of divalent ions and selectivity, is an important step towards understanding the structural base of TRPC3 channel function.
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Analysis of the Molecular Basis of Ca2+- dependent Regulation of TRPC3 Channels
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TRPC3 generates non-selective cation channels that are subject to a highly complex regulation by the permeating cation Ca2+. This Ca2+-mediated control of TRPC3 is considered to include mechanisms of negative feedback regulation (current inactivation) essential for physiological roles of the channel complex. Ca2+ ions may control TRPC channel function by interaction with regulatory proteins that are located in, or targeted to a cytoplasmic regulatory microdomain or alternatively by certain interactions with the permeation pathway. To explore the molecular basis of TRPC3 modulation by Ca2+, we tested the impact of Ca2+ permeation and of negative feedback regulation by PKC on Ca2+ sensitivity of TRPC3 channels expressed in HEK293 cells. TRPC3 currents were rapidly suppressed by elevation of extracellular Ca2+ (0.8 to 2 mM) range after activation via muscarinic receptor stimulation in nominally Ca2+-free solution, and removal of divalents from the extracellular solution activated a TRPC3-mediated conductance. Neutralization of a single negative charge within the putative pore domain (E630Q) generated channels which were largely insensitive to changes in extracellular divalents but displayed normal current decay during activating stimuli. Mutation in T573, which is required for down-regulation of channels by PKC-phosphorylation (T573A; ''moonwalker'') was associated with a strongly reduced current decay. We conclude that TRPC3 channels sense the extracellular Ca2+ concentration by a Ca2+ binding site within the permeation pathway, while current decay after stimulation is barely dependent on Ca2+ permeation through the channel but involves regulatory phosphorylation.
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Inhibition of TRPC6 Channels by TRPC1/C5 Channel Activity Through a Ca_{2+} and Pkc-Dependent Mechanism
Anthony P. Albert, Jian Shi, Min Ju, William A. Large.
Vascular myocytes express two groups of functional canonical transient receptor (TRPC) channels, which we have termed TRPC1 for TRPC1-containing and TRPC3/6/7 for non-TRPC1 containing channels. These two groups have distinct properties from each other, for example, TRPC1 channels have a single unitary conductance of 2-3 pS and require PIP2 and PKC for activation, whereas TRPC3/6/7 channels have multiple conductances between 10-70 pS and are inhibited by PIP2 and PKC. The present work investigated interactions between TRPC1/C5 and TRPC6 channels activities evoked by angiotensin II (Ang II) in freshly isolated rabbit mesenteric artery myocytes.
In low intracellular Ca2+ buffering conditions, 1 nM and 10 nM Ang II activated both 2 pS TRPC1/C5 and 15-45 pS TRPC6 channels whereas 100 nM Ang II only activated TRPC1/C5 channels in outside-out patches. Inclusion of anti-TRPC1 and -TRPC5 antibodies, raised against intracellular epitopes, in the patch pipette solution blocked TRPC1/C5 activity but increased TRPC6 activity activated by 1-100 nM Ang II. Bath application of T1E3, raised against an extracellular epitope, also potentiated Ang II-induced TRPC6 activity. These results show that inhibition of TRPC1/C5 channels increases the activity of TRPC6 channels.
With high intracellular buffering conditions, 1-100 nM Ang II-evoked TRPC6 activity was increased in outside-out patches whereas increasing intracellular Ca2+ levels reduced TRPC6 activity in inside-out patches. Pre-treatment with the PKC inhibitor chelerythrine greatly increased 1-100 nM Ang II-activated TRPC6 activity. Co-immunoprecipitation studies showed that Ang II induced phosphorylation of TRPC6 protein which was inhibited by chelerythrine, T1E3 and zero external Ca2+ levels.
These studies show novel interactions between TRPC1/C5 and TRPC6 channels, whereby TRPC1/C5-mediated Ca2+ influx and stimulation of PKC phosphorylates TRPC6 protein which leads to channel inhibition.

574-Pos Board B374
PI3 Kinase-Mediated Pathways Activate TRPC Channels in Vascular Myocytes
Anthony P. Albert, Jian Shi, Min Ju, Sohag N. Saleh, William A. Large.
It is generally thought that vasoconstrictors activate canonical transient receptor potential (TRPC) channels through stimulation of phospholipase-mediated pathways in vascular myocytes, with generation of diacylglycerol (DAG) being an essential event. TRPC1-containing channels are activated by DAG through a PKC-dependent pathway whereas non-TRPC1-containing channels (e.g. TRPC3/6/7) are gated by DAG via a PKC-independent mechanism. However, endothelin-1 (ET-1)-evoked TRPC1 and TRPC3/6/7 channels in rabbit coronary artery myocytes are not inhibited by phospholipase inhibitors, which indicates that other mechanism are involved in activating these channels. Therefore the present work investigated activation mechanisms coupling ET-1 to opening of these channels.
Stimulation of ETA receptors by ET-1 (in the presence of the ETB receptor antagonist BQ788), vascular endothelium growth factor (VEGF) and interleukin-1beta (IL-1beta) activated both TRPC1 and TRPC3/6/7 channels in cell-attached patches, which were not blocked by phospholipase C, D and A2 inhibitors but were blocked by the phosphatidylinositol-3 (PI(3)) kinase inhibitor wortmannin. Bath application of phosphoinositol-3,4,5-trisphosphate (PIP3, product of PIP3 activity) to the cytosolic surface of inside-out patches activated TRPC1 but not TRPC3/6/7 channel activity. Moreover, a PI3 kinase activator and also PI(3) phosphatase inhibitors activated TRPC1 and TRPC3/6/7 channels.
These results provide novel information that stimulation of PI3 kinase and generation of PI(3) molecules by physiological and pathological vasoactive agents also have important roles, in addition to phospholipase-mediated generation of DAG, in activating TRPC channels in vascular myocytes.

575-Pos Board B375
A Structural Model of the TRPP2/PKD1 C-Terminal Coiled Coil Complex Obtained by a Combination of Computational and Experimental Approaches
Jiang Zhu, **Yong Yu**, Maximilian H. Ulbrich, Minghui Li, Ehud Y. Isacoff, Barry Honig, Jian Yang.
Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in the genes encoding TRPP2 and PKD1, which form an ion channel/receptor complex containing three TRPP2 and one PKD1. PKD1 is a large integral membrane protein containing 11 putative transmembrane regions, while TRPP2 has 6 transmembrane regions and belongs to the transient receptor potential (TRP) channel family. TRPP2 and PKD1 associate through their C-termini, where a single PKD1 coiled coil binds to a TRPP2 coiled coil trimer. Many ADPKD pathogenic mutations result in the abolishment of the TRPP2/PKD1 coiled coil complex. Obtaining the structure of this complex would help us better understand its crucial role in the assembly and function of the full length TRPP2/PKD1 complex. By combing computational and experimental approaches, we generated a structural model for this coiled coil complex, based on a crystal structure of the TRPP2 coiled coil trimer. The structural model was constructed by a two-step docking strategy, which combines iterative rigid-body search and molecular dynamics (MD) simulations. In this structural model, the N-terminal region of the TRPP2 coiled coil remains as a trimer, but the C-terminal regions of two of the three TRPP2 helixes interact with a single PKD1 coiled coil to form a new trimer. Disruption of predicted critical TRPP2/PKD1 interface contacts abolished or greatly weakened the association between TRP2 and PKD1 coiled coils, supporting the accuracy of the structural model. Some mutations also greatly attenuated the assembly of the full-length TRPP2/PKD1 complex, providing the means to specifically disrupt this complex and study its functional importance in vitro and in vivo. The structural model also sheds light on the pathogenic mechanisms of some ADPKD-causing mutations.
*J.Z. and Y.Y. contributed equally to this work.

576-Pos Board B376
Actin-Binding Proteins Mediate Regulation by Calcium of Polycystin-2 (tRPP2)
María del Rocío Cantero, Horacio F. Cantiello.
Polycystin-2 (PC2, TRPP2) is a member of the TRP superfamily that acts as a non-selective cation channel, with permeability to calcium. PC2 is implicated in calcium transport, and therefore, its regulation by calcium is relevant to its role(s) in cell function. Recent studies (Cantero & Cantiello, BJ 98(3):340a, 2010) demonstrated that PC2 channel activity from human syncytiotrophoblast is regulated by cytoplasmic calcium, such that low calcium (< 0.3 nM) renders the channel inactive. Titration of cytoplasmic calcium recovers PC2 channel activity, with a Hill coefficient of 4 and an apparent affinity constant of 1-5 nM. Interestingly, *in vitro* translated PC2, devoid of regulatory proteins was completely insensitive to intracellular calcium (*ibid*). In this study we further explored the nature of this regulation by assessing potential calcium-sensitive target proteins in the channel complex. Several calcium sensitive and insensitive actin-binding proteins (ABP) were studied in the *in vitro* translated protein. The actin-bundling protein α-actinin (250 nM) increased PC2 channel activity in the presence of high (10 μM) cytoplasmic calcium, but instead was inhibitory in its absence. The calcium insensitive G-actin binding protein profiling (4 nM), increased PC2 channel activity both in presence and