803-Pos Board B589

Oxidized Heme - A Novel Inhibitor of Calcium-Dependent BK Channel in Rat Brain Mitochondria

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Heme is a prosthetic group that consists of an iron atom bound in the center of a porphyrin ring. It is an essential element of hemeproteins in all living organisms. Although heme is ubiquitous, its circulation is strictly controlled. Therefore it is believed that it may play as yet unknown regulatory functions.

The mitochondrial calcium-dependent BK channel (mitoBKCa) is one of the five known channels that contribute to potassium permeability of mitochondrial inner membrane. It is activated by calcium and voltage and inhibited by scorpion venom toxins such as charybdotoxin and iberiotoxin.

In the current study, we have checked the impact of the oxidized heme (hemin) on mitochondrial membrane potential and respiration rate of rat brain mitochondria. We have shown that hemin prevents the collapse of membrane potential that is normally caused by calcium-dependent BK channel openers (NS1619). A similar, though modest effect was observed in studies of oxygen consumption rate. We also report inhibitory effects of hemin on the reactive oxygen species-downregulating protperties of NS1619.

Additionally, we have studied the single channel activity of mitoBKCa by patch-clamp of mitoplasts isolated from a rat astrocyte cell line. The results that we have obtained confirm the phenomonon of reversible inhibition of mitoBKCa channel by hemin.

Our findings support the hypothesis that oxidized heme can inhibit the mitochonbdrial calcium-dependent BK channel.

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804-Pos Board B590

GSK3β Transfers Cytoprotective Signaling through Connexin 43 onto Mitochondrial ATP-Sensitive K^+ Channels

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Background: Ischemic preconditioning involves activation of signaling pathways, including activation of protein kinase C (PKC) and inhibition of glycogen synthase kinase 3beta (GSK3β). Opening of mitochondrial ATP-sensitive K^+ (mitoK_{ATP}) channels has been proposed to play a critical role in ischemic preconditioning. Recently, we demonstrated that connexin 43 (Cx43) found in the inner mitochondrial membrane, is essential for cytoprotective signal transduction onto mitoK_{ATP} channels.

Objectives: To dissect signal transduction targeted at mitoK_{ATP} channels and to possibly identify a functional link between mitoK_{ATP} channel activation, mitochondrial Cx43 and GSK3β activity.

Methods: We used direct single-channel patch-clamp recordings of cardiac mitoplasts from C57/Bl6J mice and homozygous GSK3β-S9A mice with cardiac-specific expression of a constitutively active form of GSK3β, and performed Western blots and co-immunoprecipitation of isolated mitochondria.

Results: MitoK_{ATP} channel activity was stimulated by the GSK3β small molecule inhibitor SB216763, which increased the open probability compared to control. The Cx43 inhibitor carbenoxolone and the Cx43 mimetic peptide ^{43}GAP27 significantly reduced mitoK_{ATP} channel activation by SB216763, supporting the notion that GSK3 transfers cytoprotective signaling via Cx43 onto mitoK_{ATP} channels. In GSK3β-S9A mitoplasts mitoK_{ATP} currents could be recorded with similar single-channel properties as in wildtype mitochondria. Single-channel activation of mitoK_{ATP} by the PKC activator PMA was significantly attenuated in GSK3β-S9A mitoplasts. Western blot analysis revealed reduced phosphorylation of mitochondrial Cx43 at the PKC phosphorylation site Ser368 upon PMA application in mitochondria from GSK3β-S9A mice versus wildtype. Furthermore Immunoprecipitation of Cx43 from isolated mitochondria revealed a signal for GSK3β, while immunoprecipitation of GSK3β also showed a signal for Cx43, indicating an association of these proteins.

Conclusions: PKC transmits a signal onto mitoK_{ATP} channels through GSK3β. This GSK3β signal transduction on mitoK_{ATP} channels is transferred via Cx43 within a multiprotein signaling module.

805-Pos Board B591

Two Voltage-Gated Calcium Channels Regulate Calcium Uptake in Murine Cardiac Mitochondria

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Mitochondrial Ca2+ handling controls the rate of mitochondrial energy (ATP) production, modulates the spatial and temporal profile of intracellular Ca2+ signaling, regulates mitochondrial ROS generation, and may trigger cell death. Mitochondrial Ca2+ uptake is thought to be mediated by the Ca2+ uniporter (MCU) and other non specific Ca2+ pathways. Recently, a MCU and a non-MCU-type mitochondrial Ca2+ uptake channel were documented in human myocardium. However, existence and electrophysiological properties of calcium channels in murine cardiac mitochondria remain unclear.

We isolated cardiac mitoplasts (mitochondria lacking the outer membrane) of mice and performed mitoplast-attached single-channel recordings. By patch-clamping the inner membrane of these cardiac mitochondria we identified two different murine voltage-gated Ca2+ channels, i.e. mCa1 and mCa2. Both channels differed in electrophysiological gating parameters. In the presence of Ca2+ 105 mM in the pipette solution the unitary single-channel amplitude of mCa1 (-1.16 ± 0.03 pA, at -100 mV) was higher than the amplitude of mCa2 (-0.90 ± 0.03 pA). mCa1 showed 3-5 subconductance levels, while mCa2 had only one open state. Moreover, the open probability of mCa1 (0.35 ± 0.05 %) was significantly lower compared to mCa2 (0.97 ± 0.17 %). Both channels could be inhibited by high concentrations of Ruthenium 360 (10 μM), while they were insensitive to blockers of other possibly Ca2+-conducting mitochondrial pores. Single-channel properties suggest that mCa1 underlies the murine MCU. Besides the classical MCU, mitochondrial Ca2+ uptake in mouse heart is mediated via a second voltage-gated Ca2+ channel (mCa2) with distinct properties.

806-Pos Board B592

Bax Initiates the Assembly of a Multimeric Catalyst that Facilitates Pore Formation in Mitochondrial Outer Membranes

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In vertebrates, apoptotic cell death involves a canonical ''intrinsic'' apoptotic pathway that depends on mitochondrial outer membrane permeabilization (MOMP). MOMP is mediated by the pro-apoptotic Bcl-2 family proteins Bax and/or Bak. Previous studies have modeled Bax-dependent membrane pore formation in simple in vitro systems consisting of protein-free synthetic liposomes mixed with recombinant Bax and ''direct activator'' BH3-only proteins, e.g. cBid. However, it has been unclear whether liposome systems accurately reflect the physiological events in mitochondria. To determine whether Bax requires the assistance of other membrane proteins to form pores, we analyzed the kinetics of MOMP using isolated mitochondrial outer membranes incubated with recombinant Bax, along with cBid. Compared with liposomes, native membranes were much more sensitive to Bax and displayed more complex permeabilization kinetics. Heat-labile outer membrane proteins were required for this enhanced response. A two-tiered mathematical model closely fit the kinetic data: first, Bax activation promotes the assembly of a multimeric complex, which then serves as catalyst for the second reaction, pore formation. The kinetics was affected in a reciprocal manner by [cBid] and [Bax], indicating that cBid-induced Bax activation is governed by kinetic mass action law, consistently with the ''hit-and-run'' mechanism. Strikingly, MOMP rate constants were linearly related to [Bax], implying lack of Bax cooperativity. Thus, contrary to popular assumption, pore formation kinetics does not depend on Bax oligomerization. Moreover, our data show that assembly of the catalyst complex depends on membrane fluidity and is blocked by chemical inhibitors of Drp1-dependent mitochondrial fission. However, Drp1 itself was undetectable in mitochondrial outer membranes. Thus, the data suggest that a noncanonical Drp1-like activity facilitates Bax-induced MOMP, possibly through a membrane-remodeling event.

807-Pos Board B593

AFM Study of Structure of Mitochondrial Membranes in Necrosis and Apoptosis

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The ability of mitochondria to potentiate stress-induced necrotic and apoptotic cell death is directly linked to structural changes in inner and/or outer