channels independently of Bax, the permeabilization is enhanced by the addition of less than 5nM oligomeric Bax. As much as 50nM oligomeric Bax alone did not result in any significant MOMP. The Bax enhancement occurs with an apparent affinity that increases with an increase in ceramide-induced MOMP, indicating an underlying mechanism by which Bax enhances ceramide-induced MOMP. Bax also causes apparent ceramide channel enlargement in yeast mitochondria, which lack Bcl-2 family proteins, as well as in planar phospholipid membranes, which is a defined, protein free, system. By contrast, monomeric Bax has no effect on ceramide channels in the aforementioned systems. The Bax inhibitor, Bci2 [Bruno Antonsson], prevents Bax mediated channel enlargement but does not affect permeabilization induced by ceramide alone. **Conclusions:** Both pro- and anti-apoptotic proteins regulate ceramide channels, consistent with ceramide channels being the pathway by which proteins are released by mitochondria early in apoptosis. (Supported by NSF grant: MCB-0641208)

2726-Pos Board B696

Bcl-2 Does Not Inhibit Bax Insertion During Intrinsic Apoptosis

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Mitochondrial outer membrane (MOM) permeabilization and cytochrome c release from mitochondria into the cytosol are considered to be the commitment steps of the intrinsic apoptotic pathway. Cytochrome c release is regulated by the Bcl-2 family proteins, which contains both pro-apoptotic (e.g. BAX) and anti-apoptotic (e.g. Bcl-2) members. It is now well established that after a death signal, cytosolic BAX is translocated to the mitochondrial outer membrane, inserted then in the double leaflet and activated through a conformational change. Activated BAX oligomerizes and might be associated to other mitochondrial proteins, leading to the formation of the Mitochondrial Apoptosis-induced Channel (MAC) into the MOM. This channel allows the release of cytochrome c into the cytosol. Bcl-2 inhibits MAC formation and therefore, cytochrome c release. However, the molecular mechanisms through which Bcl-2 affects earlier steps of BAX-mediated apoptosis are not fully understood.

In this study we investigated the effects of Bcl-2 over-expression on BAX-mediated apoptosis. We were able to confirm that Bcl-2 over-expression inhibits BAX translocation to the MOM and activation/oligomerization, as previously reported. Bax translocation is generally considered as the primary target of Bcl-2. Surprisingly, Bcl-2 over-expression did not alter the insertion status of BAX into the MOM. These data point out the further step, BAX activation/oligomerization, as the primary target of Bcl-2. Since Bcl-2 does not inhibit BAX insertion, we hypothesize that an event occurring after cytochrome c release triggers somehow a positive feedback on Bax expression and translocation. Thus, the inhibition of cytochrome c release by Bcl-2 could explain the further blocking on BAX translocation.

Supported by NYU Research Challenge Funds to LD.

2727-Pos Board B697

Estrogen-induced Protection of Heart Ischemia-reperfusion Injury by the Inhibition of the Mitochondrial Permeability Transition Pore (mPTP) in Isolated Heart Mouse

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Although several studies have shown that the administration of 17ß-estradiol (E2) has a cardioprotective effect during ischemia-reperfusion (I/R), the mechanisms of this action are largely unknown. In this study, we investigated the effects of E2 on the opening of mPTP and as well on the myocardial infarct size after global myocardial I/R injury. Hearts of male mice were isolated and retrograde-perfused through aorta with the Langerdoff system at 37 oC. After 20 min of perfusion, hearts were subjected to 20min global ischemia followed by 40min reperfusion. Mitochondria were isolated to measure Calcium Resistance Capacity (CRC) and mPTP installation; infarct size was evaluated by triphenyltetrazolium chloride staining (TTC). Experiments were performed in hearts perfused with Krebs Henseleit solution or with Krebs Henseleit + E2 (100 pg/ml, corresponding to E2 peak concentration at proestrus). The E2-treated group had increased CRC (0.73+0.11 μM vs. 1.2+ 0.06, $p < 0.01$) and a reduced infarct size (43 ± 3% vs. 68 ± 5%, $p < 0.01$) compared to the control group. The E2-induced infarct size reduction was abolished by the specific estrogen receptor antagonist ICI 182,780 (100 nM). These results indicate that a brief E2 exposure favors CRC by inhibiting the mPTP installation resulting in a reduction of the infarct size. We propose these actions as a mechanism for E2-induced protection during I/R in isolated hearts.

2728-Pos Board B698

In vivo Imaging of Superoxide Flashes in Skeletal Muscle

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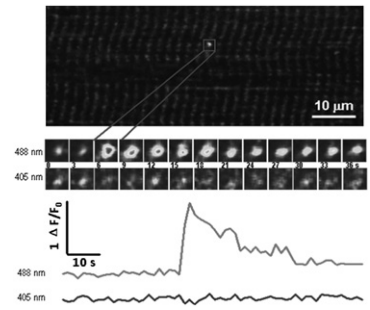
Emerging evidence suggests that reactive oxygen species (ROS) constitute a class of signaling molecules that regulate diverse cell functions including metabolism, muscle contractility and apoptosis. Recently we have developed and characterized a highly sensitive and reversible superoxide-selective probe, a circularly permuted yellow fluorescent protein (cpYFP), and demonstrated quantal and transient superoxide-producing events (superoxide flashes) within single mitochondria across multiple cell types (Wang et al, *Cell*, 132, 279).To further understand the physiological significance of flash events, we generated the pan-tissue mt-cpYFP transgenic mice expressing cpYFP in the mitochondria of multiple tissues. *In vivo* imaging of superoxide signals in gastrocnemius of transgenic mouse under anesthesia revealed mitochondrial superoxide flashes with similar properties (Fig). Further, superoxide flashes were also visualized in isolated skeletal muscle fibers transfected *in vivo* by electroporation with mt-cpYFP. Our findings support that mitochondrial superoxide flash activity is a physiologically relevant phenomenon that may participate in diverse aspects of cell function and signaling.



2729-Pos Board B699

Visualization Of Mitochondria-targeted Photodynamic Effects Of Hpph-in Coupled With Visible Laser 637 Nm In Osteosarcoma 143b Cells

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Photosensitizer HPPH cooperated with metal complex containing In (III) (HPPH-In) produces much efficient singlet oxygen production and photodynamic effects (PDE) as compared to HPPH alone. With the application of mitochondrial fluorescent probes and laser scanning imaging microscopy, mitochondrial level of PDE induced by HPPH-In coupled with visible laser 637 nm were investigated in detail. PDE of HPPH-In significantly enhances depletion of a mitochondria specific fluorescent probe MitoTracker Green at very earlier time points suggesting its primary targeting on the mitochondrial membrane. Mitochondria soon swelled and followed by plasma membrane blebing and later apoptotic condensation of nuclei and cell death. These mitochondria-associated apoptotic events induced by PDE of HPPH-In can be partially inhibited by a mitochondria antioxidant, MitoQ, and by the removal of extracellular Ca^{2+} suggesting a mROS- and Ca^{2+}-dependent mechanism is involved. When mitochondrial reactive oxygen species (ROS) formation and mitochondrial membrane potential depolarization ($\Delta\Psi$) were imaged simultaneously, PDE of HPPH-In significantly enhanced mROS formation and $\Delta\Psi$ depolarization with small delay. PDE of HPPH-In-induced increase in mROS soon propagated to adjacent non-irradiated mitochondrial population as well as that in adjacent cells and caused depolarization of$\Delta\Psi$ of these non-irradiated mitochondria. In addition to PDE of HPPH-In-enhanced mROS formation, we observed PDE of HPPH-In-induced sudden depolarization of $\Delta\Psi$effectively reduced mROS formation suggesting a possible protective preconditioning may exist. Finally, PDE of HPPH-In significantly altered mitochondrial dynamics by decreasing mitochondrial movement and enhancing fission of mitochondria. These observations suggest that multiple mitochondria-targeted devastating mechanisms provided by the PDE of HPPH-In coupled with 637 nm laser

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