absence of calcium, while the calcium-sensitive, actin-severing protein gelsolina (4 nM), regulated PC2 channel function in the presence, but not absence of calcium (10 μM). These data suggest that the formation of ABP-PC2 complexes confer distinct calcium regulatory functions to the channel, thus providing a novel cytoskeletal pathway for channel regulation. This response to cytoplasmic calcium by PC2 may provide functional diversity to the channel by choosing, and possibly exchanging ABP-structural partners.

577-Pos Board B377

Direct Activation of TRPV6 Channels by Phosphatidylinositol 4,5-Bisphosphate

Eleonora Zakharian, Chike Cao, **Tibor Rohacs**.

Transient Receptor Potential Vanilloid 6 (TRPV6) is an inwardly rectifying Ca^{2+} selective ion channel that plays a role in Ca^{2+} absorption in the intestines. The activity of these channels have been shown to depend on the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2). Increased in cytoplasmic Ca^{2+} concentrations inhibit these channels, protecting cells from toxic Ca^{2+} overload. Both Ca^{2+} Calmodulin (CaCaM) and depletion of PIP_2 have been implicated in Ca^{2+} induced inactivation. In addition, it has been proposed that cytoplasmic ATP binds directly to the channels, and this binding is important for maintaining TRPV6 channel activity in whole-cell patch clamp experiments. To evaluate whether PIP_2, CaCaM and ATP affects channel activity directly or indirectly, we have performed excised inside-out patch clamp measurements with these compounds. Channel activity upon excision showed marked current run-down, characteristic of PIP_2 dependent channels. ATP reactivated the channels, but only in the presence of Mg^{2+}. This could mean that MgATP provides substrate for lipid kinases present in the patch membrane, allowing the resynthesis of PIP_2. To test this hypothesis, we have used wortmannin and LY294002 at concentrations where they inhibit phosphatidylinositol 4-kinases, and found that they both inhibit the effect of MgATP in excised patches. At lower concentrations, where they specifically inhibit PI3Kinases, they did not inhibit the effects of MgATP. We also found that PIP_2 reactivates TRPV6 in excised patches, and this effect is inhibited by CaCaM. To further establish PIP_2 as a direct activator of TRPV6, we purified the channel protein and reconstituted it in planar lipid bilayers. We found that the reconstituted protein showed inward rectification, and its activity depended on the presence of PIP_2. Our data establish PIP_2 as a direct activator of TRPV6 and raise doubt that intracellular ATP regulates the channel through direct binding.

578-Pos Board B378

A helix-Breaking Mutation in the Epithelial Ca2+ Channel TRPV5 Leads to Reduced Ca_{2+} Dependent Inactivation

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TRPV5, a member of transient receptor potential (TRP) superfamily of ion channels, plays a crucial role in epithelial calcium transport in the kidney. This channel has a high selectivity for Ca2+ and is tightly regulated by intracellular Ca2+ concentrations. Recently it was shown that the molecular basis of deafness in varitint-waddler mouse is the result of hair cell death caused by the constitutive activity of transient receptor potential mucolipin 3 (TRPML3) channel carrying a helix breaking mutation, A419P, at the intracellular proximity of the fifth transmembrane domain (TM5). This mutation significantly elevates intracellular Ca2+ concentration and causes rapid cell death. Here we show that substituting the equivalent location in TRPV5, the M490, to proline significantly modulates Ca2+-dependent inactivation of TRPV5. The single channel conductance, time constant of inactivation (τ) and half maximal inhibition constant (IC50) of TRPV5(M490P) were increased compared to TRPV5(WT). Moreover TRPV5(M490P) showed lower Ca2+ permeability. Out of different point mutations created to characterize the importance of M490 in Ca2+-dependent inactivation, only TRPV5(M490P)-expressing cells showed apoptosis and extremely altered Ca2+-dependent inactivation. In conclusion, the TRPV5 channel is susceptible for helix breaking mutations and the proximal intracellular region of TM5 of this channel plays an important role in Ca2+-dependent inactivation.

579-Pos Board B379

Study of Ion Channel Regulation by Phosphoinositides Using Spatiotemporal Control Over PI-Phosphatase and PI-Kinase Activity

Ivan F. Gonzalez, Sharona E. Gordon.

Inositol phospholipids act as membrane-bound second messengers modulating the activity of some ion channels in the plasma membrane (PM). In eukaryotic cells, the inositol head of phosphatidylinositol (PI) can be found phosphorylated in three or fewer hydroxyl groups. PI metabolic flow is directed by the activity of PI-phosphatases and PI- kinases on the inositol head, effectively changing the relative abundance of PI species in the PM. We have adapted a recently published technique of spatiotemporal control of cell signaling that uses a light-switchable protein interaction to translocate proteins to the PM. This technique, together with novel protein constructs, allows us to reversibly translocate PI-enzymes to the PM in a light-controlled fashion, affecting the relative abundance of PI species in the illuminated PM region. The use of phosphoinositide-specific binding modules fused with fluorescent proteins during imaging provides the qualitative feedback of enzymatic activity and relative PI abundance. We report our advances on the implementation of this technique applied to test two competing models of TRPV1 channel regulation by PIP2 and PIP3. (Supported by grant EY07031 from the NEI).

580-Pos Board B380

Temperature Dependence of Mutant Vanilloid Receptor (tRPV) Channels

Jing Yao, Beiying Liu, Feng Qin.

Temperature-gated transient receptor potential (TRP) channels exhibit exceedingly large energetic changes in activation. However, the source of energy and its structural basis are not known. Several studies have recently suggested that the pore domain of the channels plays an essential role in temperature gating. In particular, multiple mutations in the outer pore of the TRPV3 channel, which is activated by heat above 30°C, were found to selectively abrogate the heat response of the channel while leaving the response to chemical agonists largely intact. Vanilloid receptors (TRPV) have a membrane topology resembling that of voltage-gated ion channels, and these mutant residues are located at the S6-linker region. The profound and yet selective effects of the region on thermal sensitivity of TRPV3 suggest that the outer pore of the channel may be responsible for heat sensing. In support of the hypothesis, the replacement of a pore turret region in TRPV1 by a glycine-based fragment was also shown to specifically eliminate the heat sensitivity of the channel without compromising its capsaicin response. Alternatively, the ''heat sensors'' of the channels, if any, may reside elsewhere, while the pore domain is involved in the downsteam pathway of activation such as allosteric coupling between the heat sensors and the gate of the channel. In this study, we will attempt to discern such two mechanisms. We will show the remaining of temperature dependence in the mutant channels and explore mechanisms and the role of the pore domain in temperature gating.

581-Pos Board B381

Extracellular Cation Gates TRPV1 via the Heat Activation Pathway

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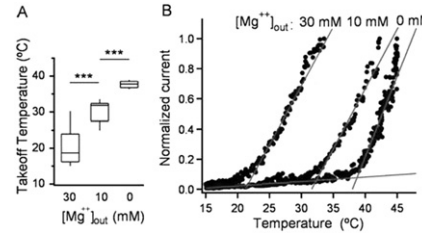
ThermoTRP channels are expressed in sensory neurons where they detect a variety of physical and chemical stimuli including temperature, ionic strength, membrane voltage and ligand binding. However, the precise mechanisms underlying such diverse modes of activation remain largely unknown. It is found that temperature and capsaicin activate TRPV1 through separate pathways. Here we investigate how extracellular cation activates the heat-sensitive TRPV1 channel.

At room temperature, Mg^{++} ions effectively and selectively activate TRPV1 from the extracellular side in a dose-dependent manner. The relative open probability induced by 100 mM $[Mg^{++}]_{out}$ is approximately 40% of that elicited by saturating capsaicin, while the same concentration of $[Mg^{++}]_{in}$ is ineffective. Mg^{++} substantially shifts the temperature sensitivity of the channel. At 10 mM, Mg^{++} shifts the takeoff temperature of TRPV1 greatly, which implies that the effects of heat and Mg^{++} are coupled tightly. Furthermore, mutant channels with an artificial pore turret, which have less temperature sensitivity but maintain normal capsaicin responses, also show reduced cation responses. In summary, our observations suggest that in TRPV1 the pore turret may detect both heat and ion strength, and effectively transfer the energy to open the activation gate.



582-Pos Board B382

TRPV1 Ionic Selectivity Dynamics are Modulated by Intracellular ATP

Clare H. Munns, Michael J. Caterina.

It was recently shown that TRPV1, a chemically- and thermally-activated cation channel highly expressed in sensory neurons, undergoes time- and agonist-dependent changes in ionic selectivity. More specifically, permeability to large cations such as N-methyl-D-glucamine (NMDG) increases during prolonged activation by capsaicin and resiniferatoxin. These changes in ionic selectivity are dose-dependent, being evoked at high, but not low, concentrations of agonist. Furthermore, protein kinase C-mediated phosphorylation of TRPV1 results in a leftward shift of the dose-response curve, inducing changes in ionic selectivity at low agonist concentrations. In addition to phosphorylation, the