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Myelin Structural Integrity in a Model for Human Early-Onset CMT1B

Michelle Crowther[1], Brian Shy[2], Adrienne M. Luoma[1], Lawrence Wrabetz[3], Michael E. Shy[2], Daniel A. Kirschner[1].
[1]Boston College, Chestnut Hill, MA, USA, [2]Wayne State University School of Medicine, Detroit, MI, USA, [3]San Raffaele Hospital, Milano, Italy.

Charcot-Marie-Tooth disease type 1B (CMT1B), a peripheral neuropathy, is caused by mutations in MPZ, the gene encoding protein zero (P0), the major integral protein of PNS myelin. An adhesive protein, P0 plays a significant role during elaboration and maintenance of multilamellar myelin. P0 mutation Arg69Cys (R69C) causes a severe early-onset form of CMT1B. To elucidate the pathogenesis of this neuropathy, an Arg69Cys knock-in mouse was generated by targeting the Arg69Cys mutation to one MPZ allele by homologous recombination in ES cells. Here we report our x-ray diffraction (XRD) measurements on the periodicity, membrane structure, and amount of myelin in unfixed, freshly-dissected nerves from wildtype (WT or +/+), heterozygous (R69C/+), and homozygous (R69C/ R69C) mice. CNS myelin (optic nerve) was also examined. The diffraction patterns showed decreasing strength of scattering intensity from myelin: WT > R69C/+ > R69C/R69C, indicating decreasing relative amounts of myelin. By contrast, optic nerves exhibited no such differences among genotypes. From the positions of the reflections the myelin periods of sciatic but not optic nerves were found to differ among the genotypes: 177.0 ± 0.4 Å for WT, 178.4 ± 0.5 Å for R69C/+, and 193.1 ± 4.2 Å for R69C/R69C. The calculated electron density profiles showed R69C/R69C's wider period derived from ~20 Å-swelling at the extracellular apposition. The extent of membrane packing distortion (Δ/d) in PNS myelin, calculated using Bragg order peak widths, was 25% greater in R69C/+ and doubled in R69C/R69C compared to WT. Differences in amount of myelin, period, and Δ/d among the genotypes were statistically significant at $p < 0.001$. Finally, comparison of R69C/+ with P0± and R69C/R69C with P0-/- suggested the small amount of mutant P0 that enters the myelin may detrimentally affect myelin-myelin interactions to produce less regular/unstable packing.

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Monolayers of a Mixed Phospholipid System

Carlos Luna[1], Helim Aranda-Espinoza[1], Amir Maldonado[2], Gerardo Paredes[2].
[1]University of Maryland, College Park, MD, USA, [2]Universidad de Sonora, Hermosillo, Sonora, Mexico.

Membranes formed with mixtures of phospholipids have interesting properties. Mixing the appropriate components can give rise to phenomena such as the rigidification of the membrane or the appearance of lipid rafs. It has been shown that hydration of a dryed SOPC:SOPS film produces vesciles whose shapes depend on the lipid composition.

This effect is probably due to different packing conditions of the phospholipid molecules in the membrane due to electrostatic interactions. In order to further understand this system, in this work we have investigated the packing of SOPC:SOPS Langmuir monolayers and AFM experiments as a function of phospholipid composition.

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Deposition of egg-PC to an Air/Water and Triolein/Water Interface

Matthew A. Mitsche, Donald M. Small.
Boston University Medical Center, Boston, MA, USA.

Phospholipid monolayers play a critical role in stabilization of biological interfaces including the alveoli of the lung, fat droplets in adipose tissue, and apolipoproteins. Behavior of phospholipids at an air-water interface is well understood. However, work at oil-water interfaces is limited due to technical challenges associated with a Langmuir trough. In this study, egg-phosphatidylcholine(PC) was deposited onto a drop of either air or triolein(TO) formed in a low salt buffer and the surface tension was measured using a drop tensiometer. The egg-PC was deposited by constituting it into SUVs and then allowing molecules to absorb to the surface. We observed that egg-PC binds irreversibly to both interfaces and at equilibrium exerts 15 and 12 mN/m at an air and triolein interface, respectively. To determine the surface concentration, which cannot be measured directly, compression isotherms from a Langmuir trough were compared to that of the drop tensiometer. The air-water interfaces had identical characteristics so the surface concentration of the drop can be determined by simply overlaying the two isotherms. Since TO is also surface active there will be triolein incorporated into the monolayer. Since TO is less surface active than PC as the pressure(Π) increases the triolein is progressively ejected. To understand the Π/area isotherm of PC on the TO drop a variety of TO-PC mixtures were spread at the air-water interface. The isotherms show an abrupt break in the curve at a specific Π caused by the ejection of TO from the monolayer into the bulk phase. A plot of these surface transition points against Π gives the monolayer surface composition at any Π. The oil drop experiment always contains bulk phase of TO, thus the 2-D phase rule predicts the monolayer composition of the droplet over a range of Π.

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Effects of Ether vs. Ester Linkage on Lipid Bilayer Structure and Water Permeability

Deren Guler[1], Dipon Ghosh[1], Jianjun Pan[1], John F. Nagle[1], John C. Mathai[2], Mark L. Zeidel[2], Stephanie Tristram-Nagle[1].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]Harvard Medical School, Cambridge, MA, USA.

The structure and the water permeability of bilayers composed of the ether linked lipid, dihexadecylphosphatidylcholine (DHPC), were studied and compared with the ester linked lipid, dipalmitoylphosphaditdylcholine (DPPC). Wide angle x-ray scattering (WAXS) on oriented bilayers in the fluid phase indicates that the area per lipid A is slightly larger for DHPC than for DPPC. Analysis of low angle x-ray scattering (LAXS) to 0.85 $Å^{-1}$ on the same fully hydrated bilayers yields area A=65.1 $Å^2$ for DHPC at 48°C and other structural quantities such as various bilayer thicknesses. The DHPC LAXS data provide the bending modulus, $K_C=4.2x10^{-13}$erg and the Hamaker parameter $H=7.2\times10^{-14}$erg for the van der Waals attractive interaction between neighboring bilayers. These quantities can be compared with the results for DPPC at 50°C: A=64.3 $Å^2$, $K_C=6.7x10^{-13}$erg and $H=8.2x10^{-14}$erg. For the low temperature phases with ordered hydrocarbon chains, use of oriented samples provides higher resolution than earlier studies. We confirm the transition from a tilted L_β, gel phase to an untilted, interdigitated $L_\beta I$ phase as the sample hydrates at 20 °C, and WAXS data suggest that the drier, gel phase is an $L_{\beta'L}$ phase. These structural results for DHPC and DPPC are compared to new measurements of Pf, the water permeability, in both the fluid and gel phases.

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Electric Field Driven Conformational Changes of Gramicidin D in a Model Membrane Supported on a Au(111) Electrode Surface

Jacek Lipkowski, Slawomir Sek, Thamara Laredo, John R. Dutcher.
University of Guelph, Guelph, ON, Canada.

In this work, we show molecular resolution scanning tunnelling microscopy (STM) images of gramicidin, a model antibacterial peptide, inserted into a phospholipid matrix supported at a gold electrode surface. The resolution of the images is superior to that obtained in previous attempts to image gramicidin in a lipid environment using atomic force microscopy (AFM). This breakthrough has allowed visualization of individual peptide molecules surrounded by individual lipid molecules. We have observed several important features: the peptide molecules do not aggregate, the peptide molecules adopt a single conformation corresponding to a specific ion channel form, and the lipid molecules adjacent to the peptide molecules are systematically longer than those in the lipid matrix. These results constitute a new approach to obtain structural characteristics of antibiotic peptides in lipid assemblies that is necessary for the understanding of their biological activity.

We then applied the polarization modulation infrared reflection absorption spectroscopy (PM IRRAS) to investigate the effect of the electric field on the conformation and orientation of gramicidin molecules in a bilayer supported at the gold electrode surface. We observed potential controlled changes in the orientation and conformation of the gramicidin molecules in the supported bilayer. Careful analysis of the IR data indicated that the potential applied to the electrode affects the bilayer structure and these changes cause reorientation and conformational transformations of gramicidin molecules.

Inward Rectifier K Channels

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An Inter-intra Subunit Salt Bridge near the Selectivity Filter Stabilizes the Conducting State of Kir1.1

Henry Sackin[1], Mikheil Nanazashvili[1], Hui Li[1], Lawrence G. Palmer[2], D. Eric Walters[1].
[1]The Chicago Medical School, North Chicago, IL, USA, [2]Weill Medical College of Cornell Univ., New York, NY, USA.

ROMK (Kir1.1) potassium channels are normally closed by internal acidification with a pKa of 6.6. If this acidification occurs in the presence of low (1mM) external K, the channels also inactivate, such that channel activity is not recovered by realkalization until high K is returned to the external solution. Mutations in an inter-intra subunit salt bridge (E118-R128-E132-Kir1.1b) in the P-loop of the channel near the selectivity filter increased the K sensitivity of

inactivation, such that the salt-bridge mutants E132Q-Kir1.1b and R128Y-Kir1.1b inactivated in 100mM K solutions after a transient acidification. However 300mM external K (but not 200mM Na +100mM K) protected E132Q and R128 from inactivation during this acidification, suggesting an altered K sensitivity in these mutants. External application of a modified honey bee toxin (TPNQ) protected wild-type ROMK from inactivation in 1mM K and protected E132Q or R128Y from inactivation in 100mM K, suggesting that TPNQ binding to the outer mouth of the channel stabilizes the conducting state. Nonetheless, TPNQ was unable to protect either E132Q or R128Y from inactivation in 1mM external K. However, both E132Q and R128Y were protected from inactivation in 1mM K either by a mutation that disrupted transmembrane helix H-bonding (K61M-Kir1.1b) or by a mutation that stabilized a selectivity-filter to helix-pore linkage (V121T-Kir1.1b). Our results are consistent with an inter-intra subunit salt bridge near the outer end of the selectivity filter that stabilizes the conductive state of the channel.

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Gating Sensitive Residues In The Pore Of An Inwardly Rectifying Potassium (Kir) Channel

Murali K. Bollepalli[1], Markus Rapedius[1], Philip Fowler[2], Man-Jiang Xie[3], Lijun Shang[3], Hariolf Fritzenschaft[1], Mark S. Sansom[2], Stephen J. Tucker[3], Thomas Baukrowitz[1].
[1]Institute of Physiology II, Friedrich Schiller Universität, Jena, Germany, [2]Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, United Kingdom, [3]Biological Physics Group, Department of Physics, University of Oxford, Oxford, United Kingdom.

Intracellular pH gates inwardly rectifying potassium (Kir) channels by controlling the reversible transition between the closed and open states. This gating mechanism underlies important aspects of Kir channel physiology and pathophysiology. H^+ inhibition is thought to be triggered by protonation of residues within the cytoplasmic domains. This then causes major conformational rearrangement of the TM helices and slide helix resulting in closure of the gate at the helix bundle crossing. To identify residues important for this gating process we performed a systematic alanine scan in Kir1.1 channels over the entire transmembrane pore structure of the channel (residues 61 - 192) and measured pH sensitivity of the individual mutants in inside-out patches. We identified gating sensitive residues in both TM1 and TM2 that appear to make up an intra-subunit gating interface as well as a cluster of residues in the proximal part of the slide helix extending into TM1. Two highly conserved phenylalanines (F84, F88) in TM1 seem be of particular importance as they had dramatic effects on the pH gating kinetics. Assuming that the mutations do not affect the cytoplasmic pH sensor directly, a change in IC50 therefore represents a change in the stability of the closed state relative to the open state. Intriguingly, most of the gating sensitive mutations (17 out of 19) increased the IC50 for pH inhibition (from 6.4 (wild-type) up to 8.5) indicating that the mutations had a marked tendency to disturb the stability of the open state more severely than the closed state. This suggests that the open state in Kir channels is structurally more optimised than the closed state.

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State-dependent Cysteine Modification during pH and PIP_2 Gating in Kir Channels

Markus Rapedius[1], Murali K. Bollepalli[1], Stephen J. Tucker[2], Thomas Baukrowitz[1].
[1]Physiology II, Jena, Germany, [2]Clarendon Laboratory; Departement of Biophysics, Oxford, United Kingdom.

Inhibition by intracellular H^+ (pH-gating) and activation by phosphoinositides such as PIP_2 (PIP_2-gating) are key regulatory mechanisms in the physiology of inwardly-rectifying potassium (Kir) channels. Our recent findings suggest that PIP_2 gating and pH gating underlie similar conformational change at the helix bundle crossing, however, little is known about the structural changes in the cytoplasmic domains. Here we explore the state-dependent changes in accessibility of three endogenous cysteines (C175, C49, C308) in Kir1.1 channels occurring during PIP_2 and pH gating. C175 in the inner pore cavity is modified by MTSET in the open state, but protected from modification in the closed state induced by either low intracellular pH or PIP_2 depletion. This confirms the concept that the helix bundle crossing represents the gate controlled by pH and PIP_2. C49 in the N-terminus is protected from modification in the open state but can be modified in the closed state induced by either low pH or PIP_2 depletion indicating a similar conformational change in this region. C308 in the C-terminus can only be modified in the closed state induced by PIP_2 depletion but is protected in the open state and as well in the pH-inhibited closed state. A homology model of Kir1.1 shows that C308 is located in close proximity to the PIP_2 binding site indicating that PIP_2 either directly, or by a conformational change at C308 protects this residue from modification. The lack of C308 modification in the pH inhibited state suggests that pH induced channel closure occurs with PIP_2 tightly bound (thereby protecting C308), which is also consistent with our measurements on the kinetics of pH and PIP_2 induced channel activation.

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Role of Kir 2-caveolin-1 interactions in the sensitivity of Kir to cholesterol

Yulia Epshtein, Richard Minshall, Irena Levitan.
UIC, Chicago, IL, USA.

Our earlier studies have shown that Kir2 channels are strongly suppressed by the elevation of cellular cholesterol and enhanced by cholesterol depletion. We have also shown that Kir2 channels partially partition into cholesterol-rich membrane domains suggesting that interactions between the channels and other components of these domains may be critical for the regulation of the channels. It is also known that cholesterol interacts with caveolin-1, a scaffolding regulatory protein residing in these domains. In this study we test whether Kir2 channels are regulated by caveolin under different cholesterol conditions. Our data shows that Cav-1 co-immunoprecipitates with both Kir2.1 and Kir2.3 channels, suggesting that Cav -1 may be involved in the regulation of Kir2 channels. Furthermore, we show here that bone-marrow derived macrophages isolated from $Cav^{-/-}$ knock-out mice have larger Kir currents than cells isolated from control animals supporting the hypothesis that Cav-1 regulates Kir channels. Finally, we also show that sensitivity of Kir currents to cholesterol in $Cav^{-/-}$ cells is weaker than in control cells providing further evidence for the role of Cav-1 in the sensitivity of Kir channels to cholesterol.

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Long Qt Syndrome Mutations In Caveolin-3 Cause Loss Of The Kir2.1-mediated Inward Rectifier Potassium Current (i_{K1})

Amanda Vega, **Lee L. Eckhardt**, Jonathan C. Makielski.
University of Wisconsin, Madison, WI, USA.

Caveolin-3 (CAV3) is a key structural protein in cardiac caveolae that serves as an anchoring protein and a regulatory element for protein signaling in human cardiac myocytes. We have demonstrated previously that mutations in *CAV3*-encoded caveolin-3 are associated with long QT syndrome (LQT9) and increase late sodium current (I_{Na}).

We postulated that CAV3 may also regulate other ion channels such as KIR2.1, the channel responsible for the cardiac inward rectifier current I_{K1} and also the channel underlying Andersen-Tawil Syndrome (ATS1/LQT7). We therefore tested the four LQT9-associated mutations (F97C, S141R, T78M, A85T) for effects on inward rectifier channel KIR2.1. Wild-type (WT) Kir2.1 was expressed transiently in HEK293 cells with either WT stably expressed or mutant CAV3 proteins with IRES-GFP transiently expressed. Kir2.1 currents were measured using whole-cell patch clamp technique. WT CAV3 had no effect on Kir2.1 current. However, F97C-, S141R-, A85T-, and T78M-CAV3 mutations abolished both inward and outward I_{K1} current density. At -120mV inward I_{K1} current density was reduced by 59% (F97C), 55% (S141R), 80% (A85T) and 41% (T78M), $p \leq 0.02$. At -40mV outward I_{K1} current density was reduced by >96% for F97C, S141R, and T78M ($p \leq 0.04$), and was reduced by 68% by A85T ($p \leq 0.04$). This marked loss of I_{K1} function, over the physiological voltage range, important for terminal repolarization, suggests that CAV3 mutations may cause the LQT phenotype by a cumulative effect on I_{K1} and I_{Na}. More generally, it suggests that caveolin-3 is a novel Kir2.1 channel interacting protein. The detailed mechanism of this interaction and the implications for cardiac electrophysiology require further investigation.

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Epidermal Growth Factor Receptor Tyrosine Kinase Stimulates Human Inward Rectifier Potassium (Kir2.3) Channels

De-Yong Zhang, Chu-Pak Lau, **Gui-Rong Li**.
The University of Hong Kong, Pokfulam, Hong Kong.

Protein tyrosine kinases (PTKs), in addition to the mediation of cellular events such as cell growth, differentiation, etc., regulate ion channels. Although Kir2.3 channel plays a crucial role in the repolarization and membrane potential stabilization of neurons and myocardium, modulation of this channel is not fully understood. The present study investigated whether/how human Kir2.3 channel is modulated by PTKs and protein tyrosine phosphatases (PTPs) in HEK 239 cells stably expressing Kir2.3 gene using approaches of whole-cell patch voltage clamp, immunoprecipitation and Western blot, and site-directed mutagenesis. We found that epidermal growth factor (EGF, 100 ng/ml) and PTPs inhibitor orthovanadate (1 mM) significantly enhanced Kir2.3 channel current, while the broad spectrum PTKs inhibitor genistein and the selective EGF receptor kinase inhibitor AG556, but not the Src-family PTK inhibitor PP2 or the platelet-derived growth factor receptor kinase inhibitor AG1295, suppressed