may be particularly useful in the eradication of malignant tumors for cancer therapy.

2730-Pos Board B700

Direct Effect of Isoflurane on Mitochondrial pH

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Introduction: A decrease in mitochondrial pH (pH_m) inhibits mitochondrial permeability transition pore (mPTP) and has been associated with cardioprotection. The volatile anesthetic isoflurane decreases mitochondrial membrane potential ($\Delta\Psi_m$) and inhibits respiratory complex I. However, the effect of isoflurane on pH_m is unknown. We hypothesized that exposure to isoflurane lowers pH_m in cardiomyocytes and isolated mitochondria. **Methods:** The direct effect of 0.5 mM isoflurane (1 MAC) was tested in cardiomyocytes and mitochondria isolated from adult male Wistar rats. We used fluorescence dyes SNARF-1 and BCECF to measure pH_m in myocytes with a confocal microscope and in mitochondria with a spectrofluorometer, respectively. In myocytes, after baseline recording, cells were superfused with isoflurane for 5 min. In mitochondria, isoflurane was added to mitochondria in the presence of pyruvate/malate (5 mM) and ADP (250 μM ADP). Respiration was recorded using a Clark-type electrode with pyruvate/malat as substrate. **Results:** Exposure of myocytes to isoflurane decreased pH_m 0.09 ± 0.03 pH units ($P<0.05$, n=5). In mitochondria, isoflurane induced decrease in pH_m was 8 ± 2% ($P<0.05$, n=5) of maximal acidification induced by mitochondrial uncoupler FCCP (4 μM). This effect was mimicked when mitochondria were exposed to rotenone (5 μM). Isoflurane inhibited pyruvat/malat-dependent oxygen consumption in the presence and absence of ADP. **Conclusion:** These results suggest that isoflurane decreases pH_m through inhibition of complex I of the electron transport chain. Isoflurane-induced acidification may contribute to the immediate protective effect provided by volatile anesthetics when applied at the onset of cardiac reperfusion after an ischemic event.

2731-Pos Board B701

Heart Ischemia: The Transition from Reversible to Irreversible Myocardial Ischemia is Governed by the Mitochondrial Permeability Transition Pore (mPTP)

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The aim of this study was to evaluate the role of mPTP in the passage from reversible to irreversible injury as function of ischemia duration followed by reperfusion. Rat hearts (n=72) were perfused with the Langendorff technique and subjected to global ischemia during 0 (sham), 10, 20, 30, 40 and 60 min at 37°C, followed by 60min reperfusion. Infarct size was evaluated by triphenyltetrazolium chloride (TTC) staining, and creatinine kinase (CK) and lactate dehydrogenase (LDH) release. Normal heart function and recovery was assessed by Rate-Pressure Product (RPP). Mitochondria function was evaluated by Ca2+ Resistance Capacity (CRC) and mPTP installation. A transition from reversible to irreversible ischemia occurred after a period of 20-30min of ischemia. The functional recovery depends on the duration of the ischemia: 10-20min ischemia RPP (10min 31264 ± 1341, 20min 28578 ± 2176 mmHg/beats/min) had a better functional recovery than longer ischemic periods that displayed dramatic RPP decrease (30min 12183 ± 1895, 40 min 7411 ± 578 and 60min 4916 ± 698 mmHg/min; $p<0.001$ vs. 20 min. ischemia). Ischemia <20 min did not elicit infarct as evaluated by TTC-staining, and CK and LDH-release. In line with this observation, CRC was significantly reduced for ischemia longer than 20 min ($p<0.001$). This study shows a parallel evolution between mitochondrial CRC and the appearance of irreversible damage in the ischemia-reperfused heart. (Values, Mean+SE).

2732-Pos Board B702

Bax C-Terminal Peptide - Insights Into Membrane Interactions

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Multi-cellular organisms eliminate unnecessary or defective cells through a process known as apoptosis. This tightly regulated series of events, leading to the concise shutting down and packaging up of the cellular machinery, is aptly coined ''programmed cell death''. The core apoptotic machinery is composed of members of the Bcl-2 family of proteins. Each protein has a specific yet apparently redundant function, as single pro-apoptotic protein deletions do not render the whole system non-functional. The members act synergistically as initiators, effectors and antagonists of apoptosis. The commitment to self-destruct hinges on Bax, the proverbial ''final straw'', facilitating the abrupt release of mitochondrial matrix proteins, setting off an irreversible avalanche of biochemical events including proteolysis and nuclear fragmentation. Though a wealth of data exists on the apoptotic process in general and the Bcl2 family in particular, the precise mechanism by which Bax interacts with and disrupts the structural integrity of the mitochondrial membrane remains elusive. Structural studies infer that the pro-apoptotic function of Bax is mediated by the insertion of the C-terminal helix as well as helices α5-α6 into the mitochondrial membrane. Conversely, the NMR structure of monomeric Bax clearly shows the hydrophobic α5-α6 helices are completely sequestered within the protein; furthermore, the putative regulatory domain is constrained by the presence of the C-terminal helix tucked solidly into its hydrophobic groove, implicating the need for a major conformational change for those interactions to occur. In order to clarify the mechanism by which Bax interacts with the mitochondrial membrane, we have measured the binding affinity, orientation, and depth of insertion of synthetic peptides, corresponding to the last 25 residues of the Bax C-terminus, to artificial mitochondrial membranes by circular dichroism (CD), resonance energy transfer (RET), fluorescence quenching and attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR).

2733-Pos Board B703

PKA Inhibited The Opening Of Mitochondrial Permeability Tansiton Pore Induced By Cytosolic GSK3β

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It has been reported that cardioprotective intracellular signalings converge on glycogen synthase kinase3β (GSK3β) and that inactivated form of GSK3β (due to the phosphorylation of Ser9 induced by kinases) inhibited the mitochondrial permeability transition pore (mPTP), a key regulator of apoptosis. Here, we hypothesized that PKA could modulate the activities of GSK3β and consequently alters mitochondrial function. To test this, we investigated (1) the opening of the mPTP (measured with fluorescent calcein), and (2) mitochondrial membrane potential (measured with TMRE) in saponin-permeabilized rat cardiomyocyte with a laser scanning confocal microscopy. Our results demonstrated that (1) Active (non-phosporylated) form of GSK3β (10 nM) accelerated calcein leakage from the mitochondria (by 82.4 ± 1.0% of the control, $p<0.01$), and this effect was blocked by CsA (an inhibitor of mPTP: 100 nM) (by 92.3 ± 1.5%, $p<0.01$). (2) SB216763 (an inhibitor of GSK3β: 3 mM) inhibited the opening of mPTP induced by active-GSK3 b (by 93.0 ± 0.9%, $p<0.01$). (3) GSK3β depolarized inner membrane potential (to 63.3 ± 7.3% of the control, $p<0.05$) and this effect was inhibited by CsA (95.9 ± 4.8%, $p<0.01$). (4) PKA catalytic subunit (PKA-cat; 10 U/ml) inhibited both the calcein leakage and membrane potential depolarization induced by active-GSK3β (by 93.6 ± 0.6%, $p<0.01$ and 93.1 ± 1.1%, $p<0.01$, respectively). From these results, we concluded that active form of GSK3β opened mPTP and depolarized inner membrane potential and that these effects were inhibited by the inactivation of GSK3β with PKA.

2734-Pos Board B704

Flash Sniper: Automated Detection and Analysis of Mitochondrial Superoxide Flash

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Mitochondrial superoxide flash is a newly discovered physiological phenomenon reflecting elemental and bursting superoxide production in mitochondria of diverse cells in culture and in vivo (Wang et al, *Cell*, **132**, 279).The discovery of superoxide flash opens a unique window to glean into the mitochondrial ROS signaling and its coupling with energy metabolism,cell fate regulation, and oxidative stress-related diseases. Because of the low frequency and long duration (~20s) of superoxide flash, time-lapse confocal imaging (*xyt*) was employed for data acquisition, resulting in huge data sets. For objective, reproducible and efficient flash identification and measurement, here we develop, validate and implement an automated detection algorithm as well as a software, *Flash Sniper*, which fulfills three basic functions – interactive data priming, flash identification, and flash parameter measurement. The data priming module consists of

correction for photobleach and photoconversion using nonlinear fitting, cell motion compensation based on hierarchical block matching, and spatiotemporal filtering. Two *xy* maps are generated to represent the maxima of fluorescent intensity change and the corresponding time, respectively. Flash events are identified via thresholding and segmentation based on these maps. Validation of the algorithm is performed on SNR-controlled synthetic data sets produced by embedding typical flash events in real images. With the aid of Flash Sniper, we examined ~1600 flashes in cardiac myocytes and revealed distinct substructures in their rising kinetics. In sum, the analytical tool developed in this study will facilitate the studies of the mechanisms of superoxide flash and its application in health and diseases.

2735-Pos Board B705

Design Of An ELISA Protocol For A Rapid Quantification Of Activated/oligomerized Bax During Apoptosis

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The release of cytochrome c from mitochondria into the cytosol is considered to be the commitment step of early apoptosis. During this step, the activation/oligomerization of the pro-apoptotic protein Bax leads to the formation of the Mitochondrial Apoptosis-Induced Channel (MAC) through which cytochrome can be released. Remarkably, Bax activation/oligomerization leads to the exposure of the previously inaccessible N-terminal domain of the pro-apoptotic protein Bax. This property was extensively used to detect activated/oligomerized Bax by immunocytochemistry and immunoprecipitation but these assays are long and tedious; and they are only qualitative or semi-quantitative at best.

During this study, we developed an ELISA protocol based on the utilization of monoclonal (6A7) and polyclonal (N-20) antibodies which specifically recognize Bax N-terminus (i.e. the activated/oligomerized form of Bax). Monomeric human recombinant Bax (hBax) was used in all the experiments. As previously described, activation/oligomerization of hBax was artificially triggered by an exposure of the recombinant protein to the detergent Triton X-100. We first confirmed that the N-20 and 6A7 antibodies were able to fully immunoprecipitate the activated/oligomerized form but not the monomeric form of hBax. We then develop a sandwich ELISA using the 6A7 and the N-20 respectively as capture and detection antibodies. Reproducible standard curves were obtained when using increasing amounts of activated/oligomerized hBax. Monomeric hBax was never detected during these assays, even when previously mixed with activated/oligomerized hBax. Taken together, these results show that this ELISA allows a specific and quantitative detection of activated/oligomerized recombinant Bax. Finally, experiments of quantification of activated/oligomerized Bax with this technique in protein extracts from control and apoptotic cells are currently underway.

Supported by NYU College of Dentistry Research Faculty Funds to LD.

2736-Pos Board B706

Cytoprotective Effects Of Mitochondrial Potassium Channel Opener BMS-191095

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Mitochondrial potassium channel openers (KCO's) were shown to be cytoprotective in models of ischemia-reperfusion induced injury in brain, heart and skeletal muscle tissue. The aim of this study was to identify the cellular events responsible for observed protection.

We have investigated the cytoprotective potential of BMS-191095, an opener of the mitochondrial ATP-regulated potassium channel (mitoK$_{ATP}$), in C2C12 myoblasts. BMS-191095 did not protect the cells against tert-butyl hydroperoxide or H_2O_2-induced injury, but prevented calcium ionophore A23187-induced cell death. A23187 caused a transient increase in cytosolic calcium levels, which was not affected by the presence BMS-191095. In contrary, the opener prevented a mild elevation of calcium levels observed after the initial peak.

This study was supported by Polish Mitochondrial Network MitoNet.pl and Polish Ministry of Science and Higher Education grants no. N30105331/1617 and P-N/031/2006.

2737-Pos Board B707

Measuring Intra-Cellular and Intra-Mitochondrial Zinc Concentrations Following Hypoxia/Hypoglycemia with an Expressible Ratiometric Fluorescence Biosensor

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Zinc is a ''trace'' metal necessary for proper cellular function. Studies have shown that the intra- and extra-cellular concentrations of labile zinc increase dramatically in models of cerebral ischemia (1) (2). Substantial evidence indicates that mitochondrial dysfunction plays a significant role in neuronal death following ischemia. Both mitochondrial dysfunction and increased intracellular zinc concentrations have been associated with increased reactive oxygen species (ROS) production and ultimately apoptosis (3, 4). We modified our fluorescent zinc biosensor (5) to be selectively expressed in the mitochondria of PC12 cells, enabling us to ratiometrically image the intra-mitochondrial concentration of labile zinc even at resting (picomolar) levels. We used this expressible biosensor and our previous sensor in cells which have undergone oxygen /glucose deprivation (OGD). Our initial results indicate that the concentration of labile, intra-mitochondrial zinc may not increase to the degree that we observed in the cytoplasm during hypoxic/hypoglycemic conditions, and may be lower than the concentrations observed in cells in more physiological conditions.

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2738-Pos Board B708

Ion Channels From Inner Mitochondrial Membrane From Rat Heart - Single Channel Properties

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Mitochondrial ion channels are objects of electrophysiological and pharmacological studies for over ten years. It is known that they are involved in cytoprotection and apoptosis.

In our study we investigated ion channels from inner mitochondrial membrane of heart mitochondria. We recorded single channel activity using patch - clamp technique. Anion channel in inner mitochondrial membrane from rat heart was observed. In symmetrical 150/150 mM KCl solution we recorded chloride channel with conductance 120 pS. The effect of different channel inhibitors and activators (DIDS, SITS, DCEBIO) on the anion channel activity was studied. We plan to characterize its electrophysiological and pharmacological properties.

This work was supported by Ministry of Science and Higher Education grant N30105331/1676 and by Polish Mitochondrial Network MitoNet.pl

2739-Pos Board B709

Bidirectional Ca^{2+}-dependent Control Of Mitochondrial Dynamics By The Mitochondrial Rhogtpase, Miro

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Mitochondrial motility (mito-motility) is regulated by cytoplasmic [Ca^{2+}] ([Ca^{2+}]$_c$) oscillations in a homeostatic manner to optimize the mitochondrial contribution to intracellullar Ca^{2+} buffering, ATP production and signal transduction. To control mitochondrial movements, Ca^{2+} targets a yet unidentified cytoplasmic factor that does not seem to be a microtubular motor or a kinase/phosphatase.

We here studied the dependence of Ca^{2+}-sensitive mito-motility on Miro (mitochondrial Rho GTPases), an integral mitochondrial membrane protein that has two Ca^{2+}-binding site of EF-hands. Mitochondrial matrix targeted YFP expressing H9c2 cardiac myoblasts were co-transfected with Miro plasmid DNA or siRNA, and then loaded with fura-2 (5 μM) to monitor the mito-motility and [Ca^{2+}]$_c$ simultaneously.

(1) At resting [Ca^{2+}]$_c$ (< 40 nM), mito-motility was enhanced by Miro overexpression irrespective of the presence of the EF-hands and was suppressed when Miro was depleted by siRNA.

(2) When [Ca^{2+}]$_c$ was increased (0.1 - 2 μM) directly or by agonist stimulation, the Ca^{2+}-induced arrest of mito-motility was also promoted by Miro overexpression and was suppressed when either the Miros were depleted or their EF-hand was mutated.

(3) Miro also enhanced the fusion state of the mitochondria at resting [Ca^{2+}]$_c$ but promoted mitochondrial fragmentation at high [Ca^{2+}]$_c$. The Miro effects on mitochondrial morphology seem to involve Dynamin related protein **1 (**Drp1). Thus, Miro serves as a [Ca^{2+}]$_c$-sensitive bidirectional regulator of both the motility and fusion-fission dynamics of mitochondria.