on the pore helix was also responsible, each of which accounted for only half of the total inhibition. Only double mutations of both residues could eliminate the full blockade. In the prototype Kcsa structure, the counter residues have been suggested to form a hydrogen bond, and the disruption of their interaction destabilizes the pore conductance. These results suggest that protons may block TRPV1 by titrating the interaction of the pore helix with the selectivity filter, and thus provides a novel model for proton-mediated pore block of ion channels.

1353-Pos Board B197

Thermally-induced Activation Of TRPV2 Channels Causes Cell Death In Airway Smooth Muscle

Peter Dyrda, Peter Malinouski, **Luke J. Janssen**.
McMaster University, Hamilton, ON, Canada.

''Bronchial thermoplasty'' — the direct application of thermal energy to the airway wall in the clinical setting — leads to reduction of the smooth muscle mass within the airway wall, reduced potential for bronchoconstriction and improvement in asthma symptoms. However the mechanism underlying this response has not yet been elucidated. We found a steep thermal sensitivity of isometric contractions in bovine airway smooth muscle: ~0%, ~50% and ~100% reduction at $<50°C$, 52.5°C and $>55°C$, respectively. These changes in contractility developed within minutes after thermal treatment. This thermal sensitivity was shifted to lower temperatures by the TRPV2 agonist 2-APB (2-aminoethoxydiphenyl borate, 10^{-4} M). Likewise, the TRPV2 agonists 2-APB and tetrahydrocannabinol (10^{-4} M) evoked a large membrane conductance with linear current-voltage relationship and reversal potential of ~0 mV. Immunohistochemistry showed TRPV2 to be distributed around the smooth muscle. These observations are all consistent with the involvement of TRPV2 in the thermal response of airway smooth muscle. Oddly, however, temperature-induced ablation of contractions was not prevented by agents which block conductance through TRPV2 channels (ruthenium red; La^{3+}, Gd^{3+}, capsaicin, removal of external Ca^{2+}). We conclude that bronchial thermoplasty activates TRPV2 channels in the muscle, and that this in some way transduces into a disappearance of the smooth muscle cell (and thus loss of contractility). Furthermore, the data suggest these changes are not dependent upon the ionic currents *per se* through those channels; instead, they may involve a direct interaction between the channels and some intracellular entity(s).

1354-Pos Board B198

Topology of the Selectivity Filter of a TRPV Channel: Rapid Accessibility of Four Contiguous Residues to the External Medium

Sebastien Wall-Lacelle, Yolaine Dodier, Francois Dionne,
Jonathan Blanchet, Alexandra Raybaud, Remy Sauve, Lucie Parent.
Université de Montréal, Montreal, QC, Canada.

TRPV5 is a six-transmembrane domain ion channel that is highly selective to Ca^{2+} ions. To study the topology of the selectivity filter using the substituted cysteine accessibility method (SCAM), cysteine mutants at positions 541 to 547 were studied as heterotetramers using dimeric constructs that couple in a tandem the control channel with a cysteine-bearing subunit. Whole-cell currents of dimeric constructs D542C, G543C, P544C, A545C, and Y547C were rapidly inhibited by positively charged MTSMT, MTSEA, and MTSET reagents whereas D542C, P544C, and A545C were inhibited by the negatively charged MTSES. In contrast, I541C dimer remained insensitive to positive and negative reagents. However the I541C / D542G and the I541C / D542N dimeric constructs were rapidly (< 30 s) and strongly inhibited by positively and negatively charged MTS reagents suggesting that removing two out of the four carboxylate residues at position 542 disrupts a constriction point in the selectivity filter. Altogether these results establish that the side-chains of contiguous amino acids in the selectivity filter of TRPV5 are rapidly accessible from the external medium in contrast to the 3-D structure of the selectivity filter in K^+ channels where the main-chain carbonyls were shown to project toward a narrow permeation pathway. The I541C data further suggest that the selectivity filter of TRPV5 espouses a specific conformation that restrains accessibility in the presence of four carboxylate residues at position 542. Several 3-D models of the TRPV5 channel (based upon KcsA, Kv1.2, KvAP, and the cationic non-selective NaK channels) will be discussed. *Supported by the Kidney Foundation of Canada.*

1355-Pos Board B199

The Role Of Phospholipase C In The Ca^{2+}-induced Inactivation Of Trpv6

Baskaran Thyagarajan, Viktor Lukacs, Bryan Benn, Sylvia Christakos,
Tibor Rohacs.
UMDNJ - New Jersey Medical School, Newark, NJ, USA.

TRPV6 is a member of the transient receptor potential superfamily of ion channels that facilitates Ca^{2+} absorption in the intestines, especially the duodenum. TRPV6 channels have been shown to be inactivated by increased cytoplasmic Ca^{2+} concentrations. We studied the mechanism of this Ca^{2+}-induced inactivation. Monovalent currents through TRPV6 substantially decreased after one minute application of Ca^{2+}, but not Ba^{2+}. We also show that Ca^{2+}, but not Ba^{2+} influx via TRPV6 activates phospholipase C (PLC) that leads to depletion of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$]. Dialysis of DiC_8 $PI(4,5)P_2$ through the patch pipette inhibited Ca^{2+} dependent inactivation of TRPV6 currents in whole-cell patch clamp experiments. $PI(4,5)P_2$ also activated TRPV6 currents in excised patches. PI(4)P, the precursor of $PI(4,5)P_2$ neither activated TRPV6 in excised patches, nor had any effect on Ca^{2+}-induced inactivation in whole-cell experiments. The PLC inhibitors U73122 and edelfosine inhibited Ca^{2+} induced $PI(4,5)P_2$ depletion and IP_3 formation, indicating effective inhibition of PLC. Both PLC inhibitors also inhibited Ca^{2+}-induced inactivation of TRPV6 and rendered Ca^{2+} signals more sustained in TRPV6 expressing cells. Inhibiting PLC with edelfosine enhanced $^{45}Ca^{2+}$ uptake in the everted duodenal gut sac assay. Our data demonstrate that PLC is involved in Ca^{2+}-induced inactivation of TRPV6 and that PLC inhibitors can be used to enhance intestinal Ca^{2+} uptake.

1356-Pos Board B200

Channel Properties Of TRPM8 In Supramolecular Complex With Inorganic Polyphosphate And Polyhydroxybutyrate

Eleonora Zakharian, Tibor Rohacs.
UMDNJ - New Jersey Medical School, Newark, NJ, USA.

Transient Receptor Potential channel of melastatin subfamily 8 (TRPM8) is a cold and menthol receptor. We have shown that the TRPM8 protein purified from HEK 293 cells is associated with inorganic polyphosphate (polyP) and polyhydroxybutyrate (PHB). In order to study the possible roles of these homopolymers we examined the channel activity of TRPM8 in *vivo* and in *vitro* experiments.

Using whole-cell patch-clamp and fluorescent calcium measurements we demonstrate that enzymatic breakdown of polyP by exopolyphosphatase (scPPX1) inhibits channel activity in human embryonic kidney cells and F11 neuronal cells expressing the TRPM8 channel. Furthermore, addition of scPPX1 completely blocked the activity of the purified TRPM8 channels reconstituted into planar lipid bilayers, where the activity of the channel was observed in the presence of phosphatidylinositol (4,5)-bisphosphate ($PtdIns(4,5)P_2$) and menthol. In the planar lipid bilayer experiments we found that two major agonists $PtdIns(4,5)P_2$ and menthol affect the channel's open probability in concentration dependent manner, while elimination of polyP from the channel diminishes the magnitude of the conductance. Biochemical analysis of the TRPM8 protein supported the idea that polyP association with the channel is insured by non-covalent interactions, while PHB binds to the TRPM8 protein covalently.

These evidences indicate that TRPM8 exists in a supramolecular complex, where its structure and function are modified by association with polyP and conjugation with PHB.

1357-Pos Board B201

Modulation Of Hepatocellular Trpm7-like Currents By A Ca^{2+}/calmodulin-sensitive Pathway

Ceredwyn E. Hill.
Queen's University, Kingston, ON, Canada.

Non-selective cation currents play integral roles in countering cell swelling, inducing apoptotic cell shrinkage, mediating Ca^{2+} influx and controlling Mg^{2+} homeostasis. The 'chanzymes' TRPM6 and TRPM7 are regulated by cytosolic Mg^{2+}, and, under specific recording conditions, Mg-ATP or cell swelling. We recently identified a Mg^{2+}-inhibited and outwardly rectifying cation current in both rat hepatocytes and the polarized rat hepatoma x human skin fibroblast cross, WIF-B. Here we investigated the regulation of these currents by cytosolic Ca^{2+}. Under standard whole-cell recording conditions in which cells were bathed and dialyzed with Na-gluconate solutions, the latter Ca^{2+}- and Mg^{2+}-free, currents reversed close to 0 mV, showed no time dependence, and were 23 times higher at +120 mV as compared with -120 mV. Current at +120 mV developed slowly over dialysis, from 17.7 ± 10.3 pA/pF at patch rupture to 106.5 ± 15.6 pA/pF at 12 min. Inward current at -120 mV did not change significantly. Pipette solution containing Ca^{2+} inhibited current development with an IC_{50} of 125 ± 35 nM. 50 μM W-7 or 200 nM staurosporine relieved the inhibition by 1 μM Ca^{2+}, implicating channel regulation by a Ca^{2+}/calmodulin-dependent kinase (CaMK). To address the nature of the kinase involved, we dialyzed the cells with more specific inhibitors of myosin light-chain kinase (MLCK) and CaMKII. 2 μM AIP, the CaMKII inhibitor, allowed full development of current, whereas 5 μM ML-7 did not affect the Ca^{2+}-dependent channel inhibition ¼ . Inspection of the rat Trpm7 primary structure identified a specific CaMKII substrate sequence in the proximal C-terminus. Conversely neither a CaM-binding domain nor a MLCK substrate consensus sequence was identified. The combined results support the conclusion that these

channels are inhibited by cytosolic concentrations of Ca^{2+} in a CaMKII-dependent manner. Supported by NSERC & the Jeanne Mance Foundation, Hotel Dieu Hospital.

1358-Pos Board B202

Characterization of Transient Receptor Potential Melastatin 7 in Bone Marrow Stem Cells

Henrique Cheng.
Louisiana State University, Baton Rouge, LA, USA.

Changes in the intracellular concentrations of Ca^{2+} and Mg^{2+} play a significant role in cell growth and differentiation. Mesenchymal stem cells (MSCs) from bone marrow are a potential source for tissue repair due to their ability to differentiate into specialized cells, including bone, fat and muscle. However, the molecular signals controlling the differentiation process remains largely unknown. In this study, we examined whether MSCs express Transient Receptor Potential Melastatin 7 (TRPM7), a member of the TRP family of ion channels and a key pathway for Ca^{2+} and Mg^{2+} entry into cells. By RT-PCR, we identified TRPM7 transcripts with the expected molecular size of 198bp, but not TRPM6 (317bp), a close family member with similar function. Electrophysiological recordings revealed that depletion of intracellular Mg^{2+} or Mg^{2+}-ATP activated TRPM7, suggesting that the channel is functionally active. Furthermore, treatment of MSCs with 2-aminoethoxydiphenyl borate (2-APB 1pM-100μM), a TRPM7 blocker inhibited TRPM7 currents in a dose-dependent manner. Our findings suggest that TRPM7 may represent an important pathway for controlling stem cell growth and differentiation by regulating the amount of Ca^{2+} and Mg^{2+} entering cells.

1359-Pos Board B203

The Type IV Mucolipidosis-Associated Protein TRPML1 is an Endolysosomal Iron Release Channel

Xian-Ping Dong[1], Xiping Cheng[1], Eric Mills[1], Markus Delling[2], Fudi Wang[3], Tino Kurz[4], Haoxing Xu[1].
[1]The Department of Molecular, Cellular, and Developmental Biology, The University of Michigan, Ann Arbor, MI, USA, [2]The Department of Cardiology, Children's Hospital Boston, Boston, MA, USA, [3]Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Science, Shanghai, China, [4]The Department of Pharmacology, Faculty of Health Science, University of Linkoping, Linkoping, Sweden.

TRPML1 (mucolipin-1/MCOLN1) is predicted to be an intracellular late endosomal and lysosomal ion channel protein belonging to the mucolipin subfamily of Transient Receptor Potential (TRP) proteins. Mutations in the human *TRPML1* gene cause mucolipidosis type IV disease (ML4). ML4 patients exhibit motor impairment, mental retardation, retinal degeneration, and iron-deficiency anemia. Since aberrant iron metabolism may cause neural and retinal degeneration, it may be a primary cause of ML4 phenotypes. In most mammalian cells, release of iron from endosomes and lysosomes following iron uptake via endocytosis of Fe^{3+}-bound transferrin receptors, or following lysosomal degradation of ferritin-Fe complexes and autophagic ingestion of iron-containing macromolecules, is the major source of cellular iron. The Divalent Metal Transporter protein (DMT1) is the only endosomal Fe^{2+} transporter currently known and is highly expressed in erythroid precursors, but genetic studies suggest the existence of a DMT1-independent endosomal/lysosomal Fe^{2+} transport protein. Here, by measuring radiolabeled iron uptake, monitoring the levels of cytosolic and intra-lysosomal iron and directly patch-clamping the late endosomal/lysosomal membrane, we show that TRPML1 functions as a Fe^{2+} permeable channel in late endosomes and lysosomes. ML4 mutations are shown to impair TRPML1's ability to permeate Fe^{2+} at varying degrees, which correlate well with the disease severity. A comparison of TRPML1$^{-/-}$ ML4 and control skin fibroblasts showed a reduction of cytosolic Fe^{2+} levels, an increase of intra-lysosomal Fe^{2+} levels, and an accumulation of lipofuscin-like molecules in TRPML1$^{-/-}$ cells. We propose that TRPML1 mediates a mechanism by which Fe^{2+} is released from late endosomes/lysosomes. Our results suggest that impaired iron transport may contribute to both hematological and degenerative symptoms of ML4 patients.

1360-Pos Board B204

Activation Mutations of the TRPML1 Channel Revealed by Proline Scanning Mutagenesis

Xiang Wang[1], Xianping Dong[1], Su Chen[1], Dongbiao Shen[1], Meiling Liu[1], Eric Mills[1], Xiping Cheng[1], Markus Delling[2], Haoxing Xu[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Children's Hospital Boston, Boston, MA, USA.

The mucolipin TRP (TRPML) proteins are a family of intracellular channels primarily localized in the late endosome and lysosome. Mutations in the human *TRPML1* gene cause mucolipidosis type IV disease, a devastating pediatric neurodegenerative disease. In wild-type TRPML1-expressing HEK293 cells, no significant channel activity can be detected at the plasma membrane, but a proline substitution (TRPML1^{V432P}) results in a large whole-cell current that allows characterization of TRPML1 as a Ca^{2+} and Fe^{2+}/Mn^{2+} dually permeable channel. As TRPML1-mediated current can be recorded in late endolysosom using our recently developed lysosome patch-clamp technique, it remains unknown whether large TRPML1^{V432P} mediated current has resulted from increased surface expression (''trafficking'' effect), increased constitutive channel activity (''gating'' effect), or both. In the current study, we systematically but individually performed the proline substitutions on 20 amino acid residues around the *432* spot, a S4-S5 linker region. These proline-substitutions were studied by whole-cell and lysosome lumenal-side-out recordings in TRPML1-expressing HEK293 cells. Several proline substitutions were identified to display gain-of-function (GOF) constitutive activity at both the plasma membrane and endolysosomal membranes, and their localizations were not restricted to late endosomes and lysosomes, while wild-type TRPML1 and non-GOF substitutions were localized exclusively in these compartments. All of the proline-substituted GOF TRPML1 channels displayed inwardly rectifying currents that were carried by Ca^{2+} or Fe^{2+}/Mn^{2+}, but not protons. As lysosomal exocytosis is known to be Ca^{2+}-dependent, constitutive Ca^{2+} permeability of proline substitutions may have resulted in stimulus-independent intralysosomal Ca^{2+} release, hence the surface expression and whole-cell current of TRPML1. We conclude that the TRPML1 channel is an inwardly rectifying proton-impermeable cation-permeable channel, which may be gated through unknown cellular mechanisms through a conformational change in the cytoplasmic face of the TM5.

1361-Pos Board B205

Dynamic Properties of the TRPML3 Pore and their Modification by the Varitint-Waddler Phenotype

Hyun Jin Kim[1], Insuk So[2], Shmuel Muallem[1].
[1]University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Seoul National University College of Medicine, Seoul, Republic of Korea.

TRPML3 is a Ca^{2+} channel expressed in intracellular vesicular compartments and is regulated by H^+ that interact with the large intravesicular loop between transmembrane domains 1 and 2. The A419P mutation in TRPML3 causes the varitint-waddler phenotype as a result of gain-of-function (GOF). The mechanism by which the A419P mutation leads to GOF is unknown. Here, we show that the TRPML3 pore is dynamic and expands when conducting Ca^{2+} to change its permeability and selectivity from a strong to a weak field strength site. Pore expansion appears to be regulated by trapping Ca^{2+}, probably within the pore. Expansion of the pore can be reversed only by conducting Na^+ through the pore. The A419P mutation, which locks the channel in an open state results in permanently expanded pore. Notably, the TRPML3(H283A) mutation that eliminates regulation of TRPML3 by H^+ and locks the channel in an open state shows the same pore properties as wild-type TRPML3. On the other hand, the pore mutation E449A also locks the channel in an open state and permanently expanded pore. Interestingly, the TRPML3 large intravesicular loop interacts with the pore domain composed of transmembranes 5 and 6. Although this interaction is enhanced by the A419P and E449A mutations, it is not affected by the loop mutation H283A, suggesting that pore expansion together with enhanced loop-pore communication is responsible for the GOF. These findings provide a molecular mechanism for GOF by the TRPML3(A419P) mutation to account for the varitint-waddler disease phenotype.

1362-Pos Board B206

Regulation by Calcium of the TRP Channel Polycystin-2 (TRPP2)

María del Rocío Cantero[1], Horacio F. Cantiello[2,1].
[1]ININCA, UBA-CONICET, Buenos Aires, Argentina, [2]Massachusetts General Hospital, Charlestown, MA, USA.

• Polycystin-2 (PC2, TRPP2) is a member of the TRP (transient receptor potential) superfamily of cation channels. Like other members of this superfamily, PC2 permeates Ca^{2+}, which is involved in both signal transduction, and Ca^{2+} entry. Previously, we showed that PC2 is normally active at intracellularly high Ca^{2+} concentrations (10-15 μM). Little is known, however, about the role intracellular Ca^{2+} plays in PC2 channel function. Here, we explored the role of physiological concentrations of intracellular Ca^{2+} in PC2-mediated channel function in reconstituted apical membranes from term human syncytiotrophoblast (hST). Addition of either EGTA (1 mM) or BAPTA (2 mM) to reach low intracellular Ca^{2+} (<5 nM) at the cytoplasmic side, elicited a complete PC2 channel inhibition. A dose response elicited by addition of increasing cytoplasmic Ca^{2+} showed that Ca^{2+} activated PC2 with an apparent half activating concentration of 4.78 nM and a Hill coefficient of ~5. Conversely, extracellular Ca^{2+} concentrations, between 0.5 mM and 5 mM, had a stimulatory effect on PC2 channel activity while higher external concentrations