1970-Pos

Mimicking Mitochondrial Cristae Dynamics and the Amyloid - beta (1- 42) Induced Failure of Mitochondrial Cristae. A Study Involving Model Lipid Membranes

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Number of studies showed, in the case of Alzheimer's disease, abnormalities in the oxidative metabolism of mitochondria, reduced ATP production, and, mitochondrial damage (broken *cristae*), which were related, amongst others, to the excessive presence of Amyloid-beta peptide in mitochondrial *cristae*. Surprisingly, the mechanisms relating the accumulation of Amyloid-beta in the *cristae* with the large number of mitochondria with broken *cristae* was even not evoked, never mind that it is widely recognized now that mitochondria function and morphology are coupled. In our previous work (Khalifat *et al*., 2008, Biophys J 95:4924), using giant unilamellar vesicles (GUVs) for modeling mitochondrial inner membrane, we offered some original insights into the factors that determine the dynamical tubular structures of the mitochondrial inner membrane *cristae*. Furthermore, we suggested a theoretical model (Fournier *et al*., 2009, Phys Rev Lett 102:018102) for elucidating the physical background of a particular membrane instability - membrane tubule formation, triggered by modulation of local pH. In the present work, using GUVs in a similar manner, we show directly (using video-microscopy) that Amyloid-beta might itself cause brutal rupturing of the model lipid membrane and make cristae-like morphology fail. Using large unilamellar vesicles we showed as well that the Amyloid-beta induces membrane dehydration and rise of membrane viscosity. Our hypothesis is: the failure of mitochondrial inner membrane morphology might be due to very basic and purely physical mechanism - the deterioration of mechanical (visco-elastic) properties of the lipid membrane. Thereby, the local strain created during *cristae* formation could provoke the inner membrane rupture. In other words, the Amyloid-beta (1-42) could induce the lipid bilayer incapacity to support the dynamics of shape changes underlying (and inherent to) mitochondrial inner membrane normal functioning.

1971-Pos

Mitochondria Structure and Function Matures During Mammalian Cardiac Development

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The adult heart requires a precise coupling between oxidative metabolism, excitation and contraction to provide sufficient energy for each heart beat. In contrast, the early embryo, due to the hypoxic environment in utero, generates energy mainly through anaerobic glycolysis. Although the heart is the first organ to become functional in the embryo, ensuring effective circulation and embryonic survival by the mid-embryonic period, little is known about mitochondria as the embryonic heart matures. To investigate the role of energetics and mitochondrial biogenesis during murine cardiac development, we examined mitochondrial structure and function in whole hearts and cultured myocytes harvested throughout the embryonic period. Primary culture of embryonic ventricular myocytes at embryonic day (E) 9.5 displayed less mitochondrial mass and mitochondria that were shorter in length and less organized, as they did not associate closely with the contractile apparatus and resided primarily around the nucleus and cell periphery. Compared to E9.5, E13.5 ventricular myocytes displayed greater mitochondrial mass, and mitochondria that were longer, branched, networked, and more closely associated with the contractile apparatus. Data from whole hearts using multiphoton and electron microscopy confirmed these findings. Functional measurements indicated that mitochondrial membrane potential was higher at E13.5 than at E9.5, suggesting higher mitochondrial activity at later stages of development. Taken together, these data suggest that mitochondrial biogenesis and function may be important in the differentiation of early cardiac myocytes and the maturation of the heart.

1972-Pos

Cyclophilin D Modulates Mitochondrial F_OF_1 ATP Synthase by Interacting with the Lateral Stalk of the Complex

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Blue-native gel electrophoresis purification and immunoprecipitation of F_OF_1 ATP synthase from bovine heart mitochondria revealed that cyclophilin (CyP) D associates to the complex. Treatment of intact mitochondria with the membrane-permeable bifunctional reagent dimethyl 3,3-dithiobis-propionimidate crosslinked CyPD with the lateral stalk of ATP synthase, while no interactions with F_1 sector subunits, the ATP synthase natural inhibitor protein IF1 and the ATP/ADP carrier were observed. The ATP synthase-CyPD interactions have functional consequences on enzyme catalysis, and are modulated by phosphate (increased CyPD binding and decreased enzyme activity) and cyclosporin (Cs) A (decreased CyPD binding and increased enzyme activity). Treatment of MgATP submitochondrial particles or intact mitochondria with CsA displaced CyPD from membranes, and activated both hydrolysis and synthesis of ATP sustained by the enzyme. No effect of CsA was detected in CyPD-null mitochondria, which displayed a higher specific activity of the ATP synthase than wild-type mitochondria. Modulation by CyPD binding appears to be independent of IF1, whose association to ATP synthase was not affected by CsA treatment. These findings demonstrate that CyPD association to the lateral stalk of ATP synthase modulates the activity of the complex.

1973-Pos

PINK1 Deficiency and Mitochondrial Dysfunction in Neurons and Skeletal Myocytes

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Mutations in the mitochondrial kinase PINK1 cause a recessive form of Parkinson's disease. Recent studies suggest that PINK1 is important for long term cell survival and mitochondrial function in midbrain neurons by regulating mitochondrial respiration, calcium homeostasis and oxidative stress. We used live fluorescence imaging to examine the effect of PINK1 deficiency on cell metabolism in primary midbrain neurons and skeletal myotubes. We found that basal mitochondrial membrane potential ($\Delta\psi m$) was decreased in PINK1 KO neurons (to $63.7 \pm 4.2\%$, $p<0.001$) compared to wild type (WT). In contrast, the $\Delta\psi m$ was increased by $98.7 \pm 40.5\%$ ($p<0.001$) in PINK1 KO myocytes compared to WT. Despite the difference in the level of $\Delta\psi m$, in both PINK1 KO neurons and myocytes, application of oligomycin induced mitochondrial depolarisation, suggesting that $\Delta\psi m$ is partially maintained by the hydrolysis of ATP by F_1F_0-ATPases, rather than solely by respiration. Using the luciferin/luciferase assay, we showed that the ATP level was 14.6 ± 2.3 ($p<0.05$) fold higher in the muscle compared to the midbrain, which may explain the selective vulnerability of PINK1 midbrain neurons to disease. Furthermore, the ATP level was 1.8 ± 0.1 ($p<0.05$) fold higher in PINK1 KO muscle compared to WT. We have also assessed the ATP metabolism in PINK1 KO neurons and myocytes, using an indirect measurement of ATP by Mag-fura and confirmed that both the PINK1 KO and WT myocytes exhibit more glycolytic activity than neurons. This accounts for the differences in $\Delta\psi m$ between neurons and myocytes, which ultimately contributes to alterations in calcium buffering and cell survival between different cell types. Our results demonstrate that PINK1 deficiency leads to impaired mitochondrial function not only in neurons but also in myocytes. Investigation of different responses in these tissues may lead to further understanding of PINK1 function and Parkinson's disease pathogenesis.

1974-Pos

Thermodynamic Analysis of Protein-Membrane Interactions: The case of Octameric Mitochondrial Creatine Kinase

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Mitochondrial creatine kinase (MtCK) is a key enzyme for bioenergetics, membrane topology and possibly also for general organelle morphology. X-ray structural analysis (1), EM (2) and mutational studies with SPR (3,4) revealed that the large MtCK octamers bind to and ''cross-link'' mitochondrial membranes by their two identical top or bottom faces (5). These expose expose four C-terminal basic interaction motifs that interact mainly with acidic cardiolipin (4). This interaction induces cardiolipin-rich domains in the membrane (5,6). However, earlier data point to additional hydrophobic interactions (7,8). Using SPR, we have performed a thermodynamic analysis of the MtCK binding process. Main results were: (i) Affinity of the MtCK-cardiolipin interaction increases with temperature, pointing to a participation of hydrophobic interactions. (ii) Rate constants of two MtCK binding sites identified earlier differed in temperature-dependence. (iii) Thermodynamic parameters revealed that the gain in free energy of MtCK binding mainly depends on the contribution of entropy, possibly due to charge neutralization and release of bound water. These data are consistent with a two-phase model of rapid electrostatic docking of MtCK to cardiolipin, and slower anchoring via a C-terminal hydrophobic MtCK stretch. This would reinforce MtCK membrane interaction, allow integration of this bulky enzyme into the narrow mitochondrial intermembrane space, and contribute to its functional coupling with adenine nucleotide translocator.