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

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''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).

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

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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.*

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

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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

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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

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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