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

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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

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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.

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

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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?

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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

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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