the ''respiratory burst'' - the coordinated activation of NADPH oxidase and proton channels. Voltage gated proton current and NADPH oxidase generated electron current were measured simultaneously in human monocytes in perforated patch configuration after one to three days in culture. Upon stimulation by 60 nM phorbol myristate acetate (PMA), electron current (reflecting NADPH oxidase activity) appeared and the proton current amplitude increased. PMA slowed the kinetics of tail currents, sped the activation of outward proton current, and shifted the g_H-V relationship negatively. The NADPH oxidase inhibitor diphenylene iodonium (DPI) inhibited the electron current but affected solely the tail current kinetics of the proton current. Thus, although monocytes differentiate from a different lineage than granulocytes, their responses to PMA resemble those of other phagocytes. Hydrogen peroxide (H_2O_2) production was recorded fluorometrically. Zinc, at concentrations that inhibit proton current, reduced H_2O_2 production in monocytes. This Zn^{2+} sensitivity resembles that seen in neutrophils and eosinophils. The dogma that extracellular glucose is necessary to support the oxidative burst of human monocytes was tested in electrophysiological and fluorescence measurements. Electron current measured in patch clamp experiments was increased 2.5 fold by adding glucose to the bath solution; proton current was unaffected. Correspondingly, H_2O_2 production was strongly increased and more sustained in the presence of glucose. In summary, the electrophysiological events during activation of monocytes resemble those in other phagocytes, but NADPH oxidase is more acutely dependent on the presence of glucose.

3447-Pos Board B494

Determining the Functional Core for Proton Transport, Ion Selectivity and Amantadine Sensitivity of the A/M2 Protein from Influenza A Virus

Chunlong Ma[1], Alexei Polishchuk[2], Yuki Ohigashi[1], William F. DeGrado[2], Robert A. Lamb[1,3], Lawrence H. Pinto[1].
[1]Northwestern University, Evanston, IL, USA, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Howard Hughes Medical Institute, Evanston, IL, USA.

Influenza continues to be an epidemic and pandemic disease. The M2 protein from influenza A virus is a pH-activated proton channel. Its function is essential for efficient replication of the virus. Moreover, the M2 protein is the target of the antiviral drug amantadine, which is one of few available antiviral drugs that inhibit influenza A replication. Although M2 protein is only a 97-amino acid protein, it possesses multiple roles by its different domains in different stages of virus life cycles. In spite of the importance of the ion channel function of the M2 protein, the part of the protein that possesses its central role-proton channel-has not been defined clearly. Moreover, recent structural studies used truncated constructs that have not yet been evaluated for proton channel function. Here we report findings from experiments designed to investigate the functional core for proton transport, ion selectivity and amantadine sensitivity of M2 ion channel protein. We constructed a series of truncation mutants, measured low-pH activated, amantadine sensitive current in oocytes of *Xenopus laevis* and also determined the relative M2 surface expression on the *Xenopus laevis* oocyte membrane. We found that a construct of residues 21-61 (''shortie''), which includes the TM domain and 18-residues of the cytoplasmic tail, has the ion channel activity indistinguishable from that of the full length M2 protein. Functional reconstitution vesicle assay also showed that this construct was sufficient for proton channel function. Further truncated peptides (residues 22-46 and residues 22-50) showed amantadine-sensitive proton fluxes similar to ''shortie'' M2 (residues 19-62), however these peptides displayed a lower proton-selectivity and some potassium ion flux.

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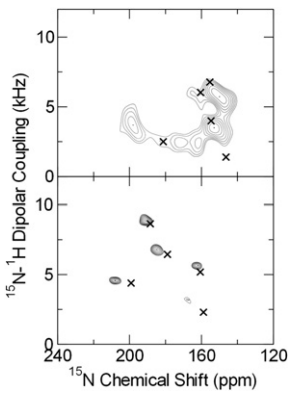
Conformational Heterogeneity Of The M2 Proton Channel: A Model For Channel Activation

Myunggi Yi[1], Timothy A. Cross[2], Huan-Xiang Zhou[1].
[1]Florida State University, Tallahassee, FL, USA, [2]National High Magnetic Field Laboratory, Tallahassee, FL, USA.

The M2 protein of influenza A is a proton selective ion channel activated by low pH. Recent structures determined by X-ray crystallography and solution NMR suggested models for open and closed states. However, these models are based on limited data and other important functional states need to be characterized. Indeed, solid-state NMR data demonstrate that the M2 protein possesses significant conformational heterogeneity. Here, we report MD simulations of the M2 transmembrane domain in the absence and presence of the anti-viral drug, amantadine. The ensembles of MD conformations for both apo and bound forms reproduce the PISEMA data well (Figure). The helices kink around Gly34, where a water molecule penetrates deeply into the backbone. The bound form exhibits a single peak around 10° in the distribution of helix kinking angle, but the apo form exhibits two peaks, around 0° and 40°. Conformations with the larger kinking angles have a wider opening around the primary gate formed by His37 and Trp41, reproducing some of the key observations on the low-pH activated state by ^{19}F NMR. We propose that this population is stabilized by low pH and leads to proton conductance.



3449-Pos Board B496

Reversibility of Amantadine Inhibition in the M2 Proton Channel of Influenza A Virus

Victoria Balannik[1], Catrin Steensen[2], Petr Obrdlik[2], Bela Kelety[2], Jun Wang[3], William F. DeGrado[3], Lawrence H. Pinto[1].
[1]Northwestern University, Evanston, IL, USA, [2]IonGate Biosciences, Frankfurt, Germany, [3]University of Pennsylvania, Philadelphia, PA, USA.

The M2 protein from influenza A virus forms a pH-activated proton channel that mediates acidification of the interior of viral particles entrapped in endosomes. M2 is the target of the anti-influenza drug amantadine. Many observations have shown that amantadine inhibits the channel only when applied extracellulary, probably by residing within the channel pore and disrupting the gating mechanism. The effect of amantadine on AM2 channel activity was found to be slowly reversible in tissue culture cells and oocytes, but the reversibility kinetics of the drug on recombinant AM2 channels has never been directly addressed. In the current work we provide evidence for significant differences in the rate of amantadine reversibility among several heterologous expression systems. We characterized the channel activity, the amantadine sensitivity and reversibility of the AM2 protein expressed in CHO-K1 cells using novel solid supported membrane technology (SURFE2R, IonGate Biosciences GmbH). SURFE2R technology allows time resolved measurements of the electrogenic activity of slowly conducting channels and provides a valuable compliment to electrophysiological studies. We also tested amantadine reversibility in AM2 expressing CHO-K1 cells using pH sensitive green fluorescent protein microscopy and in AM2 expressing oocytes using TEVC technique. We show that amantadine inhibition in AM2-expressing mammalian cells is rapidly reversible. However in oocytes the reversibility of amantadine inhibition is much slower and depends on the channel properties. These findings should be taken into consideration for future investigation of the mechanism of amantadine inhibition and show once again that the properties of recombinant proteins may be significantly influenced by the properties of the expression system.

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Study Of Gating Mechanism Of Two Pore Domain K+ Channels Gated By Extracellular Alkalinization: TASK-2 And TASK-3

Fernando D. Gonzalez-Nilo[1], Christophe Chipot[2], Alex Digenova[1], Cristell Navarro[1], Wendy Gonzalez-Diaz[1], Pablo Cid[3], Leandro Zuniga[3], Maria Isabel Niemeyer[3], Francisco Sepulveda[3].
[1]Universidad de Talca, Talca, Chile, [2]CNRS, Nancy, France, [3]Centro de Estudios Cientificos, Valdivia, Chile.

Potassium channels share a common selectivity filter that determines the conduction characteristics of the pore. Diversity in K+ channels is given by how they are gated open. TASK-2 and TASK-3 are two-pore region (2P) KCNK K+ channels gated open by extracellular alkalinization. We have explored the mechanism for this alkalinization-dependent gating using molecular simulation and site-directed mutagenesis followed by functional assay. We show that the side chain of a single arginine residue (R224) near the pore senses pH in TASK-2 with an unusual pKa of 8.0, a shift likely due to its hydrophobic environment (Niemeyer, et al., PNAS, 2007; 104(2):666-71). While, TASK-3 sense the pH through a histidine residue located at the outer part of the pore adjacent to the selectivity filter (GYG-H-) (Rajan, et al., JBC, 2000; 275(22):16650-7). R224 (TASK-2) and H98 (TASK-3) would block the channel through an electrostatic and structural effect on the pore, a situation relieved by its deprotonation by alkalinization. In this work we show a complete experimental and theoretical study about how the environment stabilizes the neutral or charged state of the sensor residue. Our free-energy profile, determinated using an Adaptive Biasing Force, together with a host of site-directed mutagenesis experiments illustrate in a physiological context the principle that the hydrophobic environment drastically modulate the pKa of charged amino acids within a protein.

3451-Pos Board B498

Dual Effect of Wogonin on TREK-2 Expression and Channel Activity

Dawon Kang, Eun-Jin Kim, Gyu-Tae Kim, Jaehee Han.
Gyeongsang National University, Jinju, Republic of Korea.

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