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