Wogonin has shown antioxidant and anti-inflammatory properties in various cell types. The aim of this study is to examine whether and how wogonin activates TREK-2, a member of the two-pore domain K^+ (K_{2P}) channel family, highly expressed in the pathological condition, such as ischemia and inflammation. Wogonin activated TREK-2 current by increasing the opening frequency. However, the wogonin-induced TREK-2 activity was decreased in a time-dependent manner, suggesting that complex signal pathway, at least two mechanisms, might be present. We first tested whether the phospholipase A2 (PLA2)- arachidonic acid (AA)-protein kinase C (PKC) signal pathways are involved in wogonin-induced TREK-2 activation. AA strongly activates TREK-2 but not TREK-2 chimera (TREK-2/TASK-3C). TREK-2/TASK-3C did not respond to application of wogonin. Wogonin failed to activate TREK-2 in the presence of PLA2 inhibitors. In the presence of PKC inhibitors, the reduction of TREK-2 activity shown after application of wogonin disappeared regardless of lapse of time. Furthermore, wogonin increased TREK-2 expression under hypoxic condition and spinal cord injury, whereas decreased the expression under nomoxic condition. These results show that wogonin has dual effect on TREK-2 channel activity and expression. Supported by R13-2005-012-01002-0 and R01-2007-000-20746-0

3452-Pos Board B499

Regulation Of The Single Channel Conductance Of K_{2p}10.1 (Trek2) By The Amino-terminus

Eric J. Cavanaugh, Dina Simkin, **Donghee Kim**.
Rosalind Franklin University, North Chicago, IL, USA.

K_{2P}2.1 (TREK-1) and K_{2P}10.1 (TREK-2), when expressed in mammalian cell lines, show several single channel conductance levels. Recent studies show that the N-terminus of TREKs controls the unitary conductance levels via alternative translation initiation mechanism that produces isoforms with long and short N-termini. For TREK-2, the isoform with the full length N-terminus (residues 1-69) has a low conductance level, whereas that with the short one (residues 55-69 or 67-69) has a large conductance level. The role of the N-terminus and the putative slide helix region in the control of the unitary conductance was studied further using deletion and substitution TREK-2 mutants. Deletion of the N-terminus up to residue 36 (out of 69 residues) had no effect on conductance levels. Further deletions up to residues 40 (~150-pS at −40 mV), 44 (~90-pS), and 49 (>30-pS) produced channels with levels that were different from the levels observed in the wild type TREK-2. A mutation within the putative slide helix region (residues 47-55) to render it non-helical resulted in formation of mainly the low conductance channel, and greatly reduced the open probability. These TREK-2 mutants also inhibited the alternative translation initiation. These results suggest that the distal region of the N-terminus (residues 37-55) including the putative helical region (residues 37-54) controls the unitary conductance of TREK-2 in an unpredictable way. The data also indicate that the putative helical region is important for the control of channel activity.

3453-Pos Board B500

A novel mechanism for inhibition of Hyperpolarization-activated Pacemaker Channels by Receptor-like Tyrosine Phosphatase alpha

Jianying Huang, Aijie Huang, Qi Zhang, Yen-Chang Lin, **Han-Gang Yu**.
West Virginia University, Morgantown, WV, USA.

We have previously reported an important role of increased tyrosine phosphorylation activity by Src in the modulation of Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels. Using a combination of whole-cell patch clamp technique, Western blot, and confocal fluorescence imaging, we assessed the hypothesis that decreased tyrosine dephosphorylation may enhance HCN channel activity as well. We discovered that the receptor-like protein tyrosine phosphatase alpha (RPTPα) significantly inhibited or even eliminated HCN2 currents expressed in HEK293 cells. Biochemical evidence showed that the surface expression of HCN is reduced by RPTPα, which was in parallel to the decreased tyrosine phosphorylation of the channel protein. Confocal imaging confirmed that the surface expression of HCN2 channel is inhibited by RPTPα. Moreover, we detected the presence of RPTPα proteins in rat cardiac ventricles and the levels of RPTPα expression changed during development. Inhibition of tyrosine phosphatase activity by phenylarsine oxide (a non-selective inhibitor for tyrosine phosphatases) shifted ventricular I(f) (generated by HCN channels) activation from non-physiological voltages to the physiological voltages associated with accelerated activation kinetics. In conclusion, we demonstrated a critical role RPTPα plays in gating of HCN channels via tyrosine dephosphorylation mediated by RPTPα. These findings are also important to neurons where HCN and RPTPα are richly expressed.

3454-Pos Board B501

Probing The Effects Of Engineered Disulfide Bonds In The Extracellular Loops Of The Porin OmpF

Beau R. Wager, Arnaud Baslé, Anne H. Delcour.
University of Houston, Houston, TX, USA.

OmpF is a 16-stranded β-barrel porin functionally found in trimers in the outer membrane of *Escherichia coli* and involved in the entry of ions, nutrients and antibiotics. OmpF contains eight extracellular loops, six of which have no known function in channel activity. Previous studies have shown that OmpF channels close more readily in acidic pH conditions, and their open probability drops from ~ 1.0 to ~ 0.4 when the pH is switched from 7.0 to 4.0. However, this effect is abrogated when Loop 1, 7, or 8 is deleted, suggesting that these loops are involved in the pH sensitivity of the channel. To further test whether the movements of these loops participate in the pH response, double cysteine mutants have been engineered to form disulfide bonds and to tether these loops to themselves or to the barrel wall, based on predictions from the SSBOND program. A thiol quantification assay utilizing papain and L-BAPNA was used to confirm the presence of the disulfide bond in each mutant. Wild-type and mutant proteins were purified and inserted into planar lipid bilayers for electrophysiological measurements. Recordings were made in 1 M KCl buffer at pH 7 and pH 4 and +/− 90mV. The open probability was calculated to determine the effect of extracellular loops tethering on OmpF closure. Alterations to the disulfide bonds were performed with the addition of reducer or cross-linker either to the protein samples or directly to the bilayer chamber. Preliminary analysis of the some of the mutants indicates that disulfide bond tethering between Loop 7 and Loop 8 has affected the rate of OmpF closure in response to acidic conditions. Supported by grant # E-1597 from the Welch Foundation.

3455-Pos Board B502

Does The Pore Diameter Of The OmpU Porin Change With pH?

Guillaume Duret, Anne H. Delcour.
University of Houston, Houston, TX, USA.

Electrophysiological recordings show that trimeric porins, such as the OmpU porin of *Vibrio cholerae*, display spontaneous closures of one third of the total conductance, corresponding to the closure of one monomer. But in acidic conditions, in addition to being more frequent and longer, the closing events of OmpU become larger while the total conductance of the trimer is unchanged. At pH 4.2, their conductance is more than half of the total conductance. In addition, only a single closing step is observed, instead of three representing each monomer closure. We have proposed three different hypotheses on the mechanism underlying these closures at acidic pH. 1) If we consider that OmpU is indeed constructed as a typical triple barrel channel, the single closure could represent the cooperative partial closures of the three monomers, and the conductance of these partial closures would increase at acidic pH. 2) The closure could involve only one monomer with a concomitant adjustment of the conductance of the other two monomers, such that the amount of current remaining through these two open monomers decreases progressively as the pH is lowered. 3) The trimer could form a single pore, and the closing events would be a partial closure with a conductance getting larger with lower pHs. In order to better understand the organization of this porin and the modifications that it undergoes at different pHs, we have performed experiments in neutral and acidic conditions to determine whether polyethylene glycol molecules of defined sizes can enter the pore or are excluded. The partitioning of PEGs will allow us to calculate the OmpU pore size in different conditions, as was shown by Bezrukov for OmpF. Supported by grant E-1597 of the Welch Foundation

3456-Pos Board B503

The Effects Of Radiofrequency Radiation On Single OmpF Channel Activity

Mehdi Mohammadzadeh[1], Hamid Mobasheri[1,*], Farokh Arazm[2].
[1]Lab. Memb. Biophys. , Inst. Biochem. & Biophys. , University of Tehran, Tehran, Iran, Islamic Republic of, [2]Antenna Lab., Dept. Comp. & Elect.l Eng., Univ. Tehran, Tehran, Iran, Islamic Republic of.

The channel behavior induced by exposure to radiofrequency electromagnetic field (RF-EMF) at 925 MHz, a frequency used in mobile communication, was studied in real time here. OmpF porin channel whose structure and dynamics have thoroughly been studied at atomic level was used as a model to study protein interactions with RF-EMF.

The activities of exposed single ion channel to RF-EMF at 20°C was compared to that of non-exposed at thermal range of 20-60°C in Montal and Muller planar lipid bilayer by means of voltage-clamp technique. These preliminary observations suggest that the exposure to radio frequency radiation affects on channel gating, conductance, and voltage sensitivity of the channel to some extent at constant medium temperature. Weather the changes are due to thermal or non-thermal effects is remained to be further evaluated. As the temperature was stably set with a decimal accuracy macroscopically, one might correlate the variations in channel activity to direct non thermal effects of the field on the intra-molecular motion of the protein. However, due to the technical limitation in defining the exact local temperature at nano scale within the channel and/or amongst the constituting amino acids, and also the difficulties in

monitoring the motion of amino acids in real time, we propose the possibility of the thermal effects on the channel molecular dynamics, what that is remained to be further studied.

3457-Pos Board B504

Novel constitutively active non-store-operated Ca2+ current in T lymphocytes

Pratima Thakur, **Sepehr Dadsetan**, Alla F. Fomina.
UC Davis, Davis, CA, USA.

The Ca^{2+}-release-activated Ca^{2+} (CRAC) channels is the only known mechanism mediating Ca^{2+} entry in T cells. However, using Mn^{2+} quench of Fura-2 fluorescence we observed a constitutive divalent cation influx in the absence of stimulated store-operated Ca^{2+} entry in Jurkat T lymphocytes. Suppression of CRAC channels activity either with blocking concentration of La^{3+} or by expression of dominant-negative Orai1 mutant did not affect the rate of constitutive Mn^{2+} quench. These data suggest the existence of an additional non-store-operated mechanism mediating Ca^{2+} entry in T lymphocytes. Consistently, a constitutively active current was recorded in metabolically intact T cells using perforated-patch technique. Whole cell and perforated patch experiments revealed that in the presence of extracellular Ca^{2+} both constitutively active and CRAC currents displayed inwardly rectifying current-voltage relationship, positive (> 50 mV) reversal potential, and were enhanced by increased concentrations of extracellular Ca^{2+}. However, when the divalent cations were removed from the extracellular solution, the monovalent CRAC current displayed fast time-dependent inactivation, whereas the monovalent constitutively active current exhibited time-dependent activation and lack of inactivation. Equimolar substitution of Na^+ with Cs^+ in Ca^{2+}-free solution reduced the amplitudes of monovalent CRAC current and constitutively-active current by > 90 % and < 40 % respectively. Taken together, these data indicate that the CRAC and constitutively active currents are carried via different types of Ca^{2+}-selective channels. We speculate that in T lymphocytes the constitutively active Ca^{2+} entry channels may supply Ca^{2+} for maintaining resting cytosolic Ca^{2+} levels and/or for store refilling at unstimulated conditions. Supported by AHA Grant-in-Aid 0755086Y to A.F.F

3458-Pos Board B505

The Role of Ion Channels in Differentiating Chondrocytes

Attila Sebe[1], Gyorgy Panyi[1], Roza Zakany[2], Tamas Juhasz[2], Csaba Matta[2], **Zoltan Varga[1]**.
[1]University of Debrecen, Dept. of Biophysics and Cell Biology, Debrecen, Hungary, [2]University of Debrecen, Dept. of Anatomy, Histology and Embryology, Debrecen, Hungary.

Cartilage is an important load-bearing component of the skeleton of vertebrates. This tissue has similar composition to other members of the connective tissue family: major constituents are cells, the chondrocytes, and the surrounding extracellular matrix; however, it is also unique being avascular. Chondrocytes are non-excitable cells and little is known about their plasma membrane ion channels. The aim of our study was to identify ion channels and establish their roles in differentiating chondrocytes.

Our *in vitro* chondrogenesis model system is a high density mesenchymal cell culture, in which chondroprogenitor cells are isolated from limb buds of chicken embryos. Using whole-cell patch-clamp we have detected voltage-dependent ionic currents in these differentiating cells, whose amplitude depended on the time elapsed since isolation. An outward current was present in chondrocytes within 1-2 days of isolation, while an inward current gradually replaced it about 2 days after isolation. Using ion substitution experiments we identified the channels responsible for the currents as voltage-gated K^+ and Na^+ channels, respectively.

The average amplitude of the Na^+ current in cells during days 3-4 following isolation was -294 +/− 22 pA at 0 mV. The current inactivated with a time constant of tau = 0.59 +/− 0.04 ms. The voltage-dependence of steady state activation and inactivation were also determined yielding $V_{1/2}$ values of -38 and -72 mV. Tetrodotoxin reversibly blocked the current with a $K_d = 12$ nM. The results of planned molecular biological experiments combined with our biophysical and pharmacological data will be used to identify the channel. The characterization of the K^+ channel is presently underway.

In many cell types changes in ion channel expression are associated with differentiation, thus our long-term aim is the clarification of the role of these channels in chondrogenesis and its potential clinical consequences.

3459-Pos Board B506

A Gadolinium-Sensitive Non-Specific Cation Channel In Canine Articular Chondrocytes

Rebecca Lewis, Richard Barrett-Jolley.
University of Liverpool, Liverpool, United Kingdom.

Non-specific cation channels are present in a number of cell membranes and can be activated by diverse cellular stimuli, allowing mono- and divalent cations to cross the cell membrane (Sanchez & Wilkins 2003).

In the present study we used both inside-out and whole-cell patch clamp electrophysiology to characterise the predominant ion channel in potassium free solutions.

Isolated chondrocytes were cultured for 7 to 9 days in Dulbeccos Modified Eagles Medium with 10% Foetal Calf Serum. Recording was carried out on first to third passage cells. For single channel data, membrane potential (*Vm*) was calculated as *Vm = -Hp-Vj* where *Hp* was the holding potential and *Vj* the calculated junction potential. Data are expressed as mean ± standard error.

Single-channel activity reversed at a membrane potential of 3 ± 2mV (n = 5) in the presence of 196mM internal and 155mM external Na^+, indicative of a non-specific cation channel. Mean slope conductance of the channel was calculated to be 67 ± 5pS (n = 5). This channel activity was seen in 53% of patches (32/61), with mean open probability of 0.6 at −40mV. 100μM gadolinium III reduced this open probability by 75 ± 9%.

In identical solutions the predominant whole-cell current showed a reversal potential of 1 ± 5mV. 100μM gadolinium III inhibited whole-cell current by 85 ± 7%. The whole-cell current exhibited weak voltage sensitivity with Boltzmann parameters for slope and half maximal activation of $k = 83$mV and $V_{1/2} = -38$mV.

The ion channels identified in these electrophysiological experiments may underlie the gadolinium-sensitive stretch-activated increases in calcium observed by Guilak *et al* (1999) in bovine tissue.

3460-Pos Board B507

Stim1 and Orai1 Mediate CRAC Currents and Store-Operated Calcium Entry Necessary for Endothelial Cell Proliferation

Iskandar F. Abdullaev, Jonathan M. Bisaillon, Marie Potier, Jose C. Gonzalez, Rajender K. Motiani, Mohamed Trebak.
Albany Medical Center, Albany, NY, USA.

Recent breakthroughs in the store-operated calcium (SOC) entry pathway have identified Stim1 as the endoplasmic reticulum (ER) calcium sensor and Orai1 as the pore forming subunit of the highly calcium selective CRAC channel. Previous studies have suggested that endothelial cell (EC) SOC is encoded by members of the Canonical Transient Receptor Potential (TRPC) channel family, either TRPC1 or TRPC4. Here we show that passive store depletion or receptor activation by thrombin or VEGF activates SOC entry pathway in primary EC with classical SOC pharmacological features. EC possess the archetypical store-depletion activated CRAC current. By amplifying currents in divalent free bath solutions, we show that EC CRAC has similar characteristics to that recorded from RBL cells, namely a similar time course of activation, sensitivity to 2-APB and low concentrations of lanthanides, the same inwardly rectifying I/V relationship, very positive reversal potential, and large sodium currents displaying the typical phenomenon of depotentiation. RNA silencing of either Stim1 or Orai1 essentially abolished SOC entry and CRAC currents in EC which were rescued by ectopic expression of either Stim1 or Orai1, respectively. Surprisingly, complete knockdown of either TRPC1 or TRPC4 proteins had no effect on SOC entry in EC. Smaller CRAC current densities in EC compared to those recorded in RBL cells were due to lower expression of Stim1. Ectopic expression of Stim1 in EC increased their CRAC currents to a size comparable to those in RBL cells. Knockdown of either Stim1, Stim2 or Orai1 inhibited EC proliferation and caused cell cycle arrest at S and G2/M phase, although Orai1 knockdown was more efficient than that of Stim1. These results are first to establish the requirement of Stim1/Orai1 in the endothelial SOC pathway necessary for proliferation.

3461-Pos Board B508

Androgens Stabilize HERG Potassium Channel Protein Via Stimulation Of Androgen Receptor Variant Ar45

Zhi-Yuan Wu, **Jin-Song Bian**.
National University of Singapore, Singapore, Singapore.

Proarrhythmic drugs induce long QT syndrome more frequently in women than men. The present study was designed to determine whether androgens regulate the function and expression of the human ether-á-go-go-related gene (HERG) encoded K^+ channel, which is largely responsible for determining the QT interval. In a concentration-dependent manner (10^{-9} to 10^{-6} M for 24 h), 5α-dihydrotestosterone (5α-DHT) increased HERG protein abundance in HEK293 cells stably expressing HERG in the presence of co-expressed cardiac androgen receptor variant (AR45). The elevation of HERG protein was seen in ER, Golgi and plasma membrane without clear preferential colocalization. Co-expression of the more common form of the androgen receptor did not confer 5α-DHT augmentation of HERG protein. Proteasome inhibitors, N-acetyl-L-leucyl-L-