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# Regulation of TRPM7 by Cytosolic Mg2+ and pH: Insights from VSP Expression

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In fluorescence spectroscopy and imaging experiments with the fluorophores laurdan and 1,6-diphenyl-1,3,5-hexatriene (DPH) we found that phenol derivatives modulate membrane properties. We propose that activation of TRPA1 by non-electrophilic compounds may arise from the induction of changes in membrane properties and suggest that chemosensation may result from primary mechanosensory mechanisms.

**1233-Pos Board B301**

**Regulation of TRPM7 by Cytosolic $Mg^{2+}$ and pH: Insights from VSP Expression**

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TRPM7 is an ion channel/protein kinase belonging to TRP melastatin and eEF2 kinase families. Under physiological conditions, most native TRPM7 channels are inactive, due to inhibition by cytoplasmic $Mg^{2+}$, protons and polyamines. $I_{TRPM7}$ is strongly potentiated when cell cytosol is depleted of $Mg^{2+}$ or alkalinized. In Jurkat T cells, $Mg^{2+}$ inhibition involves a high and a low affinity inhibitor sites, whereas proton inhibition involves only one site. Like many other TRP channels, TRPM7 is activated by $PI(4,5)P_2$ and suppressed by its hydrolysis. Here we examined $Mg^{2+}$ and pH inhibition of native TRPM7 channels in HEK293 cells overexpressing voltage-sensitive phospholipid phosphatase (VSP) or its catalytically inactive C363S mutant. Phosphoinositide depletion by VSP increased the sensitivity of channels to pH and high $Mg^{2+}$. Specifically internal pH values that were stimulatory when C363S was expressed (pH 8.2) became inhibitory in the wildtype VSP-expressing cells. 150 uM $Mg^{2+}$ or $pH_i$ 6.5 inhibited $I_{TRPM7}$ both in wildtype and C363S VSP-expressing cells but with a faster time course in the former group. Both basal and maximum currents were reduced in VSP expressing cells while the mean time to reach maximum amplitude was shortened. In order to prevent the activation of VSP by voltages reaching +85 mV used for recording TRPM7 currents, we tested the effects of VSP on inward currents in divalent cation free solutions by applying voltage ramps reaching only +20 mV. Surprisingly, this command voltage protocol produced results similar to those obtained from ramps reaching +85 mV. These observations suggest that in HEK293 cells, VSP may have significant basal activity even prior to application of depolarizing voltage pulses, possibly due to the depolarized resting membrane potential of these cells. Consistent with this scenario, a subpopulation of wildtype VSP transfected cells behaved like C363S-transfected cells, likely because their resting potential was more negative. Growing HEK cells in 25 mM KCl instead of 5 mM to shift $K^+$ equilibrium potential by ~+40 mV did not result in increased basal VSP activity, however. In summary, our experiments suggest that voltage-independent $Mg^{2+}$ and pH inhibition of TRPM7 channels is not direct but, rather, reflects electrostatic screening and disruption of $PI(4,5)P_2$-channel interactions.

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**Temperature Sensitivity of *Drosophila* Gustatory Receptor Gr28b**

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Gustatory receptors are a family of transmembrane proteins that have been extensively studied in the context of insect taste and odor sensory systems. A recent study has identified a *Drosophila* gustatory receptor, Gr28b(D), as a thermosensor expressed in peripheral ''hot cell'' neurons responsible for rapid heat avoidance. Gr28b(D) is the first gustatory receptor shown to be involved in thermosensation. However, little is known mechanistically about the protein, and previous attempts at heterologous expression were reported unsuccessful. Although proposed to have a seven-transmembrane domain topology, it is unknown whether Gr28b(D) functions as a G-protein coupled receptor (GPCR) or as an ion channel, and fundamental questions about the biophysical properties of the protein remain completely unanswered. Here, we sho successful heterologous expression of Gr28b(D) in *Xenopus laevis* oocytes and confirm temperature-sensitivity as an intrinsic property of the protein. Furthermore, we have generated Gr28b(D) single amino acid and truncation mutations to investigate structural correlates of ion selectivity and temperature sensitivity.

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**Simulated Microgravity Conditions Modulate $Ca^{2+}$ Transport through TRPV4 Channels**

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Astronauts in space tend to lose ten times as much bone mass each month as severe osteoporosis patients on Earth. Multiple reports on unloading-induced osteoporosis in bed-ridden patients indicate that bone mass correlates positively with mechanical stress. The Transient Receptor Potential Vanilloid Type 4 (TRPV4) is a $Ca^{2+}$-permeable cation channel which responds to mechanical and osmotic signals in multiple musculoskeletal tissues. This channel links mechanically triggered molecular signaling and $Ca^{2+}$ levels as contributors of the same molecular pathway leading to increased osteoclast differentiation. In this line of investigation, we simulated microgravity conditions to detect modulation of $Ca^{2+}$ transport from internal stores through TRPV4 channels using yeast luminescent reporters. The cell culture was introduced to capacity into a cylindrical High Aspect to Ratio Vessel (HARV) in a horizontal (1g, control) or vertical (simulated micro-gravity) position in the Rotary Cell Culture System. The experimental approach required design and implementation of custom optical equipment, which proved successful in determining minute changes in $Ca^{2+}$ transport by either luminescence or fluorescence measurements. The increase of cytosolic $Ca^{2+}$ upon release from internal stores was assessed using the luminescence signal generated by the aequorin-coelenterazine system in the presence of $Ca^{2+}$. Fluorescence measurements of $Ca^{2+}$ release employed the cell-membrane permeant $Ca^{2+}$ indicator Fluo-4 AM. The set of experimental data provided in this report demonstrates directly that simulated microgravity conditions induce measurable changes of $Ca^{2+}$ transport through TRPV4 channels. This modulation of $Ca^{2+}$ transport was similar to results from hypo-osmotic stress conditions previously described by several studies on the same cells. A better molecular understanding of the contribution of reduced mechanical loading to the decline in bone mass and quality during extended space flight missions and Earth-based bed-ridden conditions is crucial for mitigating pathologies which manifest as severe bone loss.

## Ion Channel Regulatory Mechanisms I

**1236-Pos Board B304**

**The Role of the N-Terminal and S1 Segments in Kv1.5 Channel Trafficking and Modulation**

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The ultra-rapidly activating delayed rectifier potassium current ($I_{Kur}$) is mediated by Kv1.5 channels. Kv1.5 channels, encoded by the KCNA5 gene, are important for atrial repolarization. The extracellular S1-S2 linker of Kv1.5 is long compared to other voltage-gated potassium channels and is susceptible to proteolytic degradation by the extracellular application of proteinase K (PK). We recently demonstrated that cleavage of mature (75-kDa) Kv1.5 channels in the S1-S2 linker with PK produced a 33-kDa C-terminal fragment and a 42-kDa N-terminal fragment. While Co-IP experiments indicate that the two fragments do not associate after PK cut, the cleaved channels are able to generate robust current. In the present study, we found that expression of the N- or C-terminal fragment alone did not generate current. However, co-expression of both the N- and C- fragments together produced a robust current similar to the WT Kv1.5 channel. Analysis of cell surface protein with biotinylation and immunocytochemistry indicated that the trafficking-competent N-fragment was required for the C-fragment to traffic to the membrane. Truncation of the first 209 amino acids from the N-terminus did not disrupt channel trafficking, indicating that the S1 segment (amino acids 248–269) and/or segment containing amino acids 210–247 are required for channel trafficking. Compared to WT Kv1.5 channels, the N-truncation Δ209 mutation displays faster activation properties. The accelerated activation of the Δ209 Kv1.5 was revoked upon extracellular cleavage of the channel with PK. Our data demonstrate that the N-terminal and S1 segments contribute to the trafficking and modulation of Kv1.5 channels.

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**ERK1/2 Mediates EGF-Dependent Kv1.3 Endocytosis**

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The voltage-gated potassium channel Kv1.3 participates in immunity, axonal growth targeting, sensory discrimination, metabolism and insulin resistance. Kv1.3 regulation by phosphorylation has been extensively studied. EGF, via