[Ca2+]o attenuates the amplitude of capsaicin (0.5 microM)-evoked single channel currents through TRPV1 receptors within a physiologically relevant concentration range (Kd = 2 mM, Hill slope = 1.1). The inhibition was observed at a range of positive and negative membrane potentials, being more pronounced at negative potentials. Use of patch clamp photometry revealed that at −60 mV in the presence of 10 mM [Ca2+]o, which is almost maximally effective for inhibiting single channel current amplitudes (56%), the fractional of the current carried by Ca2+ current was only 40%. Thus, Na+ still carries most of the cation current through the TRPV1 receptor even when Ca2+ is likely occupying the site responsible for its inhibiting cation conductance. Finally, we observed that neutralizing the charge on single amino acids located in the mouth of the putative pore and known to contribute to Ca2+ selection by TRPV1 receptors, Asp646, Glu648 and Glu651, did not alter the inhibitory effect of 2 mM [Ca2+]o. To summarize, although Ca2+ has been reported to increase the open probability of TRPV1 receptors, this divalent cation also attenuates conductance through the channel pore via an unknown mechanism seemingly discreet from that contributing to the high Ca2+ permeability of these receptors.

1779-Pos

Distinct Modulations of Human Capsaicin Receptor by Proton and Magnesium Through Different Domains

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The capsaicin receptor (TRPV1) is a nonselective cation channel that integrates multiple painful stimuli, including capsaicin, protons and heat. Protons facilitate the capsaicin- and heat -induced currents by decreasing thermal threshold or increasing agonist potency for TRPV1 activation. In the presence of saturating capsaicin, rat TRPV1 (rTRPV1) reaches full activation, with no further stimulation by protons. Human TRPV1 (hTRPV1), a species ortholog with high homology to rTPRV1, is potentiated by extracellular protons and magnesium, even at saturating capsaicin. We investigated the structural basis for protons and magnesium modulation of fully capsaicin-bound human receptors. By analysis of chimeric channels between hTRPV1 and rTRPV1,We mapped the required domain and a single amino acid residue responsible for further potentiation of capsaicin efficacy by protons. We also showed that magnesium ions could also exert similar effects for capsaicin activation of human TRPV1, but through a different functional domain. Our results demonstrate that capsaicin efficacy of hTRPV1 correlates with the extracellular ion milieu, and unravel the relevant structural basis of modulation by protons and magnesium.

1780-Pos

Interactions between DAG, IP_3 and PIP_2 Govern Activation of Heterotetrameric TRPC6/C7 Channel Activity in Rabbit Portal Vein Myocytes

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Previously we have shown that synergism between inositol-1,4,5-trisphoshate (IP_3) and diacylglycerol (DAG) mediates activation of TRPC6-like channel activity by noradrenaline (NA, Albert & Large, 2003) in rabbit portal vein myocytes. Moreover, a recent study showed that endogenous phosphatidylinositol-4,5-bisphosphate (PIP_2) produced a marked inhibitory action on TRPC6 activity in mesenteric artery myocytes (Albert et al, 2008). In the present work we investigated interactions between DAG, IP_3 and PIP_2 in regulating TRPC6-like activity in portal vein myocytes using patch clamp and immunoprecipitation methods.

In inside-out and cell-attached patches, bath application of respectively 10 μM IP_3 and the cell-permeable IP_3 analogue, 10 μM 6-IP_3, both potentiated OAG-induced TRPC6-like channel activity by 3-fold but had no effect when applied on their own. In inside-out patches, pre-treatment with 20 μM wortmannin, to deplete endogenous PIP_2 levels, increased OAG-evoked channel activity by 75-fold compared to control patches. Moreover, anti-PIP_2 antibodies activated TRPC6-like activity in quiescent inside-out patches. In wortmannin-treated inside-out patches, 10 μM diC8-PIP_2 inhibited OAG evoked channel activity (IC_{50} = 0.74 μM) which was rescued by over 50 % by co-application of 10 μM IP_3. Anti-TRPC6 and anti-TRPC7 antibodies inhibited TRPC6-like activity induced by NA by over 80%, but channel activity was unaffected by other TRPC antibodies. Co-immunoprecipitation studies showed association between TRPC6 and TRPC7 proteins and that both these channel proteins interacted with PIP_2. Pretreated with 6-IP_3, reduced association between PIP_2 and TRPC7 but not TRPC6, whereas OAG reduced PIP_2 interactions with TRPC6 but not TRPC7.

These results indicate that endogenous PIP_2 has a pronounced inhibitory action on TRPC6/TRPC7 heteromeric channels in portal vein myocytes. Moreover channel activation by DAG requires both this triglyceride and IP_3 to remove associations between PIP_2 and these channel proteins.

1781-Pos

Isoform-Selective Physical Coupling of TRPC3 Channels to IP3 Receptors in Smooth Muscle Cells Regulates Arterial Contractility

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Many vasoconstrictors bind to phospholipase C (PLC)-coupled receptors on arterial smooth muscle cells, leading to an intracellular inositol 1,4,5-trisphophate (IP_3) elevation and vasoconstriction. IP_3-induced vasoconstriction can occur independently of intracellular Ca^{2+} release and via IP_3 receptor (IP_3R) and canonical transient receptor potential (TRPC) channel activation, but signaling mechanisms mediating this effect are unknown. Here, we studied the mechanisms by which IP_3Rs stimulate TRPC channels in smooth muscle cells of resistance-size cerebral arteries. Immunofluorescence resonance energy transfer (immuno-FRET) microscopy in smooth muscle cells indicated that endogenous type 1 IP_3Rs (IP_3R1) are in close spatial proximity to TRPC3, but distant from TRPC6 or TRPM4 channels. Endothelin-1 (ET-1), a PLC-coupled receptor agonist, elevated the immuno-FRET signal between IP_3R1 and TRPC3, but not between IP_3R1 and TRPC6 or TRPM4. IP_3R1 co-immunoprecipitated with TRPC3, but not with TRPC6. An antibody targeting TRPC3 channels and TRPC3 channel knockdown with short hairpin RNA inhibited IP_3-induced non-selective cation current (I_{Cat}) activation, whereas an antibody to TRPC6 and TRPC6 channel knockdown had no effect. Biotinylation indicated that ET-1 did not alter total or plasma membrane-localized TRPC3. RT-PCR demonstrated that a calmodulin and IP_3R binding (CIRB) domain is present on the C-terminus of both TRPC3 and TRPC6 channels. A CIRB domain peptide attenuated IP_3- and ET-1-induced I_{Cat} activation. A peptide corresponding to the IP_3R region that can interact with TRPC channels activated I_{Cat}. A HIV-1 TAT-conjugated CIRB domain peptide reduced IP_3- and ET-1-induced vasoconstriction in pressurized arteries. These data indicate that IP_3 stimulates direct coupling between IP_3R1 and membrane-resident TRPC3 channels in arterial smooth muscle cells, leading to I_{Cat} activation and vasoconstriction. Data also indicate that close spatial proximity between IP_3R1 and TRPC3 establishes this isoform-selective functional interaction.

1782-Pos

Molecular and Structural Basis of Dual Regulation of a Canonical TRP Channel by Calmodulin

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The canonical transient receptor potential (TRPC) channels are widely distributed and have diverse biological functions. They are activated by stimulation of phospholipase C-coupled receptors, resulting in membrane depolarization and Ca^{2+} influx, which in turn feedback to regulate the channel activity through the Ca^{2+}-binding protein calmodulin (CaM) and other signaling pathways. Previous biochemical studies indicate that TRPC subunits contain one to four putative CaM-binding sites. One of these sites is named the ''CaM-IP_3receptor binding'' or CIRB site (because it also interacts with an IP_3 receptor fragment *in vitro*). The CIRB site is conserved in all seven TRPC subunits. CaM exerts either stimulatory or inhibitory effects on different TRPC channels. However, the molecular mechanism of CaM modulation of TRPC channels is unclear. We have solved the crystal structure of the complex of CaM and the CIRB site of TRPC5 channels, which regulate growth cone morphology and neurite growth, and require CaM for agonist-induced activation. The structure shows that the two lobes of a single Ca^{2+}-bound CaM (Ca^{2+}/CaM) bind two CIRB peptides arranged in parallel. This peptide dimerizes only in the presence of Ca^{2+}/CaM, suggesting that Ca^{2+}/CaM binding to the CIRB site may induce major conformational changes in intact channels. Structure-based mutagenesis studies show that Ca^{2+}/CaM binding to the CIRB site is not required for agonist-induced channel activation, but it safeguards the channel against inhibition produced by CaM binding to another site on the channel. We have identified this inhibitory site and found it to be a novel CaM-binding motif that can interact with not only Ca^{2+}/CaM but also CaM_{1234}, a mutant CaM deficient in binding Ca^{2+}. Our results provide new insights into the intricate feedback regulation of a canonical TRP channel.

1783-Pos

TRPC3 is Essential for Maintenance of Skeletal Muscle Cells

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During membrane depolarization associated with skeletal excitation-contraction (EC) coupling, L-type Ca^{2+} channels (dihydropyridine receptor (DHPR) in the transverse (t)-tubule membrane) undergo conformational changes that are transmitted to Ca^{2+}-release channel (ryanodine receptor

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.

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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 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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