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 $\alpha 1_S$DHPR-null muscular dysgenic myoblasts to eliminate any DHPR-mediated EC coupling-related events. Unlike $\alpha 1_S$DHPR-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 Ca2+ Handling

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TRPC channels have been identified as components of Ca2+ 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 Ca2+-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 Ca2+ influx dynamics. One of these mutants comprises an N-terminal TRPC4 fragment that we used to generate cardiac restricted transgenic mice. Intracellular Ca2+ 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 Ca2+-free buffer were store-depleted with CPA while being treated with Ang II, followed by perfusion with Ca2+ containing buffer to analyze Ca2+ influx with Fura-2 fluorescence. WT myocytes showed essentially no Ca2+ entry, however, hypertrophic myocytes that underwent TAC showed abundant Ca2+ influx. SKF96365 inhibited this Ca2+ influx, suggesting a role for TRPC channels. Indeed, isolated myocytes from dnTRPC4 transgenic mice subjected to TAC lacked this Ca2+ influx, confirming that TRPC channels mediate this previously unrecognized influx activity associated with hypertrophy in cardiac myocytes. Interestingly, Ca2+ 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 Ca2+ 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 Orai1 (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 results 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.

Supported by the FWF project P18475 and P19820

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 GsMT×4 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 Ca2+-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 homotirmer and the TRPP2/PKD1 heteromeric complex, and diminish the surface expression of both proteins. These finding 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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