lation. We hypothesized that using adeno-associated viral (AAV) delivery to overexpress the pore-forming α subunit of the BK channel in VSMCs will provide a long-term antihypertensive effect. We constructed AAV plasmids using the VSMC-specific SM22α promoter to drive expression of the BKα gene. AAV/SM22α-BKα was administered by tail vein injection to mice with angiotensin II-induced hypertension. Blood pressure decreased to basal levels and this effect was observed for 6 weeks. Overexpressed BKα mRNA was detected only in arteries but not in heart or liver. We are currently assessing the properties of transduced BK channels in VSMCs, and will use isolated, perfused vessels to verify an enhanced dilator influence of BK channels in arteries of mice treated with AAV/SM22α-BKα as antihypertensive therapy.

3030-Pos The Inhibition Of K_V Channels Mediates The 5-HT-induced Mesenteric Artery Constriction In Rat: Role Of 5-HT_{2A} Receptor

Hyun Ju Noh, Dong-Jun Sung, Sang Woong Park, Jae-Gon Kim, Young Min Bae

Konkuk University, Department of physiolog, School of Medicine, Seoul, Republic of Korea.

Board B333

Voltage-gated K^+ (K_V) current is the major regulator of resting membrane potential (E_m) in rat mesenteric artery myocytes. Recently, we reported that serotonin (5-hydroxytryptamine, 5-HT) depolarizes the mesenteric artery myocytes by decreasing K_V currents. Although it has been known that the 5-HT-induced mesenteric vasoconstriction is mediated via 5-HT_{2A} receptor in rat, the precise mechanism of the 5-HT-induced mesenteric vasoconstriction is still to be elucidated. Here, using nystatin-perforated patch-clamp technique and isometric tension measurement, we examined the hypothesis that inhibition of K_V current and subsequent membrane depolarization via 5-HT_{2A} receptor plays a role in 5-HT-induced vasoconstriction in rat mesenteric artery.

Nifedipine (1 μM) largely suppressed the 5-HT-induced mesenteric vasoconstriction, indicating that the 5-HT-induced vasoconstriction is primarily contributed by E_m depolarization. 4-aminopyridine (10 mM), a relatively selective K_V channel blocker in artery myocytes, caused marked mesenteric artery constrictions, whereas tetraethylammonium (1 mM) had no effect. These results support the hypothesis that 5-HT-induced inhibition of K_V channels and subsequent E_m depolarization contribute to mesenteric artery constriction. Next, we examined whether 5-HT-induced inhibition of K_V channels is mediated via 5-HT_{2A} receptor. Under control condition, 5-HT decreased the K_V current by ~40%. A 5-HT_2 receptor agonist α-methyl 5-HT similarly inhibited the K_V currents. However, neither anpirtoline, a 5-HT_{1B} receptor subtype agonist, nor BW-723C86, a 5-HT_{2B} receptor agonist, inhibited the K_V current. In addition, pretreatment of ketanserin, a selective 5-HT_{2A} receptor antagonist completely prevented the 5-HT-induced K_V current inhibition. The contractile responses to the 5-HT receptor subtype agonists and antagonists were well corresponded to the K_V channel response. These results suggest that 5-HT-induced inhibition of K_V current is mediated via 5-HT_{2A} receptor and this K_V inhibition plays the major role in 5-HT-induced vasoconstriction in rat mesenteric artery.

TRP Channels

3031-Pos Cation Permeability, Conductance And Block Of TRPV1 Receptors

Damien Samways, Terrance M. Egan

Saint Louis University, St. Louis, MO, USA.

Board B334

TRPV1 receptors are polymodal cation permeable ion channels. They are widely expressed in sensory neurons where they stimulate membrane depolarization and act as a conduit for Ca^{2+} entry. These receptors discriminate poorly between monovalent cations and even appear to conduct the passage of relatively large cations, such as NMDG, across the plasma membrane. Further, they exhibit a high relative permeability to divalent cations. In this study, we aimed to further investigate the characteristics of the TRPV1 receptor channel pore. First, using standard reversal potential-based methods, we determined the permeability of the TRPV1 receptors to a spectrum of cations of different sizes. Beginning with the most permeable cation, the permeability sequence was as follows: Ca>Mg>Na~K~Cs>Li>Ethylammonium>Diethylammonium, Triethyl-ammonium>Tetraethylammonium~NMDG. Plotting relative permeability against the size of the permeating cation allowed us to estimate an approximate TRPV1 receptor pore diameter of ~8Å. Next, we recorded single channel conductance from outside-out patches in the presence of different extracellular cations, and determined the following conductance sequence for small monovalent cations: K~Cs~Na>Li>Ca~Mg. Finally, we observed that the chord conductance of single channel currents at −60mV was reduced from −4.1pA to −2.3pA upon addition of 2mM extracellular Ca^{2+}. Taken together, these data concur with previous studies indicating that the pore of TRPV1 receptors can accommodate large monovalent cations. However, the channel's high relative permeability and low relative conductance to divalent cations, together with the observation that Ca^{2+} blocks single channel cation currents, suggests the presence of a divalent cation-selective site of interaction somewhere in or near the channel pore.

3032-Pos The Fractional Calcium Current Of The TRPV1 Receptor Is Attenuated By Neutralization Of Acidic Side Chains Near The Channel Pore

Damien Samways, Terrance Egan

Saint Louis University, St. Louis, MO, USA.

Board B335

TRPV1 receptors select poorly amongst small monovalent cations, but exhibit a high relative Ca^{2+} permeability (P_{Ca}/P_{Na}) comparable

to that reported for other non-selective cation channels such as the glutamatergic NR1/NR2A, purinergic $P2X_1$, and nicotinic α 7 receptors. In the present study, we used patch clamp photometry to determine the fraction of the agonist-evoked inward cation current carried by Ca^{2+} (the "Pf%") in HEK293 cells expressing recombinant TRPV1 receptors. We observed that the Pf% of the proton-activated current evoked by reducing pH from 7.4 to 5 was significantly smaller (6.8 ± 0.5%) than that evoked by capsaicin (9.9 ± 0.4%) despite the fact that the P_{Ca}/P_{Na} determined from reversal potential measurements was the same for the two agonists. To test the hypothesis that the smaller Pf% resulted from the partial titration of acidic side chains contributing to Ca^{2+} influx, we investigated the effect on the Pf% of mutating two glutamate residues (Glu^{648} and Glu^{651}) located near the putative selectivity filter of the TRPV1 channel. Neutralizing individual fixed negative charge by mutation to glutamine produced two mutants, E648Q and E651Q, which displayed significantly reduced Pf% values of 4.2 ± 0.8% and 5.5 ± 0.5% respectively for the capsaicin-evoked current. In contrast, conserving charge by substituting aspartate for glutamate produced two mutants, E648D and E651D, whose values for the capsaicin-evoked current did not differ significantly from the wild type TRPV1 channel. However, consistent with previous studies, the P_{Ca}/P_{Na} was not significantly reduced by removal of charge from Glu^{648} or Glu^{651}. In conclusion, the Pf% technique revealed two acidic residues that enhance Ca^{2+} influx through the TRPV1 receptor via a mechanism not detectable using conventional reversal potential-based techniques for determining relative Ca^{2+} permeability.

3033-Pos The Molecular Basis For TRPV1 Activation By Pungent Compounds

Héctor Salazar[1], Itzel Llorente[1], Andrés Jara-Oseguera[1], León D. Islas[2], Refugio García-Villegas[3], Sharona E. Gordon[4], Tamara Rosenbaum[1]

[1] *Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Mexico, Mexico,*
[2] *Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico, Mexico,*
[3] *CINVESTAV, Mexico, Mexico,*
[4] *University of Washington, Seattle, WA, USA.*

Board B336

Some members of the TRP superfamily of ion channels are expressed in sensory neurons which are in charge of sensing noxious stimuli. TRPV1 channels mediate the response to a variety of stimuli such as high temperatures, tissue damage and exposure to pungent compounds such as capsaicin, all of which converge on these channels and underlie the common perceptual experience of pain. Recent studies have demonstrated that other agents such as the pungent compounds found in garlic extracts activate TRPV1. Interestingly, the same compounds have also been shown to activate the TRPA1 channel by a mechanism involving covalent interaction with cysteine residues. However, in the case of TRPV1channels, activation of the channel by these compounds remains controversial.

In this study we have explored the mechanism of activation of TRPV1 by the active compound found in garlic and onion extracts as well as other cysteine-modifying reagents on DRG neurons and HEK293 cells expressing TRPV1. Our data show that the TRPV1 channel is in fact a target of these compounds which are capable of activating the channel by means of covalent modification of cysteine residues. These findings point to a conserved mechanism of activation in TRPV1 channels which may provide new insights into the molecular basis of noxious stimuli detection.

3034-Pos TRPV2 Knockdown Supress The Stretch-induced Ca^{2+} Increase And Subsequent Cellular Responses In HUVEC

Yuki Katanosaka, Keiji Naruse

Okayama Univ, Okayama, Japan.

Board B337

Mechanical stimulation to vascular endothelial cells plays a physiologically important role in cardiovascular system. We previously reported that intracellular Ca^{2+} increased transiently in response to stretch through the activation of mechanosensitive (MS) cation channel in human umbilical vein endothelial cells (HUVEC). However, neither the identity nor the mechanism of activation of the plasma membrane influx pathway that mediates stretch-induced change in $[Ca^{2+}]_i$ is known. Since HUVEC expresses several types of mechanosensitive TRP channels including TRPV2, we examined the effects of the targeting suppression of TRPV2 protein expression in HUVEC using TRPV2-specific morphorino-oligo. Western blot analysis and immunocytochemical staining confirmed that TRPV2 was efficiently knocked down by transfection with the morphorino-oligo. In these cells, the stretch induced Ca^{2+} increase measured by fluorescence imaging using fura2 was completely abolished. After the cells were subjected to 20% uni-axial cyclic stretch at 1Hz for 1h, neither a stretch-enhanced stress fiber formation nor a shift in the cell orientation transverse to the strain direction could not be observed. Finally, TRPV2-knocked down HUVEC did not show stretch-induced NO production. These observations strongly suggest that TRPV2 is a responsible ion channel for stretch-induced Ca^{2+} increase, which leads the cytoskeletal reorganization and NO production in HUVEC. Thus, TRPV2 would be a key component of MS channel complex in HUVEC. Furthermore, the result that the production of vasorelaxing factor NO depends on TRPV2 suggests that TRPV2 is involved in hypertrophic heart disease caused by change in blood pressure.

3035-Pos PI(4,5)P2 is the Endogenous Phosphoinositide Regulating TRPV1 Channels

Carmen A. Ufret-Vincenty, Rebecca Klein, Sharona E. Gordon

University of Washington, Seattle, WA, USA.

Board B338

Phosphoinositides are known to be important in cell signaling, but identification of the endogenous phosphoinositides that modulate

ion channels and transporters has proven elusive. Here we show that although PI(4,5)P_2 can potently regulate activation of TRPV1 by capsaicin, PI(4)P and PI(3,4,5)P_3 can as well. To identify the endogenous phosphoinositide regulating TRPV1, we used recombinant pleckstrin homology (PH) domains in inside-out excised patch experiments. We found that the PH domain from PLCδ1 (PI(4,5)P_2 specific) inhibited TRPV1 and the PH domain from GRP1 (PI(3,4,5)P_3 specific) had no effect. Use of a rapamycin-inducible lipid phosphatase in simultaneous electrophysiology-imaging experiments further demonstrate that depletion of PI(4,5)P_2 prevented activation of TRPV1 by capsaicin. We conclude that PI(4,5)P_2 is the endogenous phosphoinositide regulating TRPV1 channels.

3036-Pos Proximity of the S1-S2 Linker to the Pore of Vanilloid Receptors TRPV1 ?

Beiying Liu, Jing Yao, Feng Qin

SUNY Buffalo, Buffalo, NY, USA.

Board B339

TRP ion channels are believed to have a membrane topology similar to voltage-gated channels, consisting of six transmembrane segments with a reentrant pore loop between S5 and S6. Our previous study suggests that the linker between S1 and S2 of the vanilloid receptor TRPV1 has a broad impact on the activation of the channel. Here we report that the seemingly distant linker is also capable of influencing the permeation properties of the channel. Charge reversal in the region reduced the unitary current and increased the binding affinity of ruthenium red, a slow cation pore blocker of the channel. The effects were most prominent on the inward current at low pH. They were relieved by increasing the ionic strength of the extracellular solution, and could be mimicked by introducing negatively charged cysteine-labeling compound (MTSES) into the region. Although quantitative modeling is still underway for a more precise understanding of the mechanism, our present data support an electrostatic interaction between the S1-S2 linker and the pore of the channel, where the basic residues in the S1-S2 linker counteract the acidic residues on the permeation pathway that give rise to proton block of the pore.

3037-Pos TRP Channels In Sensory Neurons As Novel Targets Of The Antimycotic Clotrimazole

Victor Meseguer[1,2], Yuji Karashima[1], Karel Talavera[1], Dieter D'Hoedt[1], Felix Viana[2], Bernd Nilius[1], Thomas Voets[1]

[1] *KU Leuven, Leuven, Belgium,*

[2] *Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, San Juan de Alicante, Spain.*

Board B340

Clotrimazole (CLT) is a widely used drug for the topical treatment of yeast infections of skin, vagina and mouth. Common side effects of topical CLT application include irritation and burning pain of the skin and mucous membranes. Here we provide evidence that TRP channels in primary sensory neurons underlie these unwanted effects of CLT. We found that clinically relevant CLT concentrations activate heterologously expressed TRPV1 and TRPA1, two TRP channels that act as receptors of irritant chemical and/or thermal stimuli in nociceptive neurons. In line herewith, CLT stimulated a subset of capsaicin-sensitive and mustard oil-sensitive trigeminal neurons, and evoked nocifensive behavior and thermal hypersensitivity upon plantar injection in mice. Notably, CLT-induced pain behavior was suppressed by the TRPV1-antagonist BCTC and absent in TRPV1-deficient mice. In addition, CLT inhibited the cold and menthol receptor TRPM8, and blocked menthol-induced responses in capsaicin- and mustard oil-insensitive trigeminal neurons. The concentration for 50% inhibition (IC_{50}) of inward TRPM8 current was ~200 nM, making CLT the most potent known TRPM8 antagonist and a useful tool to discriminate between TRPM8- and TRPA1-mediated responses. Taken together, our results identify TRP channels in sensory neurons as molecular targets of CLT, and offer means to develop novel CLT preparations with less unwanted sensory side effects.

3038-Pos Temperature Jump Studies Of Trpv1 Kinetics

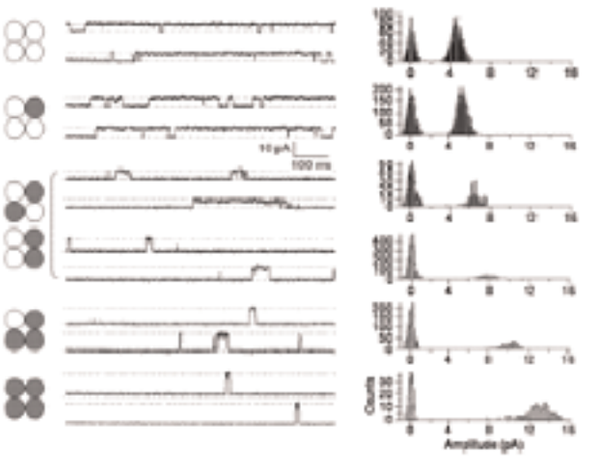
Frederick Sachs, Thomas Pennell, Thomas Suchyna, Susan Z. Hua

SUNY, Buffalo, NY, USA.

Board B341

We constructed a microfabricated heater with a channel depth of 25 μm in with ohmic heating via a Pt film zig-zag resistor ≈ 1500 μm long and 12 μm wide and we measured the outflow temperature with a separate platinum film thermometer on the chip. In response to a step (<100ms) increase in temperature, channels opened slowly reaching a kinetically busy steady state and when the temperature dropped to room temperature, the current rapidly decreased to baseline. At high temperatures the channels inactivated (Fig. 1).

Figure 1. A cell-attached patch recording in response to a temperature step from 295K to 328K. The lower traces show a scaled trace of T and 1/T use for the curve fit shown in red. Without inactivation, the kinetics fit a three state linear model: $C_1C_2O_3$ where k_{12} and k_{23} were explicitly temperature dependent. The fit provided the enthalpy of activation for k_{12} and k_{23} as ΔH~ 20kcal.M^{-1} and ΔS_{12} ~ −8cal.$M^{-}1K^{-1}$ and ΔS_{23} ~−16 cal.$M^{-1}K^{-}1$ assuming that the preexponential term was 6.10^{12}/s. A mole of what is not obvious. ΔH contained a voltage dependent energy term with an effective sensing charge of about 1e.



3039-Pos Remote Control of Ion-channels and Cells by AC Magnetic Fields

Heng Huang, Monica Zugravu, Savas Delikanli, Feng Qin, Hao Zeng, Arnd Pralle

SUNY Buffalo, Amherst, NY, USA.

Board B342

We are developing remote activation of temperature sensitive TRP channels using magnetic nanoparticles in alternating magnetic fields. Remote control of ion-channels provides a powerful tool to analyze neurological circuits and cellular communication. Recently, several groups have developed methods to activate ion-channels by light. However, many cells lie too deep inside the body to be accessed by the near UV light used. Magnetic fields however penetrate the body easily, making magnetic control of ion channels a very exciting possibility.

We are using manganese iron oxide nanoparticles to convert energy from an alternating magnetic field to local heat. The nanoparticles are coated to be water soluble and functionalized to target the membrane of cells expressing the temperature sensitive channel TRPV1.

In super-paramagnetic nanoparticles heat is supplied through Neel relaxation in an alternative magnetic field. Based on the measured hysteresis of the material we have computed the power dissipation of a handful of particles on the membrane to be sufficient to open the TRPV1 channels without causing damage to the cells.

Our experiments with TRPV1 expressed in HEK293 cells show that it is possible to activate VR1 completely within seconds by applying alternating magnetic field. The field strength is low enough to be compatible with application in whole body experiments.

Expressed in a neuron cell, the opening of the channel will depolarize the cell's membrane potential enough to trigger an action potential. TRPV1 may be expressed in a subgroup of cells and trigger that group of cells specifically. The size of the magnetic particles determines the optimal frequency of the alternating field for efficient energy coupling, allowing the possibility of multiple specific stimulations.

3040-Pos TRPC1 Modulates TRPV6 Channel Activity

Marc Fahrner[1], Rainer Schindl[1], Heike Kahr[1], Annarita Graziani[2], Ursula Utzmann[1], Alexander Wagner[1], Marlene Hack[1], Reinhard Fritsch[1], Klaus Groschner[2], Christoph Romanin[1]

[1] *Institute for Biophysics, Linz, Austria,*
[2] *Institute for Pharmacology, Graz, Austria.*

Board B343

The mammalian TRP proteins assemble to form mainly homomeric channels, but also heteromultimers are possible predominantly within the six subfamilies. We report here, a novel interaction between TRPC1 and TRPV6, members of the canonical respectively vanilloid TRP subfamily, as analysed by both co-immunoprecipitation as well as confocal Förster Resonance Energy Transfer (FRET) microscopy. The latter technique clearly revealed colocalisation of TRPC1 and TRPV6 as well as increased FRET , although interaction was not as robust as respective homomeric interactions. TRPC1-TRPV6 interaction led to significantly reduced membrane expression of TRPV6 as analysed by biotinylation assay and consequently to reduced currents or Ca2+ entry in comparison to TRPV6 overexpressing HEK cells. Remaining currents of TRPC1 and TRPV6 coexpression are reminiscent of TRPV6 in current-voltage relationship. FRET microscopy narrowed the interaction domains down to the N-terminal ankyrin-like repeats for both TRPC1 and TRPV6 proteins. The inhibitiory role of TRPC1 on TRPV6 was also maintained when only an N-terminal fragment of TRPC1 including the ankyrin-like repeats was coexpressed with TRPV6. This inhibitory role on TRPV6 was not observed for the ankyrin-like repeat domain of either TRPC3 or TRPC4. We suggest that TRPC1 is able to reduce TRPV6 channel activity due to a direct interaction via their N-terminal strands.

Supported by Austrian Science Foundation FWF-16537, FWF-18169, NB-10878.

3041-Pos Calcium Influx Induces Adaptation of Pain Receptor TRPV1

Jing Yao, Feng Qin

SUNY-Buffalo, Buffalo, NY, USA.

Board B344

Adaptation is a common feature of many sensory systems. But its occurrence to pain sensation has remained largely enigmatic. Here we address the problem at the receptor level, and show that the capsaicin ion channel TRPV1, which mediates nociception at the peripheral nerve terminals and responds to a variety of noxious stimuli including heat, capsaicin and protons, possesses properties characteristic to adaptation of sensory receptors. In particular, after full desensitization upon initial application of saturating capsaicin, the channel loses function only to the stimulus at the same concentration or below. It remains fully reactivated by subsequently elevated concentration. The desensitization primarily down-regulates the agonist sensitivity. We further show that this adaptation of

agonist sensitivity correlates with the depletion of PIP2 induced by calcium entry. With simultaneous recording of the current and the fluorescence change of PIP2 on the plasma membrane, we observed that the depletion of PIP2 proceeds in a time course similar to the desensitization of current. Furthermore, the extent of the depletion reaches a level adequate for the change of the agonist sensitivity. The depletion of PIP2 alone thus can account for the adaptation of agonist sensitivity occurring during desensitization. Our results suggest the adaptation as a physiological function for the atypical desensitization of the channel and underscore the capability of adaptation of nociception at the receptor level.

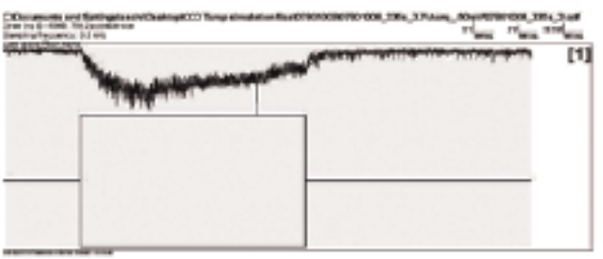
3042-Pos Assembly and Functions of Heteromeric Heat-Sensitive TRPV Channels

Wei Cheng[1], Shuang Liu[2], Ping Liang[2], Jie Zheng[1], KeWei Wang[2]

[1] *University of California, Davis, CA, USA,*
[2] *Peking University Department of Neurobiology, Beijing, China.*

Board B345

TRPV1–4 are the major cellular sensors for the increase in temperature. Co-expression of different TRPV subunits yields heteromeric channels with intermediate conductance and gating properties. Biochemistry experiments with isolated protein segments using in vitro pull-down assays suggested that the N-terminal ankyrin repeat domain (ARD) does not mediate subunit assembly. In addition, no interaction between ARD and the intracellular C-terminal region was observed. In contrast, robust interactions were observed between the C-terminal regions of like subunits as well as different TRPV subunits. Pharmacological tests further indicated that heteromeric assembly gave rise to channels with graded sensitivities to ligands as well as inhibitors, adding new flavors to the family of heat-sensitive channels.



3043-Pos Examination of Proposed C-terminal PI(4,5)P_2 Binding Sites in TRPV1

Rebecca M. Klein, Carmen Ufret-Vincenty, Sharona E. Gordon

University of Washington, Seattle, WA, USA.

Board B346

Many ion channels, including TRPV1, are modulated by phosphoinositides. We have recently shown that PI(4,5)P_2 is the endogenous phosphoinositide that regulates TRPV1. Although there is consensus that phosphoinositide regulation of TRPV1 is important, it is not clear whether PI(4,5)P_2 acts by binding to the channel directly. PI(4,5)P_2 binding sites in both the proximal and distal C-terminal domain of TRPV1 have been proposed. To examine whether either part of the C-terminal domain is required for PI(4,5)P_2 regulation we generated a number of deletion and truncation mutants. Using inside-out patches, we found that deletions in the distal C-terminal domain produced channels that were inhibited by poly-lysine, a PI(4,5)P_2 scavenger. Like we have previously reported for wild-type TRPV1, inhibition by poly-lysine was rescued by PI(4,5)P_2 applied to the bath. We expressed the soluble C-terminal domain fused to YFP and examined its cellular localization using confocal and TIRF imaging. This isolated C-terminal domain did not localize to the plasma membrane using confocal microscopy, raising the question of whether the C-terminal domain indeed constitutes the PI(4,5)P_2 binding site. We are currently examining whether other regions of the TRPV1 sequence may be involved in binding PI(4,5)P_2.

3044-Pos Hydrolysis Of Phosphatidylinositol 4,5-bisphosphate Mediates Calcium Induced Inactivation Of TRPV6

Baskaran Thyagarajan, Viktor Lukacs, Tibor Rohacs

UMDNJ - New Jersey Medical School, Newark, NJ, USA.

Board B347

TRPV6 is a member of the transient receptor potential superfamily of ion channels that facilitates Ca^{2+} absorption in the intestines. These channels display high selectivity for Ca^{2+}, but in the absence of divalent cations they also conduct monovalent ions. 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 TPV6 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]. The PLC inhibitors U73122 and edelfosine, and 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. Conversion of PI(4,5)P_2 to PI(4)P by a rapamycin-inducible PI(4,5)P_2 5-phosphatase inhibited TRPV6 currents in whole-cell experiments. Inhibiting phosphatidylinositol 4-kinases with wortmannin decreased TRPV6 currents and Ca^{2+} entry into TRPV6 expressing cells. Our data demonstrate that PI(4,5)P_2 activates TRPV6 channels and we propose Ca^{2+} induced PI(4,5)P_2 hydrolysis as a mechanism for the inactivation of TRPV6 by Ca^{2+}.

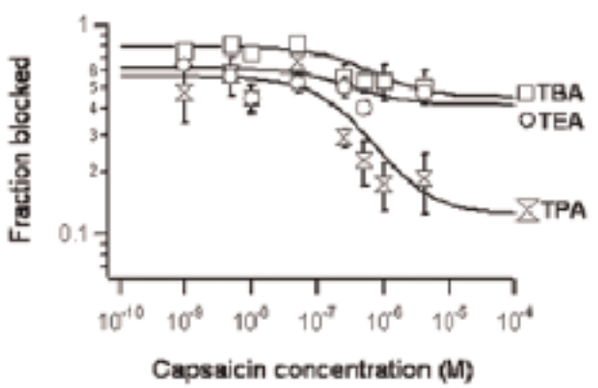
3045-Pos Open-state Blockers Of TRPV1 Reveal An Asymmetry Among Multiple Open States

Andres Jara-Oseguera[1], Tamara Rosenbaum[2], Leon D. Islas[1]

[1] *Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,*
[2] *Intituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.*

Board B348

We have tested the effects of quaternary ammonium ions on TRPV1 channels and characterized their blocking effects. These molecules act as open-state blockers, occupying all of the multiple open states of TRPV1 channels. Since their affinity for the different open states is not the same, the blocking mechanism is complex. As the molecule's size increases, the overall affinity also increases, reflecting a hydrophobic interaction with the channel pore. The larger molecules show a decreased affinity for the open states located further along the activation pathway and larger affinity for earlier open states, while the smaller blocker (TEA) have roughly the same affinity for all open states. This implies that the smaller size blockers discriminate poorly among open states, while bigger blockers prefer open states populated earlier in the activation pathway. These results point to an asymmetry in the structure of the different open states, perhaps reflecting a more hydrophobic and larger sized inner pore when the channel occupies earlier open states.



3046-Pos Voltage Sensor Movements During Activation of the TRPM8 Channel

Sebastian Brauchi, David Clapham

Children's Hospital Boston, Harvard Medical School, Boston, MA, USA.

Board B349

Temperature transduction in mammals is mediated in part by temperature-dependent transient receptor potential (TRP) ion channels in dorsal root ganglia neurons and skin cells. Several TRP ion channels have unusually high temperature sensitivity ($Q10 > 10$). The structural basis for this unusual temperature sensitivity is not understood. TRPM8 channel activity is increased by cooling below ~22°C. Application of menthol shifts the conductance vs voltage curve (G/V) to more negative voltages. In this study, we attempt to measure movement of TRPM8s' voltage sensor domain after changes in voltage. The hTRPM8 subunit has only 2 positively charged arginine residues in its S4 segment (842, 851; histidine residue at 845). Cysteine residues were introduced into the S4 segment (TRPM8-I831C) near the putative extracellular lipid-water interface. HEK-293T transfected with the modified TRPM8 constructs were labeled with a sulphorhodamine-maleimide conjugated dye. Fluorescence quenching was measured while voltage pulses were applied to the cell. The fluorescence vs voltage curve (F/V) was fitted to a Boltzmann relation. Mean V1/2 of the F/V curves were always negative to the G/V curves. These results imply that voltage changes alter fluorescence emission of the dye, which we interpret as voltage-dependent movement of the S4 segment.

3047-Pos Inhibition Of TRPM5 By Quinine Underlies Bitter-sweet Taste Interactions In The Gustatory Periphery

Karel Talavera[1], Keiko Yasumatsu[2], Ryusuke Yoshida[2], Robert F Margolskee[3], Voets Thomas[1], Yuzo Ninomiya[2], Bernd Nilius[1]

[1] *KU Leuven, Leuven, Belgium,*
[2] *Kyushu University, Fukuoka, Japan,*
[3] *Mount Sinai School of Medicine, New York, NY, USA.*

Board B350

The prototype bitter compound quinine is known to inhibit the perception of sweet taste. Here we investigated the effects of this compound on TRPM5, a Ca-activated non-selective cation channel expressed in taste receptor cells and known to have an important role in the transduction of sweet taste. Patch-clamp experiments in HEK-293 cells show that TRPM5 is inhibited by quinine and its stereoisomer quinidine with an IC_{50} ~40–50 μM at −50 mV. Quinine reduces the maximal whole-cell TRPM5 conductance and accelerates channel closure. Electrophysiological recordings in mouse chorda tympani nerves indicate that both quinine and quinidine inhibit the gustatory responses to sweeteners in wild type but not in *Trpm5* knockout mice. Quinine induces a dose- and time-dependent inhibition of TRPM5-dependent responses of single sweet-sensitive fibers to sucrose, according to the restricted diffusion of the drug to the site of TRPM5 expression in the basolateral membrane of taste receptor cells. The inhibitory effect of quinine on CT nerve responses to different taste stimuli correlates with the degree to which these responses depend on TRPM5. The inhibition of TRPM5 by quinine constitutes the molecular basis of a novel mechanism of peripheral taste processing, whereby the bitter tastant inhibits directly the sweet transduction pathway.

3048-Pos Role Of The Conserved Asp^{987} Residue In The Ca^{2+} Permeation Of hTRPM2 Channel

Rong Xia, Zhu-Zhong Mei, Hong-Ju Mao, Lin-Hua Jiang

Institute of Membrane and system biology, Leeds, United Kingdom.

Board B351

TRP cation channels show significant variation in Ca^{2+} permeability (Owsianik et al., 2006, Ann Rev Physiol 68: 685–717), but the principle governing this property is still poorly understood. The conserved aspartic acid (or glutamic acid) residue in the putative ion selectivity filter is known to be crucial in the Ca^{2+} permeation of several TRPV channels (Owsianik et al., 2006). However, a recent study has argued against the importance in the Ca^{2+} permeation of the conserved aspartic acid residue in the putative ion selectivity filter of the TRPM7 channel (Li et al., 2007, J Biol Chem *282*:25817–25830). In this study combining site-directed mutagenesis and subunit concatenation with patch clamp recording, we examined the role in the Ca^{2+} permeation of the corresponding Asp^{987} residue of the TRPM2 channel. Replacement with alanine and several other residues except glutamic acid abrogated the channel function. The D987E mutant channel was functional but the Na^{+} mediated channel currents were strongly inhibited by Ca^{2+}, which was not observed for the wild type channel. Furthermore, the D987E channel showed significantly increased Ca^{2+} permeability relative to the wild type channel. Expression of concatenated subunits (two WT subunits, or one WT and one D987E mutant subunits, or one WT and one D987N mutant subunits) resulted in functional channels. The channel composed of wild type and D987E mutant subunits, but not the channel of wild type subunits or wild type and D987N mutant subunits, showed significant sensitivity to Ca^{2+} inhibition and increase in the Ca^{2+} permeability. Our results together have provided consistent evidence supporting an important role of the Asp^{987} residue in the putative ion selectivity filter domain in the functional expression and in particular in determining the Ca^{2+} permeation of the TRPM2 channel.

3049-Pos Redox Regulation of TRPM8

Cristian A. Zaelzer[1,2], Ramon Latorre[1]

[1] *Centro de Estudios Científicos, Valdivia, Chile,*
[2] *Universidad Austral de Chile, Valdivia, Chile.*

Board B352

TRP channels mediate numerous sensory transduction processes. A number of TRP channels respond to combinations of stimuli and function as polymodal signal detectors that assess changes in the chemical and physical environment of the cell.

In TRPV1, three cysteine residues located in the putative pore region, are specifically linked with the extracellular modulation of TRPV1 by reducing agents (Susankova et al., Mol. Pharmacol., 2006). TRPM8, another thermo TRP, is activated by cold and the cooling compound menthol. The structure of TRPM8 contain 22 cysteine residues, but only two (C929 and C940) are present in the extracellular side of the channel, in the putative pore region. These two cysteine residues are linked to correct channel function. Mutation of either or both of these cysteines creates non-functional channels (Dragoni et al., J. Biol. Chem., 2006). Here, we show that exposure to the oxidizing agent H2O2 (58 mM) allows a remarkable reduction in currents (25 to 50%) in TRPM8 that were not completely reversed by washout, suggesting modification of covalent sulfhydryl residues. This effect was only reversed by exposure to the reducing agent DTT (10 mM). DTT exposure alone causes minimal enhancement in the response of the channel to decrease in temperature. On the contrary, exposure to external 10 mM GSH (IC50 2.5 mM) causes a dramatic decrease of current (70 to 80%) with decay time constant of 13.14 +/− 1.9 s. Washout and treatment with DTT (10 mM) only allow partial recovery of currents. The activation and deactivation time constant analysis shows notable differences in the fast component of activation. The internal exposure to GSH is ineffective. These results suggest that the active site for GSH is in the extracellular side of channels and indicate that an optimal redox state is crucial for proper function of TRPM8 channels.

3050-Pos Transient Receptor Potential channel TRPM8 is associated with Polyphosphate and Polyhydroxybutyrate

Eleonora Zakharian[1], Baskaran Thyagarajan[1], Evgeny Pavlov[2], Robert J. French[2], Tibor Rohacs[1]

[1] *University of Medicine & Dentistry of NJ, Newark, NJ, USA,*
[2] *University of Calgary, Calgary, AB, Canada.*

Board B353

The transient receptor potential channel, TRPM8, of the melastatin subfamily, is the cold and menthol receptor. Regulation of the activity of TRPM8 is a complicated process which can be driven by several factors, including temperature, chemical reagents, and phosphoinositides. Here, we show that TRPM8 expressed in human embryonic kidney cells (HEK-293) is associated with two widely distributed homo-polymers - inorganic polyphosphate (polyP), and the amphiphilic, solvating polyester, poly-(R)-3-hydroxybutyrate (PHB), which has frequently been associated with polyP. The presence of these polymers was revealed, in a purified preparation of TRPM8, by using chemical and immunological analyses. PolyP was resolved by SDS-PAGE with subsequent staining using Toluidine blue. In addition, PHB was detected by Western blot analysis of SDS-PAGE gels with anti-PHB IgG raised in rabbits to a synthetic 8-mer of R-3-hydroxybutyrate. Both polymers have been found in association with a variety of membrane proteins, including some ion channels. Furthermore, it has been proposed recently that both of these polymers are required for normal functioning of certain ion channels (Negoda et al., 2007 PNAS 104:4342 and Zakharian and Reusch, 2007 Biophys. J. 92:588). In the present study, we demonstrate a critical role of polyP in supporting the activity of TRPM8 channels. The channels were inhibited upon the hydrolysis of polyP with the exopolyphosphatase, scPPX1, in both whole-cell patch clamp studies on HEK-293 cells, and in planar lipid bilayer experiments on reconstituted channels. This suggests that polyphosphate association with TRPM8 protein is essential for channel function.

3051-Pos TRPM7-like, Non-selective Cation Currents In Hepatocytes

Ceredwyn E. Hill, Ravi Mishra, Navid Shobeiri

Queen's University, Kingston, ON, Canada.

Board B354

Non-selective cation currents play integral roles in countering cell swelling, inducing apoptotic cell shrinkage, mediating Ca^{2+} influx and controlling Mg^{2+} homeostasis. A number of 'TRP' (Transient Receptor Potential) channels are candidates for these currents in different tissues and organs. Here we identified an outwardly rectifying cation current in both rat hepatocytes and the polarized rat hepatoma, human skin fibroblast cross, WIF-B. Under standard whole-cell recording conditions in which cells were bathed and dialyzed with Na-gluconate-rich solutions, the latter Mg^{2+}-free and Ca^{2+}-EGTA buffered, currents reversed at 0 mV, showed no time dependence, and were ten-fold higher at +120 mV as compared with −120 mV. Current at +120 mV developed slowly over dialysis, from 7.8±3.1 pA/pF at patch rupture to 35±6.5 pA/pF at 12 min. Inward current at −120 mV (−5.2±2.2 pA/pF) did not change significantly over the time course of the dialysis. The inclusion of 1mM $MgCl_2$ in the pipette solution caused the outward current to decrease to a stable value of 4.0±2.5 pA/pF within 2 min. 1mM EDTA addition to the same solution (0.1mM Mg^{2+}) completely blocked the decay, allowing the current to develop as under standard conditions. Similarly, ATP addition to chelate all but 0.1mM Mg^{2+} also permitted outward current to increase. Additional $MgCl_2$ to the ATP-Mg^{2+} containing solution (1mM Mg^{2+}) blocked current development. These results suggest that ATP allows current development by chelating Mg^{2+} rather than via a kinase-mediated phosphorylation event. RT-PCR of WIF-B and rat hepatocyte RNA demonstrated expression of both TRPM6 and TRPM7 channels. However, 2-aminophenylborate blocked current development (IC_{50} = 110±20μM, range tested 10μM to 2mM). The functional and expression data suggest that homologous TRPM7 channels, rather than heterologous TRPM6/TRPM7 channels, are responsible for the whole-cell currents in rat hepatocytes.

Supported by NSERC & Hotel Dieu Hospital, Kingston.

3052-Pos Pungent Chemicals, Calcium And Tetrahydrocannabinol Activate Different Conformations Of TRPA1

Donghee Kim, Eric J. Cavanaugh, Dina Simkin

Rosalind Franklin University, North Chicago, IL, USA.

Board B355

Thiol-reactive pungent chemicals from mustard oil, garlic and cinnamon activate TRPA1 by a covalent modification at cysteine residues located at the N-terminus. Our recent study shows that pungent chemicals can activate TRPA1 in cell-attached, but not in inside-out patches, suggesting that either a cytosolic factor is required for activation or that TRPA1 has shifted to a conformation whose cysteine residues are no longer accessible. In inside-out patches, activation by TRPA1 by pungent chemicals can be restored by polyphosphates, suggesting that polyphosphate-like molecules in the cell keep TRPA1 in the sensitive conformation by making cysteine residues accessible. The presence of different conformations was further tested using THC and Ca2+, two non-electrophilic molecules that are structurally different from those of pungent chemicals, and therefore should not act on cysteine residues. TRPA1 was transiently expressed in HeLa cells. Activation of TRPA1 by THC was very weak from the extracellular side (cell-attached; >20 microM), but very potent from the intracellular side (inside-out; K1/2, ~2 microM), even in the absence of polyphosphates. Increasing the extracellular [Ca] from 0 to 1 mM activated TRPA1 in cell-attached patches. Elevation of cytosolic [Ca] using thapsigargin and histamine (that release Ca from SR) also activated TRPA1, showing that Ca acts on the cytosolic region of TRPA1, presumably at the Ca-binding EF-hand domain. Similar to pungent chemicals, Ca (1–5 microM) failed to activate TRPA1 in inside-out patches, unless polyphosphates were present. These findings show that TRPA1 can exist in two functional conformations, and that structurally different molecules bind to different conformations to activate the channel.

3053-Pos Molecular Determinants of Species-specific Activation or Block of TRPA1 Channels

Jun Chen[1,2], Xu-Feng Zhang[1], Michael E. Kort[1], Chaohong Sun[1], Laura J. Miesbauer[1], Steven C. Cassar[1], Torben Neelands[1], Victoria E. Scott[1], Regina M. Reilly[1], Philip J. Hajduk[1], Philip R. Kym[1], Charles W. Hutchins[1], Connie R. Faltynek[1]

[1] *Abbott Labs, Abbott Park, IL, USA,*
[2] *Abbott Laboratories, Abbott Park, IL, USA.*

Board B356

As cellular sensors, the transient receptor potential (TRP) ion channels open and close ion conduction pathways in response to various stimuli such as temperature, osmolarity and chemical ligands. However, the molecular mechanisms underlying these processes are largely unknown. Here we utilize species-specific pharmacological properties to explore the gating mechanism of TRPA1, a channel implicated in sensory functions and pain states. We report thioaminal compounds (e.g., CMP1) that covalently modify cysteine residues within the channels and produce pronounced species-specific effects, i.e., activation of rat TRPA1 (rTRPA1) but block of human TRPA1 (hTRPA1). The opposite gating effects can be attributed to residue differences between rTRPA1 and hTRPA1 in the upper portion of the S6 domains: Ala-946 and Met-949 of rTRPA1 determine channel activation, while residues located in equivalent positions of hTRPA1 (Ser-943 and Ile-946) determine channel block. Furthermore, a small side-chain at position 946 of rTRPA1 is essential for channel activation. These data, corroborated with homology modeling, indicate that these S6 residues dictate channel opening or closing by affecting a gate localized in the selectivity filter. Thus, our findings reveal a molecular basis for species-specific TRPA1 channel functions and provide novel insights into how TRPA1 channels respond to stimuli. Given its emerging role as a major target for the relief of pain,

understanding the structure-function relationship of TRPA1 will facilitate drug discovery efforts.

3054-Pos Analysis Of Expression And Function Of TRP Channels In Atrial Myocardium

Michael Poteser[1], Petra Eder[1], Zora Saad[1], Johanna Astner[1], Christoph Romanin[2], Klaus Groschner[1]

[1] *Karl Franzens University Graz, Graz, Austria,*
[2] *Kepler University Linz, Linz, Austria.*

Board B357

Recent evidence suggests a pivotal role of TRPC-related cation channels in cardiac physiology and pathophysiology, with TRPC3 and TRPC6 as potential key players in cardiac remodelling and pathogenesis of hypertrophy. In an attempt to further delineate the role of TRPC3/6 channels in atrial myocytes, we adopted the murine HL-1 cell model. In standard culture conditions HL-1 cells were found to express both TRPC3 and TRPC6 at low levels along with TRPC1 as well as typical signalling partners such as NCX1. Stimulation of angiotensin-2 (Ang II, 1 μM) or endothelin-1 (ET-1, 100 nM) receptors resulted in significant promotion of TRPC3 expression within 6–12h. Electrophysiological consequences of TRPC3 overexpression were characterized in both Ang II and ET-1 stimulated cells as well as in HL-1 cells transiently and stably expressing a YFP-TRPC3 fusion protein. Our results demonstrate that TRPC3 is overexpressed in response to excessive stimulation of receptor-PLC pathways and results in substantial distortion of basic electrophysiological properties, indicating a role of TRPC channels in remodelling-associated atrial dysfunction.

3055-Pos TRPC6 Channel Activation by PKC Induced Phosphorylation

Christina V. Floreani, Takeharu Kawano, Yasuko Nakajima, Shigehiro Nakajima

University of Illinois at Chicago, Chicago, IL, USA.

Board B358

TRPC6 channels, of the transient receptor potential channel superfamily, have emerged as significant players in diverse physiological and pathophysiological processes. Despite their importance, the details of the signal transduction pathway activating TRPC6 conductance have yet to be elucidated. In the present study, we investigate the role of protein kinase C (PKC) induced phosphorylation of TRPC6 in the signal transduction activation of TRPC6 conductance. The whole-cell patch-clamp method of electrophysiology was applied to primary neuron cultures of rat VTA as well as to CHO-K1 cells, transfected with TRPC6, neurotensin receptor (NTR), and GFP cDNAs. NTR is coupled to the Gq signal transduction pathway, known to activate TRPC6 conductance. NT application, within a few seconds, activated TRPC6 conductance in CHO-K1 cells and rat VTA DA neurons. The reversal potential of TRPC6 current averaged −15 mV at 0.5 mM external Ca2+. TRPC6 current amplitude in CHO-K1 cells, as in rat VTA DA neurons (see Farkas et al, 1996; J Neurophysiol. *76*(3):1968–81), decreased with increasing extracellular Ca2+ concentration. PKC inhibition by bisindolylmaleimide I, 1uM for 10 minutes in CHO-K1 cells and 3uM for 20 minutes in rat VTA DA neurons, completely abolished the TRPC6 conductance activation by NT. Incubation with the PKC peptide inhibitor, PKC(19–36), 40 uM for 10 minutes, inhibited NT-induced TRPC6 conductance. A serine to alanine mutation of TRPC6 residue 768, a putative PKC phosphorylation site, rendered the channel unresponsive to NT. Mutant TRPC6 was expressed at normal levels, observed by Western blot analysis, and it localized to the plasma membrane, observed by confocal microscopy. These results suggest an essential role for PKC phosphorylation of TRPC6 in activation of the channel.

This investigation was supported by a Whitehall Foundation Grant and NIH grants NS43239 and T32-HL07692.

3056-Pos Junctophilin 2 as a TRPC3-interacting protein in skeletal muscle

Jin Seok Woo[1], Jianjie Ma[2], Eun Hui Lee[1]

[1] *Dept of Physiology, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea,*
[2] *UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.*

Board B359

Canonical type transient receptor potential cation channel type 3 (TRPC3) allows the entry of Ca^{2+} and Na^{+} into various cells. Ryanodine receptors (RyRs) functions as Ca^{2+} release channels in the sarcoplasmic reticulum (SR) and are an essential protein for excitation-contraction (EC) coupling in striated muscles. Recently, by knock-down study using retroviral delivered small interference RNAs and single cell cloning, we found that expression of TRPC3 was tightly regulated during skeletal muscle cell differentiation and functional interaction between TRPC3 and RyR1 could regulate the gain of SR Ca^{2+} release during EC coupling (*J. Biol. Chem. 281*: 10042–10048, 2006). In this study, by co-immunoprecipitation assay with whole cell lysate of mouse skeletal primary myotubes using anti-TRPC3 antibody and by MALDI-TOF mass spectrometric analysis of a cross-linked triadic protein complex from rabbit skeletal triad vesicles, junctophilin 2 (JP2) was identified as a TRPC3-interacting protein and a possible linker protein between TRPC3 and SR Ca^{2+} store. To identify critical region(s) of JP2 participating in interacting to TRPC3, GST-fused various fragments of JP2 were expressed in E. coil and were subjected to co-immunoprecipitation assay with TRPC3. A fragment of JP2 from 143 to 234 amino acids was the most efficient region for binding to TRPC3.

3057-Pos Costameric Localization and Trafficking of TRPC3 Cation Channels in Normal and mdx Mouse Muscle Fibers

Christiane Kunert-Keil[1], Silke Lucke[1], Markus Beckmann[1], Karlhanns Endlich[2], Heinrich Brinkmeier[1]

[1] *Institute of Pathophysiology, University of Greifswald, Germany,*
[2] *Institute of Anatomy and Cell Biology, University of Greifswald, Germany.*

Board B360

The transient receptor potential (TRP) channel TRPC3 is a non-voltage gated Ca^{2+}-permeable cation channel that is expressed in skeletal muscle. It is assumed that TRPC3 is important for the cellular Ca^{2+} homeostasis and that the channel is involved in Ca^{2+} dependent signal transduction. To study the role of TRPC3 in skeletal muscle we investigated gene expression and cellular localization of the TRPC3 protein. We further tested whether expression and localization of TRPC3 are altered in murine muscular dystrophy (mdx), a muscle disease characterized by abnormal cellular Ca^{2+} regulation. Using RT-PCR and Western blot techniques, we did not find differences in TRPC3 gene expression in limb muscles and diaphragm between mdx and control mice. Immunofluorescent staining of isolated interosseus fibers with an anti-TRPC3 antibody revealed a cross striation pattern near the sarcolemma and a faint cytoplasmic fluorescence. Double labelling experiments showed co-localization of TRPC3 with vinculin and dystrophin, but not with the ryanodine receptor or the dihydropyridine receptor. The latter results were confirmed for both genotypes, however, mdx fibers showed a more prominent cytoplasmic TRPC3 staining. The strong cytoplasmic TRPC3 signal diminished, while the sarcolemmal staining increased, after incubation of mdx fibers with Gd^{3+} (50 μM), nifedipine (50 μM), epidermal growth factor, 2-aminoethoxydiphenly borate (2-APB) or a Ca^{2+}-free solution. In control fibers, the effects of the ion channel blockers were lesser. Only Gd^{3+} and nifedipine stimulated the translocation of TRPC3 to the sarcolemma. Our data suggest a costameric localization of TRPC3 in skeletal muscle and a Ca^{2+}-dependent trafficking of the channel from cytoplasmic pools to the sarcolemma. In dystrophin-deficient mdx fibers TRPC3 seems to be displaced to the cytoplasm, an effect that can be reverted by inhibition of Ca^{2+} influx.

Supported by BMBF (MD-NET, project R14).

Synaptic Transmission

3058-Pos Monomeric Helical α-Synuclein Forms Highly Conductive Ion Channels

S. D. Zakharov[1], Y. N. Antonenko[2], A. A. Sobko[2], E. A. Dutseva[2], J. D. Hulleman[1], J. C. Rochet[1], W. A. Cramer[1]

[1] *Purdue Univ., West Lafayette, IN, USA,*
[2] *Moscow St. Univ., Moscow, Russian Federation.*

Board B361

Monomeric wild type α-synuclein (αS) and two mutants associated with familial Parkinson's disease, E46K and A53T, form ion channels with well defined conductance states in planar bilayer membranes containing 25–50% anionic lipid (PG) and 50% phosphatidyl-ethanolamine (PE) in the presence of a *trans*-negative potential. In contrast, another familial mutant, A30P, known to have a lower membrane affinity, did not form ion channels. Membrane permeabilization by oligomeric αS, compared to that by the monomer, differed by a low probability of discrete channel formation, no requirement for curvature-inducing lipid, and a pronounced decrease in the dependence of channel activity on anionic lipid and trans-membrane potential.

Circular dichroism (CD) analysis revealed a significant increase in helical content of the oligomeric form of αS upon binding to liposomes with optimum for channel formation lipid composition. This implies partial conversion of oligomer from β-strand to α-helix conformation. Fluorescence correlation spectroscopy was employed to measure the lateral mobility of αS after binding of monomeric aS to planar bilayer membranes under conditions favorable for channel formation. It was found that the lateral mobility of αS is similar to that of 19 kDa channel-forming domain of colicin E1, which is known to function as a monomer in the membrane-bound state, and is slightly larger then that of lipids. Together with the CD data, this implied the absence of oligomerization of αS upon membrane-binding.

It was inferred that discrete ion channels with well defined conductance states were formed by one or several molecules of monomeric αS in an α-helical conformation and that such channels have a role in the normal function and pathophysiology of the protein

[Supported by NIH grants: GM-38323, NS-049221 and Fogarty TW-01235]

3059-Pos Kv Channels Ion Independent Effect on Frequency and Characteristics of Fusion Events

Lori Feinshreiber[1], Reut Friedrich[1], Ulf Matti[2], Jens Rettig[2], Uri Ashery[1], Ilana Lotan[1]

[1] *Tel Aviv University, Ramat Aviv, Tel Aviv, Israel,*
[2] *Universität des Saarlandes, Homburg/Saar, Germany*

Board B362

Kv2.1 channel is commonly expresses in the soma and dendrites of neurons, where it could influence the release of neuropeptides and neurotrophins and in neuroendocrine cells, where it could influence hormone release. The traditional role of this channel is to inhibit neurotransmitter release by influencing the membrane potential and thereby inhibiting voltage-gated calcium channels activation. Recently (Singer-Lahat *et al.*, 2007), we suggested an unexpected role for Kv2.1 of facilitating neuropeptide release from rat PC12 cells expressing recombinant fluorescent atrial natriuretic factor polypeptide. The enhancement was independent of the channel ion conducting function and occurred through a direct interaction with syntaxin 1A, a member of the SNARE proteins which are component of the vesicle release machinery. These data implied a complex role for this channel in the regulation of exocytosis, and raised the need for understanding the mechanism underlying this enhancement effect.

Here, we further investigated the enhancement effect on release from bovine chromaffin cells using amperometry, which allows direct measurements of single secretion events. We show that over expression of Kv2.1 wild-type or a non-conducting mutant channel enhances release (~ two fold) of catecholamines triggered by external application of high potassium solution, compared with control cells. Detailed single spike analysis reveals that the enhancement can be attributed mostly to a higher fusion events