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