Pulmonary hypertension results in RV hypertrophy, fibrosis and impaired function resulting in RV failure. PH is associated with impaired heart metabolism and mitochondrial respiration. Mitochondrial supercomplexes (mSC) are assemblies of multiple electron transport chain (ETC) complexes that consist of physically associated complex I, III and IV to enhance respiration. We induced PH in rats by Sugen-Hypoxia (3 weeks) followed by normoxia (4 weeks). Control (n=11) and PH (n=10) were subjected to echocardiography, CN-PAGE to assess SC abundance and activity. RV function (PAT and TAPSE) was reduced in PH and there was significant RV hypertrophy. Activity (100+/- 3.6 vs 64.0+/-3.8 %, p<.01) and abundance (100+/-1.8 vs 49.3 +/-1.1, p=.02) of complex IV in mitochondrial SCs was severely reduced in PH animals compared to control. There were no differences in total CIV activity or abundance in smaller ETC assemblies (100 vs 97.5%, p=.78) TAPSE and RV Wall thickness significantly correlated with CIV SC activity (r=.71 and -.80 respectively P<.01). CIII abundance was lower in SCs (P=.01) but total CIII levels were unchanged. Overall levels of CI were unchanged. CI activity in SC assemblies was mildly reduced in PH but this was not significant (100+/-12 vs 77.4 +/-8.2, p=.14). There were no changes in CII activity or abundance in RV. When expressed as % total activity/complex, there was a small but significant shift in SC complex I activity in females (6) with PH but not males (4) (91% of non-PH for females vs 97% of non-PH for males, p=.02). There were no sex-dependent changes in RV supercomplexes in CIV activity with PH. In conclusion PH-induced RV dysfunction is associated with reduced assembly and activity of mitochondrial SCs with major disruption of CIV.

2458-Pos

Mitochondrial ROS signaling during synaptic potentiation

Irina Stavrovskaya[1], Erna Mitaishvilli[1,2], Bethany Kristi Morin[1], Aria Walls[2], Grace Terry[2], **Pablo M. Peixoto**[2,3].
[1]Research Foundation - CUNY, New York, NY, USA, [2]CUNY Graduate Center, New York, NY, USA, [3]Natural Sciences, Weissman School of Arts and Sciences, Baruch College - CUNY, New York, NY, USA.

Exacerbated emission of reactive oxygen species (ROS) from presynaptic mitochondria is a well-studied hallmark of several neurodegenerative diseases, including amyotrophic lateral sclerosis. Outside the context of pathology, the potential physiological role of mitochondrial ROS in presynaptic function and plasticity remains largely understudied. Here, we investigated this potential role by combining optogenetic techniques, electrophysiological recordings, confocal microscopy, and a well-established protocol for induction and measurement of synaptic potentiation in drosophila neuromuscular preparations. We observed an expected increase in spontaneous miniature excitatory junction potentials (mEJP), accompanied by a temporary increase in ROS emission seen by confocal imaging of presynaptic motor neuron mitochondria expressing roGFP2-Orp1. Furthermore, we were able to replicate this increase in mEJP frequency after optogenetic induction of ROS emission from pre-synaptic mitochondria expressing mito-killer red. These preliminary but exiting results may indicate a potential role of mitochondrial ROS signaling in synaptic potentiation. Further studies will inquire about this role, as well as the potential signaling targets of mitochondrial ROS in the presynaptic structure of drosophila neuromuscular junctions.

2459-Pos

Low intensity magnetic fields stimulate the electron transport chain in heart mitochondria

Shey-Shing Sheu[1], Gisela Beutner[2], Huoy-Jen Yuh[1], Ilan Goldenberg[3], Arthur Moss[3].
[1]Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA, [2]Pediatrics, University of Rochester, Rochester, NY, USA, [3]Medicine, University of Rochester, Rochester, NY, USA.

Recent studies suggested that low intensity magnetic fields (MF) exerted a stimulatory effect on the mitochondrial metabolic activity, when applied to the whole animals, organs, or single cells. However, the mechanisms and the ranges of MF strength for this stimulation are still unclear. Here we hypothesize that static low intensity MF can regulate mitochondrial electron transport chain activity as such enhance mitochondrial respiration. Nearly uniform low-strength magnetic fields were generated by Helmholtz coils, which were mounted into the water jacket of the measure chamber of an oxygraph. Application of 0-1.93 milli Tesla (mT) MF to mitochondria isolated from adult rat hearts produced a bell-shape increases in the respiratory control ratio that peaked at 0.50 mT and returned to baseline at 1.50 mT. The activity of the complexes 2, 3 and 5 but not 1 of the mitochondrial electron transport chain and several enzymes of the tricarboxylic acid cycle also showed a bell-shape increase after similar intensity of MF exposure. We further explained these results by applying the physical chemistry principles of magnetic Zeeman effect on radical pair redox in electron transport chain complexes. Taken together, our data show that low intensity MF can increase the mitochondrial respiratory activity via Zeeman splitting on the radical pair mechanism by a narrow range of small MF.

2460-Pos

In search for peptide therapeutics to reduce α-synuclein-induced mitochondrial dysfunction in Parkinson's disease

Megha Rajendran.
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA.

α-Synuclein, a neuronal protein implicated in Parkinson's disease (PD), is known to cause mitochondrial dysfunction. We have shown that α-synuclein binds to the outer mitochondrial membrane and reversibly blocks the Voltage-Dependent Anion Channel (VDAC) with nanomolar efficiency. Our results also suggest that α-synuclein uses VDAC as an entryway to mitochondria, where it can target specific enzymes of the electron transport chain. According to our proposed model, the N-terminal of α-synuclein binds to the lipid membrane followed by the capture of its disordered C-terminal tail by the VDAC pore, thus reducing the transport of ATP/ADP and other metabolites mediated by this channel. The binding of α-synuclein to the lipid membrane is the crucial step. Here we report the inhibitory effect of a small membrane-binding peptide (MBP) on the α-synuclein interaction with VDAC as a proof of principle. Using single-channel electrophysiology, we show that additions of MBP in micromolar concentrations to the same side of the membrane as α-synuclein results in a progressive reduction of the frequency of VDAC blockage events with IC_{50} of 30 μM. Using two independent methods of measuring protein-membrane interaction, bilayer overtone analysis, and fluorescence correlation spectroscopy, we found that MBP induces detachment of α-synuclein from lipid membranes with $k_d \sim 30$ μM, independently of the presence of VDAC. We further confirmed these in vitro results in HeLa cells treated with MBP linked to a cell-permeable peptide (CPP) using proximity ligation assay. The addition of 5 μM MBP-CPP to the cells decreased the accumulation of α-synuclein at the inner membrane of mitochondria. Our results demonstrate that it is possible to regulate α-synuclein blockage of and translocation through VDAC by rationally designed peptides suggesting potential therapeutics to reduce α-synuclein-induced mitochondrial toxicity in PD and other synucleinopathies.

2461-Pos

Cardiomyocyte-specific AMPK double-KO impairs mitochondrial function and performance at high workload

Malgorzata Tokarska-Schlattner[1], Laurence Kay[1], Pascale Perret[2], Raffaella Isola[3], Stéphane Attia[1], Frédéric Lamarche[1], Cindy Tellier[1], Cécile Cottet-Rousselle[1], Amjad Uneisi[1], Marc Foretz[4], Hervé Dubouchaud[1], Cathérine Ghezzi[2], Christian Zuppinger[5], Benoit Viollet[4], Uwe Schlattner[1,6].
[1]Laboratory of Fundamental and Applied Bioenergetics, Inserm U1055, Université Grenoble Alpes, Grenoble, France, [2]Radiopharmaceutiques Biocliniques, Université Grenoble Alpes & Inserm U1039, Grenoble, France, [3]Dipartimento di Scienze Biomediche, University of Cagliari, Monserrato, Italy, [4]Centre Cochin, Inserm U1016, CNRS UMR 8104 and Université Paris Descartes, Paris, France, [5]Klinik und Poliklinik für Kardiologie, Klinische Forschung, Inselspital Bern, Bern, Switzerland, [6]Institut Universitaire de France, Grenoble, France.

AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis under conditions of energy stress. Though heart is one of the most energy requiring organs and depends on a perfect match of energy supply with high and fluctuating energy demand to maintain its contractile performance, the role of AMPK in this organ is still not entirely clear, in particular in a non-pathological setting. Here we characterized cardiomyocyte-specific, inducible AMPK α1 and α2 knockout mice (KO), where KO was induced at the age of 8 weeks, and assessed their phenotype under physiological conditions. In the heart of KO mice, both AMPK α isoforms were strongly reduced and thus deleted in a large part of cardiomyocytes already two weeks after tamoxifen administration, persisting during the entire study period. AMPK KO had no effect on heart function at baseline, but alterations were observed under increased workload induced by dobutamine stress, consistent with lower endurance exercise capacity observed in KO animals. AMPKα deletion also induced a decrease in basal metabolic rate (oxygen uptake, energy expenditure) together with a trend to lower locomotor activity of AMPK KO mice 12 months after tamoxifen administration. Loss of AMPK resulted in multiple alterations of cardiac mitochondria: reduced respiration with complex I substrates as measured in isolated mitochondria, reduced activity of complexes I and IV, and a shift in mitochondrial cristae morphology from lamellar to mixed lamellar-tubular. A strong tendency to diminished ATP and glycogen level was observed in older animals, one year after tamoxifen administration. Our