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.

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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.

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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.

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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.

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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.

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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