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.

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

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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

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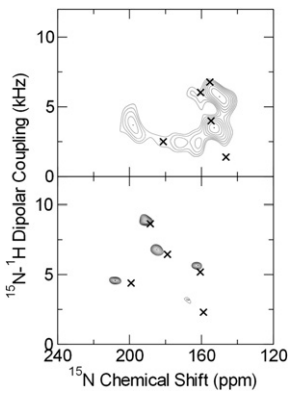
The M2 protein of influenza A is a proton selective ion channel activated by low pH. Recent structures determined by X-ray crystallography and solution NMR suggested models for open and closed states. However, these models are based on limited data and other important functional states need to be characterized. 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.



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

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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

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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

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