by mutations in $Ca_V2.1$ (P/Q-type) Ca^{2+} channels. $Ca_V2.1$ channels play a key role in initiating action potential-evoked neurotransmitter release at central synapses. FHM1 mutations shift channel activation to lower voltages and increase Ca^{2+} influx through single recombinant human $Ca_V2.1$ channels. Knockin mice carrying a human FHM1 mutation show an increased P/Q-type Ca^{2+} current in cerebellar and cortical neurons and a reduced threshold for and increased velocity of cortical spreading depression (CSD), the phenomenon that underlies migraine aura and may activate migraine headache mechanisms. To investigate the mechanisms of CSD facilitation, we studied neurotransmission at synapses of cortical pyramidal cells in microculture and in connected pairs of layer 2/3 pyramidal cells and fast-spiking interneurons in acute thalamocortical slices. Our data show increased strength of excitatory neurotransmission due to enhanced action potential-evoked Ca^{2+} influx through synaptic $Ca_V2.1$ channels and increased probability of glutamate release at pyramidal cell synapses of FHM1 KI mice. At the same synapses, short-term depression during trains of action potentials was enhanced. There was no evidence of homeostatic compensatory mechanisms at synapses onto pyramidal cells. To investigate possible alterations of the cortical excitation-inhibition balance in FHM1, we studied inhibitory neurotransmission between fast-spiking interneurons and pyramidal cells in thalamocortical slices. At this inhibitory synapse the strength of neurotransmission was unaltered in KI mice. Our findings may explain CDS facilitation in FHM1 mice, and point to tipping the finely tuned dynamic balance between excitation and inhibition during cortical activity towards excitation as the basis for CSD propensity and abnormal processing of sensory information in migraine.

1047-Wkshp
Presenilins Function as ER Calcium Leak Channels: Implications for Alzheimer's Disease
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Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder. Mutations in presenilins are responsible for approximately 40% of all early onset familial Alzheimer's disease (FAD) cases in which a genetic cause has been identified. FAD mutations and genetic deletions of presenilins have been linked with calcium (Ca2+) signaling abnormalities, but mechanistic basis for these results has not been clearly determined. Presenilins are highly conserved transmembrane proteins that support cleavage of the amyloid precursor protein by gamma-secretase. In our studies we discovered that in addition to acting as a gamma-secretase, presenilins also function as passive endoplasmic reticulum calcium (Ca2+) leak channels. We demonstrate that wild type PS1 and PS2 proteins form low conductance divalent cation-permeable ion channels in planar lipid bilayers. In experiments with PS1/2 double knockout (DKO) mouse embryonic fibroblasts (MEFs) we discovered that presenilins account for ~80% of passive Ca2+ leak from the endoplasmic reticulum. The ER Ca2+ leak function of presenilins is independent from their gamma-secretase function In additional experiments we demonstrated that ER Ca2+ leak function of presenilins is impaired by M146V, L166P, A246E, E273A, G384A and P436Q FAD mutations in PS1 and N141I mutation in PS2. In contrast, FTD-associated mutations (L113P, G183V and Rins352) did not appear to affect ER Ca2+ leak function of PS1 in our experiments, indicating that the observed effects are disease-specific. Our data uncover a novel Ca2+ signaling function of presenilins and provide support to the potential role of disturbed Ca2+ homeostasis in AD pathogenesis. We are in the process of expanding these findings to neuronal system. Our latest findings will be discussed.

1048-Wkshp
Mutations in skeletal muscle
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Mutations in skeletal muscle sodium channels ($Na_V1.4$) cause periodic paralysis. Paramyotonia congenita and hyperkalemic periodic paralysis are caused by gain-of-function mutations spread widely through the protein, which increase channel activity and lead to repetitive firing or depolarization block. In contrast, mutations that cause hypokalemic (HypoPP) and normokalemic (NormoPP) periodic paralysis are localized in the outermost three gating-charge-carrying arginine residues (R1-R3) in the S4 segment in domain II, and they do not have major effects on sodium channel function as typically measured. Site-directed mutations of these residues cause gating pore current, a voltage-gated leak current through the voltage sensor (Sokolov et al., 2005); mutations of R1 and R2 cause gating pore current in the resting state, whereas mutation of R3 causes gating pore current in the activated state. Similar studies of the HypoPP mutant R2G revealed gating pore current of approximately 1% of peak current at the resting membrane potential, which was decreased by depolarization (Sokolov et al., 2007). This gating pore current was selective for Cs>K>Na and blocked by mM concentrations of divalent cations, Zn>Ba>Ca. A gating pore current of similar size was observed in the resting state for the HypoPP mutants R1H and R2H, but this current is selective for protons. In contrast to HypoPP, the mutations that cause NormoPP are in R3 (R3G/Q/W). All of these mutations cause gating pore conductance for sodium in the activated and slow-inactivated states, in which the voltage sensors are in their outward position. The common pathogenic feature of these mutations is likely to be depolarization and sodium overload, which are observed in patient biopsies. Dominant gain-of-function pathogenic effects may arise directly from excess sodium entry for R2G and R3G/Q/W and indirectly from excessive Na-H exchange for R1H and R2H.

1049-Wkshp
Gating Pore Currents from S4 Mutations of NaV1.4: A Common Pathomechanism in Hypokalemic Periodic Paralysis
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The mechanism whereby missense mutations in charged residues of the S4 segments of CaV1.1 and NaV1.4 cause the skeletal muscle disorder hypokalemic periodic paralysis (HypoPP) remains poorly understood. Recent work suggests a possible common functional defect, in which HypoPP mutations produce aberrant ionic conductances flowing through the aqueous gating-pore in which the mutant S4 segment resides. We observed low-amplitude gating-pore currents for HypoPP mutations in the R1 and R2 positions of S4 in domain II in NaV1.4. Several features of these HypoPP-associated gating pore conductances were unexpected, and may provide insight into S4 segment function. For instance, gating pores exposed by mutations at the R2 site exhibited marked current saturation at hyperpolarized voltages. Saturation can be accounted for by a model with a single cation binding site very near the external surface of the electrical field. The ionic selectivity of different HypoPP gating pores is dependent on the substituted residue: histidine substitutions causing proton-selectivity, whereas other substitutions result in limited selectivity among monovalent cations. The pathophysiological significance of this dichotomy remains unclear. In addition, the low amplitude of the disease-associated gating pore currents (~.1% of the peak Na current through the central pore) is probably insufficient to directly cause the large depolarization of affected muscle fibers during a paralytic attack. These small currents might predispose to episodic paralysis by potentiating the normal sarcolemmal propensity to depolarize upon reduction of external K^+. This paradoxical depolarization is a consequence of the K^+ dependence for the inward rectifier K^+ conductance, which causes Vrest to deviate from Nernstian behavior. Thus, muscle fibers with an inward gating-pore current may function normally at most times, but may be poised for massive depolarization in the setting of minor perturbations of extracellular $[K^+]$.

Workshop 3: Enzymes in Energy Metabolism

1050-Wkshp
Enzymes in Energy Metabolism, Introduction
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Several years ago, the bioenergetics subgroup decided to broaden the more narrow definition of bioenergetics to include all pathways involved in energy production. The importance of studying them in combination can be illustrated by the very different metabolic behavior of cancer cells that, in contrast to healthy cells, derive most of their energy from glycolysis and at the same time show a lack of apoptosis. While each presentation in this workshop will be focused on specific topics, glycolysis, the mitochondrial membrane system, the pyruvate dehydrogenase as part of the TCA cycle and the fumarate reductase as one of the enzymes in the mitochondrial respiratory chain, emphasis will be given to the interconnection between the different systems.

1051-Wkshp
Organization and Structural Features of Phosphofructokinase and other Glycolytic Enzymes to Meet their Role in Energy Metabolism
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Although glycolytic enzymes can be isolated as cytosolic components, some of them are known to bind to proteins from the cytoplasm and other cellular compartments, such as the mitochondria, the nucleus, or the plasma membrane. In some cases these protein-protein interactions are considered significant for the regulation of energy metabolism, as well as for the modulation of other cell functions. Among glycolytic enzymes, phosphofructokinase (PFK) is thought to play a fundamental role in the control of this pathway, because of the number of metabolic signals that can regulate its complex allosteric behavior. No X-ray structure of a characteristic eukaryotic PFK is available yet. However, sequence data indicated that the eukaryotic enzyme originated by duplication, fusion and divergence of an ancestral prokaryotic gene, such that the duplicated fructose 6-phosphate catalytic site in the C-terminal half became an allosteric site for the activator fructose 2,6-bisphosphate. It has been suggested that both sites are shared in the interface between subunits aligned in an antiparallel orientation. To test the contribution of each terminal domain to these two binding sites, chimeric mammalian PFKs involving exchange of their terminal domains have been found to exhibit affinity properties for fructose 6-phosphate and fructose 2,6-bisphosphate that resembled those of the native isozyme that donated the N-terminal half and the C-terminal half, respectively. Further mutational analyses of muscle PFK led us to investigate the composition of the fructose phosphate binding sites and to gain insight into the structural organization of eukaryotic PFK. Data will also be presented evidencing the relationship between mitochondrial function and the operation of glycolysis, as observed after impairment of mitochondrial DNA transcription.

1052-Wkshp
How Mitochondrial Structure Can Affect Energy Metabolism: Insights From Electron Microscopic Tomography
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The idea that the internal structure of mitochondria might influence energy metabolism has its origins in electron microscopic studies in the 1960s and 1970s of morphologic changes during respiratory state transitions and ligand binding to adenine nucleotide translocator. In the ensuing years, the variety of known morphologic transitions displayed by mitochondria, in test tubes and in cells, has increased, as has information about the corresponding three-dimensional membrane topologies, provided by electron tomography. Experiments and simulations indicate that mitochondrial inner-membrane topology influences mitochondrial metabolic rates and other functions by altering internal diffusion pathways for ions, metabolites and soluble proteins. At the same time, the roles of certain proteins (e.g. ATP synthase) and lipids (e.g. cardiolipin) in determining inner-membrane topology are being uncovered. The emerging hypothesis is that mitochondrial inner-membrane topology is regulated by the cell to optimize mitochondrial performance in response to different stimuli (such as metabolite concentrations, apoptotic signals, and reactive oxygen). As techniques for cryo-specimen preparation (e.g. using focused ion beams) and imaging improve, it will become possible to apply electron tomography to whole cells and tissues in near-native, frozen-hydrated state. These enhanced imaging capabilities should provide new information about not only in-situ membrane topology, but also the nature of the physical contacts between mitochondrial outer and inner membranes; the nature of the tethers that regulate the spacing between mitochondria and endoplasmic reticulum (which in turn regulates mitochondrial calcium uptake, which in turn activates matrix dehydrogenases); the organization of the mitochondrial matrix; and possibly even the structure of respiratory supercomplexes in the mitochondrial inner membrane. (The Resource for Visualization of Biological Complexity is supported as an NCRR Biomedical Technology Research Resource by NIH grant P41-RR01219.)

1053-Wkshp
Molecular Architecture of Pyruvate Dehydrogenase Complexes
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Pyruvate dehydrogenase multienzyme complexes are among the largest multifunctional protein assemblies within cells, catalyzing the four-step conversion of pyruvate to acetyl CoA using an assembly comprised of multiple copies of E1, E2 and E3 enzymes, Using cryo-electron microscopy, we have shown that the outer shell enzymes (E1 decarboxylase and E3 pyruvate dehydrogenase) are separated from the inner icosahedral core (E2 acetyl transferase) by an annular gap that is about ~ 100 Å wide. The presence of the gap is a key structural element in the spatial organization of the three enzymes in the complex, and allows the ''swinging arm'' lipoyl domain to shuttle between the active site of the E2 at the inner core and the active sites of E1 and E3 enzymes at the outer shell to carry out synthesis of acetyl CoA. Using a combination of circular dichroism, analytical ultracentrifugation and solution NMR studies we have also demonstrated that the peptide corresponding to the linker region has an extended conformation with a persistence length of ~75-89 Angstroms, consistent with the observed size of the gap. Cryo-electron tomography of individual complexes with varying occupancies of enzymes in the outer shell confirmed unequivocally that the annular gap between the core and the outer shell was maintained even at very low E1 or E3 occupancies. These studies demonstrate unambiguously that it is the linker, rather than interactions between the outer shell enzymes, that are responsible for holding the subunits above the core. The prospect of using cryo-electron tomography to map the locations of individual enzymes within single multi-enzyme complexes could be a powerful approach to obtain structural information, without molecular averaging, on large and structurally heterogeneous biological assemblies that are not amenable to analysis by NMR or X-ray crystallographic techniques.

1054-Wkshp
Electroneutral And Electrogenic Catalysis By Diheme-Containing Succinate:Quinone Oxidoreductases
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Membrane proteins can support both the generation and utilisation of a transmembrane electrochemical proton potential (''proton-motive force''), either by transmembrane electron transfer coupled to protolytic reactions on opposite sides of the membrane or by transmembrane proton transfer. In the case of the diheme-containing quinol:fumarate reductase (QFR) of *Wolinella succinogenes* [1], both theoretical and experimental results, reviewed in [2], supported, but did not prove, a previous hypothesis [3] that both of these mechanisms are combined in a single membrane protein complex, so as to facilitate transmembrane electron transfer by transmembrane proton transfer. Results of measurements on proteoliposomes will be presented, providing evidence for the presence of this unprecedented transmembrane proton transfer pathway (''E-pathway'') in the wild-type enzyme and its non-functionality in a variant QFR where a key glutamate residue has been replaced [4]. The ''E-pathway'', discussed on the basis of the 1.78-Å-resolution crystal structure of QFR, is essential for life under the conditions of fumarate respiration. Results will be compared to those obtained with the diheme-containing succinate:menaquinone reductase from the Gram-positive bacterium *Bacillus licheniformis* [5,6].
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