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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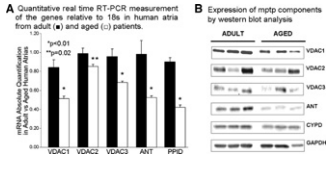
Cardiac vulnerability to injury is increased with aging and is associated with enhanced susceptibility to opening of mitochondrial permeability transition pore (mPTP), a nonspecific high conductance channel in the inner mitochondrial membrane, however the basis for this is not fully understood. The effect 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



VDAC1 and ANT proteins was significantly reduced ($p<0.05$), while VDAC2, VDAC3 and cyclophillin D were not significantly altered at protein level (Fig B). Downregulation of VDAC1 and ANT expression in the aging human heart may underlie the increased predisposition of the atria to injury during stress.

1956-Pos

Ranolazine Reduces Mitochondrial Tyrosine Nitration During Cardiac Ischemia and Reperfusion Injury

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Excess superoxide ($O_2^{\bullet-}$) and nitric oxide ($NO^{\bullet}$) generate peroxynitrite ($OONO^-$) during cardiac ischemia-reperfusion (IR) injury. $NO^{\bullet}$ alone may be cardioprotective whereas $OONO^-$ has deleterious effects. Tyrosine nitration by $OONO^-$ may lead to dysfunctional mitochondrial proteins. Ranolazine (RAN), a slow Na^+ channel blocker and anti-ischemic drug, may also attenuate mitochondrial complex I respiratory activity. We tested if the tyrosine nitration of mitochondrial proteins that occurred during IR was reduced when RAN was given just before ischemia. **Method:** Guinea pig hearts were perfused with Krebs -Ringer solution and subjected to one of six treatments: (i) control (no ischemia), (ii) 30 min global ischemia alone, (iii) 30 min ischemia + 10 min reperfusion, (iv) ischemia reperfusion plus RAN given for 10 min before, but not during ischemia, (v) ischemia plus RAN (no reperfusion), (vi) RAN control perfusion (no ischemia). Mitochondria were isolated immediately after each treatment. Tyrosine nitration was measured by Western blotting using 3nitro-tyrosine (3-NT) antibody. **Result:** RAN markedly improved cardiac function. Two bands positioned at about 25 kDa and 15 kDa were 3-NT immunopositive in all experiment groups. Compared to the control, mitochondria after ischemia reperfusion displayed increased 3-NT immunopositivity at the 25 kDa and 15 kDa positions by approximately 100% and 28%, respectively. Treating hearts with RAN before ischemia reperfusion decreased the 3-NT immunopositive 25 kDa band density to non-ischemia levels and the 15 kDa band density to 10% of the ischemia reperfusion alone level. The nitrated proteins require further identification. **Conclusion:** Cardiac injury increases the tyrosine nitration of selected mitochondrial proteins. Inhibition of complex I may underlie the cardiac injury-induced increase in mitochondrial protein tyrosine nitration. This reduction in mitochondrial protein nitration may correlate with the improved cardiac function we observed previously with RAN.

1957-Pos

Modulation of the Mitochondrial Permeability Transition Pore of Cardiac Myocytes by Inorganic Polyphosphate

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Background: Inorganic polyphosphate (polyP) is a long polymer made of up to several hundred orthophosphates linked together by phosphoanhydride bonds.Previously we found that polyP of rat liver mitochondria participates in formation of a channel with properties similar to the mitochondrial permeability transition pore (mPTP) suggesting a possible role in pathophysiology. The aim of this study was to investigate the role of polyP in the regulation of mitochondrial Ca homeostasis and Ca-induced opening of mPTP in cardiac myocytes. **Methods:** We used primary cultures of adult rabbit ventricular myocytes with enzymatically reduced levels of mitochondrial polyP achieved by adenoviral expression of polyP hydrolyzing enzyme from yeast (scPPX). Cytosolic Ca ($[Ca]_i$), mitochondrial Ca ($[Ca]_m$), mitochondrial membrane potential, and mPTP activity were measured using the fluorescent dyes indo-1, rhod-2, TMRM, or calcein red, respectively. **Results:** 1) No difference was detected in amplitude, rise and decay time of $[Ca]_i$ transients induced by electrical field stimulation (1 Hz) in control and scPPX expressing intact myocytes. 2) In permeabilized cells under conditions of mitochondrial Ca overload, mitochondrial Ca uptake in control cells was followed by fast Ca release which was prevented by the mPTP inhibitor cyclosporine A. The rate of mitochondrial Ca release was significantly slower in scPPX cells. 3) Similar levels of basal mitochondrial membrane potential were observed in both cell types, however Ca-induced mitochondrial membrane depolarization was more pronounced in control cells. 4) Mitochondria of permeabilized myocytes expressing scPPX were less sensitive to Ca-induced mPTP opening as estimated by the kinetics of calcein red release and the degree of Ca-induced mitochondrial membrane depolarization. **Conclusion:** Our data indicate that reducing of the mitochondrial polyP levels decreases Ca-induced opening of the mPTP in cardiac myocytes.

1958-Pos

Hydroxide Ion Channel Controls Uncoupling and Thermogenesis of Brown Fat Mitochondria

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Uncoupling proteins (UCP1- UCP5) are six-transmembrane-domain transport proteins of the inner mitochondrial membrane (IMM). They increase electrical conductance of the IMM, thus dissipating the electrochemical proton gradient across this membrane and uncoupling mitochondrial respiration and ATP synthesis. By controlling mitochondrial membrane potential, UCPs can affect many aspects of mitochondrial function and have been implicated in regulation of body's energy efficiency, reducing fat depositions, thermogenesis, diabetes, and protecting the cell against oxidative damage and ageing. The founding member of the family, UCP1, is specifically expressed in brown adipose tissue (BAT) and is responsible for adaptive thermogenesis mediated by this tissue. Due to its unusually high level of expression, upon activation UCP1 completely uncouples BAT mitochondria and converts the energy of the substrate oxidation into heat. Since UCP1 can dissipate large amounts of energy, it has attracted attention as a potential target to treat obesity. In spite of their physiological and therapeutic significance, the mechanism of operation of uncoupling proteins including their ionic selectivity has long remained unknown due to the lack of direct methods to study their activity in their native membrane environment. Here, by applying the patch-clamp technique to the whole inner membrane of BAT mitochondria and for the first time directly measuring transmembrane currents produced by UCP1, we show that UCP1 is a ligand-gated hydroxide (OH-) ion channel activated by fatty acids. UCP1 is the only hydroxide ion channel reported to date. Thus, BAT thermogenesis involves the outward transport of protons by the electron transport chain along with the outward transport of OH- by UCP1, thereby amounting to cycling of water across the IMM and not to futile cycling of protons as was largely considered before.

1959-Pos

Characterization of an Anion Channel on the Inner Membrane of Heart Mitochondria

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Preconditioning is a powerful form of cardioprotection whereby a brief ischemic episode, or a brief exposure to drugs such as volatile anesthetics, can protect the myocardium from a subsequent prolonged ischemia. It triggers an intracellular signaling cascade that leads to the delay in the opening of the mitochondrial permeability transition pore (mPTP). Depolarization of the inner membrane of mitochondria (IMM) can delay mPTP opening. Ion channels have been identified on the IMM that may play key roles in this depolarization. Yet their molecular identities and detailed electrophysiological characterizations have been elusive. In the present study, we recorded ion channel activities on the IMM isolated from guinea pig hearts. Mitoplasts (mitochondria sans the outer membrane) were formed by incubating mitochondria in a hypotonic buffer. The inside-out configuration of the patch clamp technique was utilized. We have identified a channel with a primary conductance of 109 ± 5 pS (n=9) in equimolar 150 mM KCl. The channel exhibited voltage-dependent behavior, with activity being more prominent at positive membrane potentials. When the 150 mM KCl bath solution that corresponded to the mitochondrial matrix side was replaced with 150 mM K-glutamate, channel activity was abolished. When TEA-Cl substituted for KCl, channel activity was not significantly affected. These results suggested an anion channel permeable to chloride. This was confirmed by DIDS (100 μM), a chloride channel blocker, which abolished channel activity. However, bongkrekic acid (100 nM), a specific inhibitor of the mitochondrial adenine nucleotide translocase, failed to inhibit channel activity. In addition, the presence of 2 mM Mg2+ in the buffer solution, a concentration that blocks IMAC, the inner membrane anion channel, did not prevent channel opening. Experiments are currently underway to further characterize and identify this anion channel on the IMM.

1960-Pos

Upregulation Leads Bcl2 to Behave as a Mitochondrial Decoy Receptor for Bax

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Cytochrome c release, the commitment step of apoptosis, is regulated at the mitochondria through protein-protein interactions between the Bcl2 family proteins. An imbalance of this interaction network due to the upregulation of the proto-oncogene *Bcl2* leads to a resistance to apoptosis and is associated with tumor formation. Bcl2 overexpression inhibits BAX oligomerization and mitochondrial outer membrane (MOM) permeabilization. However, the molecular