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
Juan J. Aragón, Cristina Ferreras, Cristina Sánchez, Valentina Sánchez, Eloy D. Hernández, Carmen Hermida, Cristina Adan, Rafael Garesse, Oscar H. Martínez-Costa.