[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