Mitochondria in Cell Life & Death

1946-Pos

Mechanisms Underlying Fructose-Induced Oxidative Stress in the Cytosol and Mitochondria

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High levels of dietary fructose are increasingly recognized as an important nutritional factor in the development of nonalcoholic fatty liver disease and intestinal inflammation in both humans and animal models. The signals linking excessive fructose intake on one hand and the onset of these pathologies are not known. We have employed genetically encoded biosensors sensitive to changes in superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2) concentrations to test the hypothesis that fructose metabolism increases the production of reactive oxygen species (ROS) in hepatocytes or intestinal cells. The data indicate that acute treatment with physiological concentrations of fructose (1 - 10 mM) significantly increased the formation of mitochondrial O_2^- as well as mitochondrial and cytosolic H_2O_2. The xanthine oxidase inhibitor, allopurinol, inhibited fructose-induced increases in cytosolic H_2O_2, but was unaffected by apocynin, a NADPH oxidase inhibitor. These data are consistent with the well known effects of acute fructose treatment to reduce ATP levels and stimulate the breakdown of purines. Fructose-induced ROS production in the mitochondria was not altered by allopurinol treatment, whereas apocynin strongly suppressed mitochondrial derived ROS. Fructose administration transiently increased ROS formation in all mitochondria regardless of the subcellular localization and this was paralleled by a sustained rise in mitochondrial membrane potential and an increase in pyridine nucleotide fluorescence. The activation of mitochondrial metabolism was followed by large amplitude O_2^- bursts in a subset of mitochondria. The addition of mitochondrial respiration inhibitors blocked the effects of fructose on mitochondrial, but not cytosolic ROS production. Taken together, these data indicate that fructose treatment stimulates mitochondrial metabolism leading to an increase in ROS production through an apocynin sensitive pathway. Finally, we show that chronic consumption of fructose results in markedly higher baseline levels of mitochondrial ROS in hepatocytes.

1947-Pos

Uncoupling of Mitochondrial Inner Membrane Potential has Opposite Effects on ROS balance in Heart Mitochondria in Situ Versus in Vitro

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The balance between reactive oxygen species (ROS) production and scavenging is determinant of cell survival and electrical and contractile recovery of the heart during ischemia-reperfusion. How ROS balance is affected by electron transport rate during oxidative phosphorylation and changes in mitochondrial membrane potential ($\Delta\Psi_m$) is still controversial. Here, we investigate the effect of $\Delta\Psi_m$ uncoupling on ROS balance in isolated mitochondria and in mitochondria in intact cardiomyocytes in forward electron transport mode. Opposite effects of the protonophore FCCP on ROS balance were observed when either intact cardiomyocytes or isolated mitochondria from guinea pig hearts were subjected to increasing (10-50nM) FCCP concentrations while monitoring mitochondrial $\Delta\Psi_m$, NADH, and ROS (O_2^-; and H_2O_2). Low FCCP concentrations increased both O_2^- and H_2O_2 in intact cardiomyocytes, whereas it decreased their levels in 5mM glutamate/malate energized isolated mitochondria. In cardiomyocytes imaged with two-photon laser scanning fluorescence microscopy, a faster accumulation of both O_2^- and H_2O_2 (indicated by MitoSOX or CM-DCF, respectively) was noted for FCCP concentrations up to 20nM: further increases of protonophore elicited either slower ROS production or hypercontracture and death. In isolated mitochondria, ROS levels decreased by $\approx$60% in parallel with $\Delta\Psi_m$ and NADH, at FCCP concentrations similar to those utilized in intact cells. In cells, the effects of FCCP on ROS balance could be prevented by preincubation with dithiothreitol, indicating that oxidation of the thiol pool was involved. In isolated mitochondria, the ROS signals were increased significantly with exogenous H_2O_2 exposure, or depleting the GSH pool with monochlorobimane. The findings highlight the important role played by the redox environment, particularly with respect to GSH levels, in determining the net effect on mitochondrial ROS balance in response to uncoupling oxidative phosphorylation.

1948-Pos

Mitochondrial Superoxide Flashes in Skeletal Muscle are Linked to Metabolism

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Reactive Oxygen Species (ROS) constitute important intracellular signalling molecules. Mitochondria are a well known source of ROS production, especially through the electron transport chain. In the present work, the Ca^{2+} biosensor mt-pericam, kindly provided by Pr Miyawaki (RIKEN, Saitama, Japan) was used as a mitochondria-targeted specific superoxide biosensor, by appropriate choice of the excitation wavelength. Mt-pericam was transfected *in vivo* into mouse *flexor digitorum brevis* adult muscles. Fibers were isolated and studied by confocal microscopy. Targeting of the biosensor was specific to the mitochondrial network with little, if any, cytosolic contamination. Fluorescence flashes were detected using excitation at 477 nm which was isosbestic for calcium. Flashes corresponded to superoxide production as shown from simultaneous records of either Mitosox (a mitochondrial superoxide probe) or Rhod-2 fluorescence signals. In addition, the superoxide scavenger Tiron decreased the flash frequency by 85%. On average, flashes have a 20 sec duration, and a F/F0 amplitude of 2. Flashes were recorded in subsarcolemmal, and intermyofibrillar mitochondria. Intermyofibrillar flashes presented three different spatial patterns: longitudinal, transversal or patch-shaped. Flash frequency was increased by application of glucose/pyruvate and decreased by inhibition of the electron transport chain with antimycin A. This is strong evidence for quantal superoxide production being intimately linked to mitochondrial metabolism. Superoxide flashes were also found to cause a decrease of the mitochondrial -potential and -free calcium, as shown by simultaneous measurements of TMRM and Rhod-2/X-Rhod-1, respectively. Together, my results show that superoxide flashes are a physiological phenomenon linked to mitochondrial metabolism that occurs in all subcellular populations of mitochondria in adult skeletal muscle fibers.

1949-Pos

Mitochondria Function in Arterial Smooth Muscle Cells

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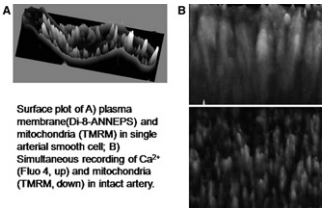
Mitochondria are organelles that play a central role in the cellular metabolic regulation of diverse cells including vascular smooth muscle cells. Reactive oxygen species (ROS) produced from mitochondria was reported to activate Ca^{2+} sparks in arterial smooth muscle cells, but the underlying mechanisms are still unclear. This work was designed to investigate the ROS production, mitochondria organization, and the interplay of cellular Ca^{2+} ($[Ca^{2+}]_i$) and mitochondrial Ca^{2+} ($[Ca^{2+}]_{mito}$) with the mitochondrial membrane potential, $\Delta\Psi_{mito}$. Tetra methyl rhodamine methyl ester (TMRM) was used to identify mitochondria in both single arterial smooth muscle cells and intact artery to track $\Delta\Psi_{mito}$, and Di-8-ANNEPS was used as a plasma membrane marker. ROS was monitored by transfecting cpYFP into intact cerebral arteries, and mitochondria Ca^{2+} was tracked using Rhod-2. The spatial organization of the arterial smooth muscle mitochondria in isolated cells and intact perfused arteries have been examined (Figure). In cannulated cerebral arteries, we have monitored the $\Delta\Psi_{mito}$ with TMRM and local and global Ca^{2+} with fluo 4 simultaneously (Figure). The interaction of ROS, $\Delta\Psi_{mito}$, $[Ca^{2+}]_i$ and $[Ca^{2+}]_{mito}$ will be discussed.



Surface plot of A) plasma membrane(Di-8-ANNEPS) and mitochondria (TMRM) in single arterial smooth cell; B) Simultaneous recording of Ca^{2+} (Fluo 4, up) and mitochondria (TMRM, down) in intact artery.

1950-Pos

Methylglyoxal Increases Mitochondrial Superoxide Production in Rat Colony-Forming Endothelial Progenitor Cells (cf-EPCs)

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Endothelial progenitor cells (EPC) play an important role in replenishing the vasculature and its inability will lead to endothelial dysfunction. It is known that endothelium becomes dysfunctional during diabetes and that production of reactive carbonyl species (RCS) increases. What remains undefined is the impact RCS have on the function of EPC. This study was designed to determine effects of the potent RCS methylglyoxal (MGO) on EPC function and viability. Colony-forming EPCs (cf-EPC) were isolated from whole blood of male Sprague-Dawley rats using Ficoll density gradient centrifugation and RT-PCR was used to confirm the presence of cf-EPC markers. After 7 days in culture, cf-EPC (10,000 cells per well) from controls were incubated with MGO for 24 hr at 37°C. Thereafter mitochondrial dehydrogenase activities were determined using the MTT assay. Cf-EPC were isolated from rats overexpressing glyoxalase 1 (AAV2/9-Glo1), the enzyme that degrades MGO. Changes in cytoplasmic Ca^{2+} and mitochondrial superoxide were determined using Fluo-3 and MitoSOX with confocal microscopy. Approximately 2-2.5 million cells were isolated per mL of rat blood. MGO induced a dose-dependent decrease in mitochondrial dehydrogenase activities in control cf-EPC. In cf-EPC from rats overexpressing glyoxalase 1, low concentrations of MGO (5-20 μM) enhanced mitochondrial dehydrogenase activities. Higher

concentrations (30-500 μM) inhibited this activity. Acute exposure of control cf-EPC to 100 μM MGO increased basal cytoplasmic Ca^{2+} and this was followed by an increased production of mitochondrial superoxide. These new data suggest that MGO whose production is increased shortly after the onset of hyperglycemia is inducing cf-EPC demise by mechanisms that involve perturbations in intracellular calcium homeostasis and increased production of mitochondrial superoxide. Overexpression of glyoxalase 1 minimizes the effects of MGO. This work was funded in part by NIH HL085061 and the Nebraska Redox Biology Center.

1951-Pos

Effect of Transient and Permanent Permeability Transition Pore Opening on NAD(P)H Localization in Intact Cells

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In order to study the effect of mitochondrial Permeability Transition Pore (PTP) opening on NAD(P)H localization, intact cells were exposed to the Ca2+ ionophore A23187. PTP opening, mitochondrial membrane potential, mitochondrial volume and NAD(P)H localization were assessed by time-lapse laser confocal microscopy using the calcein-cobalt technique, TMRM, MitoTracker and NAD(P)H autofluorescence respectively. Concomitant with PTP opening, NAD(P)H fluorescence increased outside mitochondria. These events occurred in all cells and were prevented by cyclosporin A. Mitochondrial membrane potential was not systematically collapsed while mitochondrial volume did not change, confirming that A23187 induced transient PTP opening in a subpopulation of cells, and suggesting that mitochondrial swelling did not immediately occur after PTP opening in intact cells. NAD(P)H autofluorescence remained elevated after PTP opening, particularly after membrane potential had been collapsed by an uncoupler. Extraction of nucleotide for NAD(P)H quantification confirmed that PTP opening led to an increase in NAD(P)H content. Because the oxygen consumption rate decreased while the lactate/pyruvate ratio increased after PTP opening in intact cells, we conclude that PTP opening inhibits respiration and dramatically affects the cytosolic redox potential in intact cells.

1952-Pos

mtDNA T8993G-Augmented Mitochondrial Stresses Upon Mca^{2+} Overload and its Protection by Melatonin in a Narp Cybrid

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Mitochondrial DNA (mtDNA) T8993G mutation inhibits specifically mitochondrial F1F0-ATPase (complex V) for severe ATP deficiency and is clinically associated with neurological muscle weakness, ataxia, and retinitis pigmentosa so called NARP mutation. At present, detail T8993G-associated mitochondrial mechanisms as well as its therapeutic strategies are limited. Using time-lapse laser scanning dual fluorescence imaging microscopy, this study investigated T8993G-altered apoptotic mitochondrial pathology particular upon mCa2+ stress and protection by melatonin, previously reported to protect mCa2+ stress-mediated apoptosis (Hsu et al., 2009 JPR in press). In comparison to its parental osteosarcoma 143B and mtDNA less (ρ^0) cells, T8993G induced significant hyperpolarization of mitochondrial membrane potential ($\Delta\Psi m$) and potentiated greatly ionomycin-induced mCa2+ stress. T8993G-augmented mCa2+ stress subsequently elicited rigorously generation of mitochondrial oxygen species (mROS), depletion of cardiolipin (CL) and activation of the mitochondrial permeability transition (MPT). In contract, ρ^0 cells, with much depolarized $\Delta\Psi m$, suffered less mCa2+ stress, mROS formation, CL depletion and the MPT opening. Interestingly, melatonin reduced significantly peak amplitude of the ionomycin-induced mCa2+ transient and antagonized efficiently mCa2+-augmented mROS generation for a reduced depletion of CL and activation of the MPT. In addition, melatonin prevented ''oxidation free mCa2+''-mediated MPT suggesting its direct targeting on the MPT. Melatonin-enhanced tail amplitude of mCa2+ transient possibly due to the reduced MPT-dependent depolarization of $\Delta\Psi m$, however, did not enhance mCa2+ stress-mediated pathology in NARP cybrids possibly as melatonin-elevated mCa2+ improved mitochondrial respiratory. Thus, the administration of melatonin may provide potential improvement for the treatment of mtDNA T8993G-associated NARP syndromes and diseases.

1953-Pos

Visualization of Melatonin's Multiple Mitochondrial Levels of Protection Against Mitochondrial $Ca2^{+}$-Mediated Permeability Transition and Beyond in Rat Brain Astrocytes

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Melatonin protects cells against oxidative stress-induced apoptosis due primarily to its ability to effectively scavenge pathological condition-augmented generation of mitochondrial reactive oxygen species (mROS). Once produced, mROS in addition to indiscriminately damage mitochondrial components they crucially activate directly the mitochondrial permeability transition (MPT), one of the critical mechanisms for initiating post mitochondrial apoptotic signaling. Whether or not melatonin targets directly the MPT, however, remains inconclusive, particularly during oxidative stress. Thus, we investigated this possibility of an ''oxidation free Ca^{2+} stress'' in the presence of vitamin E after ionomycin exposure as a sole Ca^{2+}-mediated MPT in order to exclude melatonin's primary antioxidative effects as well as Ca^{2+}-mediated oxidative stress. With the application of laser scanning fluorescence imaging microscopy, we visualized for the first time multiple mitochondrial protections provided by melatonin during Ca^{2+} stress in cultured rat brain astrocytes RBA-1. Melatonin, due to its primary antioxidative actions, completely prevented mCa^{2+}-induced mROS formation for a reduced mROS-activated MPT during ionomycin exposure. In the presence of vitamin E, melatonin, significantly reduced cyclosporin A (CsA) sensitive mitochondrial depolarization and MPT during ionomycin exposure suggesting its direct targeting of the MPT. Moreover, when the MPT was inhibited by CsA, melatonin reduced further MPT-independent mitochondrial depolarization and apoptosis suggesting its targeting beyond the MPT. As astrocytes play active role in regulating neuronal pathophysiology, these multiple mitochondrial protections provided by melatonin against mCa^{2+}- and/or mROS-mediated apoptosis may thus be crucial for the future therapeutic prevention and treatment of astrocyte-mediated neurodegeneration in the CNS.

1954-Pos

High-Frequency Photoconductive Stimulation Reveals Central Role of Mitochondrial Permeability Transition Pore in Activity-Driven Neuronal Cell Death

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Loss of the ability to regulate calcium is a central event leading to neuronal cell death during a wide range of pathological conditions including stroke and seizure. Here we present a new dissociated hippocampal cell culture model of acute electrical activity which incorporates the photoconductive stimulation of neuronal networks grown on silicon wafers. This technology allows precise modeling of user defined neuronal activity patterns, and the study of their effect on neuronal physiology. Here, seizure-like conditions were created by continuous stimulation, causing hundreds of neurons to fire synchronously at 50 Hz for 4 minutes. This stimulation protocol induced cell death as monitored by propidium iodide staining. The number of dead cells per stimulation region increased from 3.6 ± 2.1 preceding stimulation to 81 ± 21 30 minutes following stimulation. Excitotoxicity primarily affected excitatory rather than inhibitory neurons, and was preceded by an increase in intracellular calcium as well as changes in the mitochondrial morphology and membrane potential as measured by a tetramethylrhodamine methyl ester (TMRM) assay. Cyclosporin A (CsA), a mitochondrial permeability transition pore (PTP) blocker, was effective in preventing cell death. We propose that photoconductive stimulation is a useful tool for investigating the pathogenesis of excitotoxicity in vitro.

1955-Pos

Aging Results in Downregulation of Putative Components of mPTP in Human Atria

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Cardiac vulnerability to injury is increased with aging and is associated with enhanced susceptibility to opening of mitochondrial permeability transition pore (mPTP), a nonspecific high conductance channel in the inner mitochondrial membrane, however the basis for this is not fully understood. The effect of aging on the expression of putative components of mPTP in human myocardium was determined in atrial tissue obtained from elderly (76 ± 6 yrs) and adult (49 ± 5 yrs) patients undergoing coronary artery bypass surgery using microarray, Quantitative RT-PCR and Western blot. Aging was associated with a significant reduction in the expression of genes coding for the voltage-dependent anion channel isoforms, *VDAC1, VDAC2* and *VDAC3* ,adenine nucleotide translocase (ANT) and Cyclophilin-D (*PPID*) in atria from the elderly patients (Fig A, $p<0.01$). The expression of