with TRPC3 and TRPC6. We observed differential expression of these two channels during development of primary erythroid progenitors. We also showed with immunoprecipitation that endogenous TRPC3 and TRPC6 interact in the erythroid cell line TF-I. The increasing TRPC3/TRPC6 ratio during differentiation of erythroid cells correlated with increased Epo-stimulated calcium influx. We investigated the identity of the domains involved in Epo-stimulated TRPC3 activation and determined that the TRPC3 carboxyl terminus (C-domain) is required and sufficient for TRPC3 response to Epo. Furthermore, substitution of the TRPC3 TRP domain with that of TRPC6 eliminated the Epo-stimulated rise in $[Ca^{2+}]_I$, but substitution of TRPC6 TRP domain with that of TRPC3 did not reconstitute activity. In summary, our observations indicate that the TRPC3/TRPC6 ratio is physiologically relevant, suggesting that TRPC6 plays an important role in the proliferation and differentiation of erythroid cells through its role in modulating Epo-stimulated activation of TRPC3. In addition, the TRPC3 TRP-domain is critical in TRPC3 activation by Epo.

1348-Pos Board B192

Cellular Targeting And Function Of Trpc4 Channels In Human Vascular Endothelium

Annarita Graziani[1], Martin Krenn[1], Michael Poteser[1], Werner Baumgartner[2], Klaus Groschner[1].
[1]University of Graz, Graz, Austria, [2]RWTH Aachen University, Aachen, Germany.

TRPC4 has been suggested as a Ca^{2+} entry channel, which governs endothelial permeability. In an attempt to identify mechanisms that link TRPC4 function and cell adhesion, we tested the hypothesis that TRPC4 is part of the local signal transduction machinery within adherens junctions. In HEK293 cells transiently co-transfected with VE-Cadherin and TRPC4 constructs, we observed a co-localization of the two proteins within cell-cell contacts. In human microvascular endothelial cells (HMEC), endogenous TRPC4 was found to co-precipitate with two essential components of junctional complexes, VE-cadherin and β-Catenin. Membrane presentation of TRPC4 strongly promoted the formation of cell-cell contact and modified the response to pro-inflammatory stimuli. We observed that both basal- as well as agonist-stimulated Ca^{2+} influx were substantially augmented by the formation of cell-cell contact in HMEC. Furthermore, we found a significant increase in TRPC4-mediated Ca^{2+} signals and membrane currents in response to the formation of cell-cell contacts in TRPC4 and VE-cadherin-expressing HEK293 cells. We propose recruitment of TRPC4 proteins into cell-cell contacts as a key mechanism for control of endothelial Ca^{2+} signalling.

1349-Pos Board B193

Amino Acid Residues Within The Putative Pore Region Of TRPC3 As Determinants Of Channel Regulation

Michael Poteser[1], Hannes Schleifer[1], Cristoph Romanin[2], Klaus Groschner[1].
[1]University of Graz, Graz, Austria, [2]Johannes-Kepler-University Linz, Linz, Austria.

TRPC3 channels are typically activated in response to stimulation of PLC-coupled receptors and are considered to play a role in a variety of tissues. So far little information is available on structural determinants of channel function. In this study we set out to modify putative permeation-relevant residues in this ion channel by mutagenesis. The impact of the mutations on TRPC3 function was characterized in HEK293 cells by patch-clamp experiments as well as calcium imaging. A triple mutation (E630A, D639A, E644A) within the putative pore region resulted in enhanced basal activity and in a more linear IV-relation. Substitution of charge polarity at these positions (E→Q, D→N) failed to induce detectable changes in PLC-dependent activation, rectification or selectivity. Similarly exchange of a single negative residue in this region (D639A) failed to affect channel function as well. Surprisingly, double substitution of E to Q near the putative external vestibule (residues 615 & 616) generated a TRPC3 channel that no longer responds to PLC-mediated stimulation, while substitution of a single charged residue (E616) did not induce functional consequences. Furthermore, we tested for the localisation of particular regions of the protein in the outer vestibule and/or the permeation pathway by a cysteine scanning strategy.

In summary, we identified critical amino acid residues within the putative pore region which may be important determinants of channel regulation and/or gating.

Supported by the FWF, P18475, P19820.

1350-Pos Board B194

Trpc3 Encodes Native Constitutively-active Cation Channels Controlling The Resting Membrane Potential In Airway Smooth Muscle Cells

Junhua Xiao, Yunmin Zheng, Yong-Xiao Wang.
Albany Medical College, Albany, NY, USA.

Native constitutively-active cation channels have been proposed to play an important role in physiological and pathological cellular responses in a variety of cells. In the present study, we aimed at determining the molecular identity and functional role of native constitutively active cation channels remain in smooth muscle cells (SMCs). Using Western blot analysis, we have shown that TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6 proteins were expressed in airway SMCs. Single channel recordings indicate that anti-TRPC3 antibodies blocked the activity of constitutively-active cation channels, while anti-TRPC1, TRPC4, TRPC5 and TRPC6 antibodies had no effect. Anti-TRPC3 antibodies, but not anti-TRPC1, TRPC4, TRPC5 and TRPC6 antibodies, significantly hyperpolarized the resting membrane potential. Similarly, siRNA-mediated TRPC3 gene knockdown greatly diminished the constitutively-active cation channel activity and hyperpolarized the resting membrane potential, whereas TRPC1 and TRPC6 gene knockdown did not affect either the channel activity or the resting membrane potential. Intriguingly, we have also found that in asthmatic Airway SMCs, the activity of constitutively-active cation channels was significantly augmented, the resting membrane potential was depolarized, and TRPC3 protein expression was increased. Anti-TRPC3, but not ani-TRPC1 and TRPC6 antibodies prevented the constitutively-active cation channel activity and hyperpolarized the resting membrane potential in asthmatic airway SMCs. Taken together, these findings demonstrate that TRPC3 encodes the native constitutively-active cation channels, playing an important role in controlling the resting membrane potential in SMCs. Moreover, TRPC3-encoded channels may contribute to asthma and other smooth muscle diseases.

1351-Pos Board B195

Characterization Of A Novel TRPC6 Mutant Identified In FSGS Patients

Jianyang Du[1], Saskia F. Heeringa[2], Clemens C. Möller[3], Jochen Reiser[3], Friedhelm Hildebrandt[2], Lixia Yue[1].
[1]University of Connecticut Health Center, Farmington, CT, USA, [2]University of Michigan, Ann Arbor, MI, USA, [3]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

TRPC6 is a Ca^{2+}-permeable non-selective cation channel. Gain of function mutations of TRPC6 have been shown to cause focal segmental glomerulosclerosis (FSGS). Among six mutants of TRPC6 indentified in FSGS, three of them cause increase in Ca^{2+} influx. It appears that the enhanced Ca^{2+} influx underlies TRPC6 mutation associated FSGS. However, it is unclear how different mutations lead to gain of channel function and increase in Ca^{2+} influx. Here we report a novel TRPC6 mutant, M132T, which causes early-onset FSGS. Whole cell patch clamp experiments showed that current amplitude of M132T was 3- to 5-fold larger (476.9 ± 55.9 pA/pF) than that of wild-type (wt) TRPC6. Interestingly, while the wt TRPC6 exhibited apparent time-dependent inactivation, M132T did not show inactivation or only minor time-dependent decline of inward current. Inward Ca^{2+} current of M132T measured in 10 mM Ca^{2+} external solution was 10-fold larger than that of wt TRPC6. Moreover, Ca^{2+} influx of M132T was also significantly bigger than wt TRPC6. To understanding the mechanism of slow inactivation kinetics of M132T, we applied various intracellular Ca^{2+} concentrations and compared inactivation processes of M132T and wt TRPC6. We found that higher Ca^{2+} concentration was required to induce M132T inactivation in comparison with wt TRPC6, suggesting that M132T is less sensitive to intracellular Ca^{2+} induced inactivation. Taken together, our results indicate that the lack of inactivation may confer the enhanced Ca^{2+} influx in M132T. Further investigation is required to understand the mechanism of enhanced channel functions of TRPC6 mutants in FSGS.

1352-Pos Board B196

Pore Helix Mediates Proton Block of Vanilloid Receptors

Beiying Liu, Jing Yao, Feng Qin.
SUNY Buffalo, Buffalo, NY, USA.

Tissue acidosis occurs during inflammation and injury, and modulates many receptors and ion channels on the pain pathway including the capsaicin ion channel TRPV1. Extracellular low pH exerts several effects on the function of TRPV1. Extreme acidification leads to direct activation of the channel, while mild acidic pH potentiates its response to other stimuli. Paradoxically, protons also inhibit the unitary conductance of the channel. This inhibitory effect confers TRPV1 a similar maximum response at low pH in spite of increased agonist sensitivity, thereby limiting the ion flux into cells. Proton-mediated pore block has been studied extensively in other ion channels. Two representative mechanisms have been proposed, one involving competitive inhibition with permeating ions, and the other by reducing the surface potential of membranes. We have examined these mechanisms for proton block of TRPV1. Surprisingly, we found that neither mechanism could adequately account for the full blocking effect of protons. Mutagenesis experiments revealed that, in addition to a residue at the pore entrance, another residue

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