC mutants and examined the ensuing effect on cardiac hypertrophy and Ca^{2+} influx dynamics. One of these mutants comprises an N-terminal TRPC4 fragment that we used to generate cardiac restricted transgenic mice. Intracellular Ca^{2+} signals were measured in adult cardiac myocytes of wildtype (WT) and dn-TRPC4 mice that underwent TAC (transverse aortic banding) or a sham procedure. Isolated myocytes maintained in Ca^{2+} -free buffer were store-depleted with CPA while being treated with Ang II, followed by perfusion with Ca^{2+} containing buffer to analyze Ca^{2+} influx with Fura-2 fluorescence. WT myocytes showed essentially no Ca^{2+} entry, however, hypertrophic myocytes that underwent TAC showed abundant Ca^{2+} influx. SKF96365 inhibited this Ca^{2+} influx, suggesting a role for TRPC channels. Indeed, isolated myocytes from dnTRPC4 transgenic mice subjected to TAC lacked this Ca^{2+} influx, confirming that TRPC channels mediate this previously unrecognized influx activity associated with hypertrophy in cardiac myocytes. Interestingly, Ca^{2+} transients from myocytes of dn-TRPC4 mice showed increased peak amplitudes, which might indicate an increased cardiac contractility and cardiac performance when dn-TRPC4 is expressed. More importantly, dnTRPC4 mice showed significant attenuation of cardiac hypertrophy following TAC stimulation. These data show that inhibition of TRPC channels exerts ameliorative effects on the development of cardiac hypertrophy by decreasing Ca^{2+} signals that likely regulate pro-hypertrophic pathways.

1785-Pos

Analysis of the Role of TRPC3 in Ca^{2+} Signaling of RBL-2H3 Mast Cells

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Direct or indirect involvement of TRPC channels in store-operated Ca^{2+} entry (SOCE) has repeatedly been proposed. In this study, we explored the role of TRPC3 in SOCE-associated Ca^{2+} signaling of RBL-2H3 mast cells by employing both genetic and pharmacological strategies. Mast cells overexpressing a fluorescence-tagged, functional TRPC3 fusion protein displayed enhanced Ca^{2+} entry in a classical thapsigargin-induced store depletion/calcium re-entry protocol. A well-characterized dominant-negative, n-terminal fragment of TRPC3 (aa 1-302) reduced SOCE significantly down to basal entry. A similar extent of inhibition was observed with a dominant negative mutant of Orail (E106Q). Two pore mutants of TRPC3 (E616K and E630Q), which represent a non-functional, dominant negative protein and a protein with distinctly altered cation permeability, respectively, failed to affect SOCE in RBL-2H3 cells. The pyrazol compound Pyr3 (ethyl-1-(4-(2,3,3-trichloroacrylamide)-phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate), which was recently proposed as a selective inhibitor of TRPC3 channels, effectively suppressed SOCE in wild-type controls as well as TRPC3 over-expressing cells. Our re-

sults argue against a role of TRPC3 as part of the store-operated Ca^{2+} permeation pathway in RBL-2H3 cells and point towards an indirect link between TRPC3 and SOCE.

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1786-Pos

Role of TRPC1 in Myoblasts Differentiation and Muscle Development

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Myoblasts migration is a key step in myogenesis. It allows myoblasts alignment and their fusion into myotubes. The process has been shown to involve m- or μ -calpains, two calcium-dependent cysteine proteases. Fluorometric measurements of calpain activity in cultured cells showed a peak at the beginning of the differentiation process. We also observed a concomitant and transient increase of the influx of Ca^{2+} and of the expression of TRPC1 protein. After repression of TRPC1 in myoblasts by siRNA and shRNA, this transient influx of calcium was significantly reduced and the concomitant peak of calpain activity was abolished. Interestingly, myoblasts fusion into myotubes was significantly slowed down, due to a reduced speed of cell migration. Accordingly, migration of control myoblasts was inhibited by 2 to 5 μM GsMTx4 toxin, an inhibitor of TRP channels or by 50 μM Z-Leu-Leu, an inhibitor of calpain. These effects were not observed in TRPC1 knocked down cells. Moreover, TRPC1 knocked down myoblasts also accumulated of myristoylated alanine-rich C-kinase substrate (MARCKS), an actin-binding protein, substrate of calpain. We therefore suggest that an entry of calcium through TRPC1 channels induces a transient activation of calpain, a subsequent proteolysis of MARCKS, allowing in its turn, myoblasts migration and fusion. The role of TRPC1 in muscle regeneration, a process involving myoblasts migration and differentiation, is under study. To further characterize the role of TRPC1 in muscle development, we compared morphological and mechanical parameters of muscles from TRPC1^{+/+} and TRPC1^{-/-} mice. We observed that muscles from TRPC1^{-/-} mice display a smaller fibre cross-sectional area and generate less force per cross section area. They do not present other major signs of myopathy but were more sensitive to muscle fatigue.

1787-Pos

Structural and Molecular Basis of the Assembly of the TRPP2/PKD1 Complex

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases in human and is caused by mutations in PKD1 and TRPP2 proteins. PKD1 (also known as polycystin-1 or PC1) is a large integral membrane protein with 11 putative transmembrane regions, a large extracellular N terminus and a short intracellular C terminus. PKD1 is generally thought to function as a cell surface receptor that couples extracellular stimuli to intracellular signaling. TRPP2 (also known as polycystin-2, PKD2 or PC2) is a member of the transient receptor potential (TRP) channel family. It has 6 putative transmembrane segments and a pore-forming loop and forms a Ca^{2+} -permeable nonselective cation channel. How mutations in PKD1 and TRPP2 lead to ADPKD is unclear but these two proteins likely share some common functions since mutations in them produce similar pathological manifestations. These two proteins associate physically through their C-termini and form functional complexes. However, the subunit composition of this complex and the molecular mechanism of its assembly are unknown. By combining biochemistry, X-ray crystallography, and a single molecule imaging method to determine the subunit composition of proteins in the plasma membrane of live cells, we find that this complex contains 3 TRPP2 and 1 PKD1. A C-terminal coiled coil domain of TRPP2 is critical for the assembly of this complex. This coiled coil domain forms a homotrimer and binds to a single coiled coil domain in the C-terminus of PKD1. Mutations that disrupt this coiled coil trimer abolish the assembly of both full-length TRPP2 homotrimer and the TRPP2/PKD1 heteromeric complex, and diminish the surface expression of both proteins. These findings have significant implications for the assembly, regulation and function of the TRPP2/PKD1 complex, and for the pathogenic mechanism of some ADPKD-producing mutations.

1788-Pos

Immunodetection and Oligomerisation of TRPM1

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To study expression and assembly of TRPM1 channels we cloned seven full lengths TRPM1 cDNAs from mouse eye which represent splice variants and encode proteins of 1462, 1506, 1512, 1578, 1584, 1622 and 1628 amino acid residues. These TRPM1 protein variants vary in their N terminus, in a putative extracellular loop and the predicted pore region. For expression analyses we prepared polyclonal and monoclonal antibodies directed against various antigenic epitopes of TRPM1. All antibodies detect proteins of the size expected for TRPM1 in the eye, in lung and skeletal muscle. They immunoprecipitate the TRPM1 protein from mouse retina and specifically label postsynaptic dendrites of bipolar cells adjacent to the ribbon synapse. All TRPM1 proteins contain a coiled-coil domain in their C termini and isolated fragments comprising this coiled-coil domain have been shown to form dimers (1). We show that after disruption of this coiled-coil domain by site directed mutagenesis the full-length TRPM1 proteins still assemble to dimers and higher molecular protein complexes and interact with each other, apparently by additional intermolecular interactions of TRPM1 sequences within the N-terminus. Similarly, a related TRPM protein, TRPM4, still forms dimers after destruction of a C-terminal coiled-coil domain, most probably via interactions of N-terminal protein sequences.

1 Tsuruda PR, Julius D, Minor DL Jr (2007) *Neuron* 51, 201-212.

1789-Pos

A Reduction of Glucose-Induced Bursting Frequency in Pancreatic Islets Correlates with Decreased Insulin Release and Impaired Glucose Tolerance in TRPM5^{-/-} Mice

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¹K.U.Leuven, Leuven, Belgium, ²Mount Sinai School of Medicine, New York, NY, USA, ³Universite Catholique de Louvain, Bruxelles, Belgium. Glucose homeostasis is critically dependent on insulin release from pancreatic beta cells, which is strictly regulated by glucose-induced simultaneously oscillations in membrane potential (V_m) and cytosolic calcium concentrations $[Ca^{2+}]_{cyt}$. We propose that TRPM5, a Ca^{2+} -activated monovalent cation channel, is a positive regulator of glucose-induced insulin release. Micro-array screening and immunostaining reveal high and selective expression of TRPM5 in pancreatic islets. Whole cell current measurements in WT pancreatic islet cells demonstrate a Ca^{2+} -activated non-selective cation current with properties comparable to TRPM5 measured in over-expression, including the bell-shaped dependency on intracellular Ca^{2+} , time constant of activation and permeation properties. This current is significantly reduced in *Trpm5*^{-/-} cells. Ca^{2+} -imaging and electrophysiological analysis show that glucose-induced oscillations of V_m and $[Ca^{2+}]_{cyt}$ have a reduced frequency in *Trpm5*^{-/-} islets. WT islets display either slow or fast oscillations, or mixed oscillations, consisting of fast oscillations superimposed on slow ones. In contrast, *Trpm5*^{-/-} islets never show a fast oscillation pattern. Fast oscillations in V_m show a shorter burst interval, due to a higher slope of depolarization towards the threshold potential for burst initiation. Our results indicate that TRPM5 accelerates the depolarization during the interburst interval, initiating rapid oscillations and higher insulin release. As a consequence, glucose-induced insulin release from *Trpm5*^{-/-} pancreatic islets is significantly reduced, resulting in an impaired glucose tolerance in these mice. Pharmacological modulation of TRPM5 activity may represent a novel means to adjust insulin release in diabetic patients.

1790-Pos

Physiological Role of the Oxidative Stress-Sensitive TRPM2 Ca^{2+} Channel in Immunocytes

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It is known that a large amount of reactive oxygen species (ROS) exist at inflamed sites. ROS induce chemokines responsible for the recruitment of inflammatory cells at inflamed sites. Here, we demonstrate that the plasma membrane Ca^{2+} -permeable channel TRPM2 controls ROS-induced chemokine production in monocytes/macrophages. In monocytes from *Trpm2*-deficient mice, H_2O_2 -induced Ca^{2+} influx and production of the macrophage inflammatory protein-2 (CXCL2), which exhibit potent neutrophil chemotactic activity, were impaired. In the inflammation model dextran sulfate sodium-induced colitis, CXCL2 expression was attenuated by *Trpm2* disruption. Interestingly, the number of recruited neutrophils was significantly reduced in DSS-treated *TRPM2* KO mice, whereas that of DSS-induced macrophages after infiltration into inflamed sites, was indistinguishable in WT and *TRPM2* KO mice. Importantly, *TRPM2* deficiency failed to impair important aspects of CXCL2-evoked neutrophil chemotaxis, including Ca^{2+} response, *in vitro* migration, and *in vivo* infiltration after DSS administration. Thus, *TRPM2*-mediated Ca^{2+} influx

induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. We propose functional inhibition of *TRPM2* channels as a new therapeutic strategy for treating inflammatory diseases.

1791-Pos

Multi-Dimensional Characterization of the Desensitization Behavior of Temperature- and H⁺-Activated Human TRPV1 Channels using Microfluidics

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This work describes the pH- and temperature-dependence of acute desensitization and tachyphylaxis in human TRPV1 channels. We use an in-house developed microfluidic device and associated methods, which independently can control the temperature and the solution environment (e.g. the pH) around patch-clamped cells, as well as the time a cell is exposed to different solutions. Thus, cells can be stimulated and controlled in a multi-dimensional parameter space. Our results show that if TRPV1 channels are exposed repeatedly to low pH applications, the rate of desensitization becomes progressively slower, whereas the rate of channel activation remains unchanged. Also, both the rate of activation and the rate of acute desensitization increase at higher temperatures. The extent of tachyphylaxis is found to be dependent on pH, temperature, and exposure time. We also show that both the desensitization rate and the extent of tachyphylaxis are correlated to current density. This could be due to the fact that Ca^{2+} is an important factor for both acute desensitization and tachyphylaxis, and since TRPV1 is permeable to Ca^{2+} , the current density is proportional to Ca^{2+} influx.

1792-Pos

A Novel Tarantula Toxin Irreversibly Activates TRPV1

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Spider venoms contain an evolutionarily honed pharmacopeia of natural toxins that target membrane receptors and ion channels to produce shock, paralysis, pain, or death. Toxins evolve to interact with functionally important protein domains, including agonist binding sites, ion permeation pores, and voltage-sensing domains, making them invaluable reagents with which to probe mechanisms underlying channel activation or modulation. We have identified a novel tarantula peptide toxin that serves as an irreversible agonist for the heat/capsaicin-activated channel, TRPV1. The toxin contains two independently functional Inhibitor Cysteine Knot (ICK) domains, endowing it with an antibody-like bivalency that results in extremely high avidity for its multi-meric channel target. We are using this new toxin as a tool to help dissect the unique properties of TRPV1.

Muscle: Fiber & Molecular Mechanics & Structure II

1793-Pos

Passive Force Augmentation in Actively Stretched Myofibrils and Sarcomeres

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Single skeletal muscle myofibrils were stretched actively and passively ($pCa^{2+} = 3.5$ and 8 respectively) from optimal length to beyond myofilament overlap (4.0 μm) and individual sarcomere lengths and the associated stretch forces were measured. Actively stretched myofibrils produced much more force than passively stretched myofibrils at all sarcomere lengths, including when all sarcomeres were beyond myofilament overlap (Figure 1). In order to confirm that cross bridge based acto-myosin forces were not present at sarcomere lengths beyond myofilament overlap, passively and actively stretched myofibrils were deactivated and activated, respectively at 5 μm . In both cases,

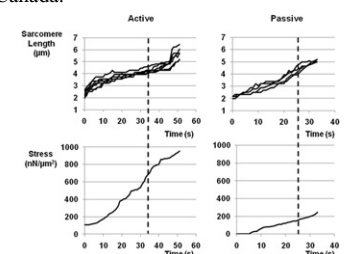


Figure 1. Individual sarcomere length and force traces for single active and passive myofibrils. Actively stretched myofibrils show much greater force than passively stretched myofibrils at sarcomere lengths beyond myofilament overlap where actin-myosin based cross-bridge forces are zero. Dashed vertical line denotes when all sarcomeres are beyond myofilament overlap.