dimeric tubulin and its charged CTT. We propose a model for tubulin-VDAC channel interaction in which the tubulin CTT penetrates into the channel lumen, potentially reaching through the channel and interacting with a positively charged domain of VDAC. We found that tubulin/VDAC interaction is greatly dependent on the state of VDAC phosphorylation. Remarkably, phosphorylated VDAC is more than an order of magnitude more sensitive to tubulin-induced closure than dephosphorylated VDAC.
Tubulin addition to isolated mitochondria increases Km for ADP and lowers oxygen consumption, most likely by restricting flux of ADP through VDAC. Thus interaction of tubulin CTT with VDAC blocks nucleotide passage into and out of mitochondria, thereby regulating oxidative phosphorylation. Examination of the evolution of CTT sequences in mitochondria-containing cells reveals high conservation of charge and length of the tails. Considering the known conservation of VDAC folding pattern throughout mitochondria-containing eukaryotes, we propose that this interaction is widespread and ancient.

2745-Pos Board B715
Spontaneous Oscillations in Mitochondrial Membrane Potential of Cultured Neurons Did Not Correlate With Cytosolic Calcium Concentration
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We used two-photon imaging of the fluorescent dye tetramethyl-rhodamine methyl ester (TMRM) to visualize *spontaneous oscillations* of mitochondrial membrane potential in cultured rat hippocampal neurons. TMRM-loaded cells displayed two distinct forms of spontaneous oscillation when imaged in artificial cerebral spinal fluid (ACSF) at room temperature: fast oscillation of individual mitochondria (flickering) corresponding to periods of depolarization lasting several seconds, and slow oscillation of groups of mitochondria (wave) corresponding to depolarizations lasting 1-3 minutes. Similar types of spontaneous oscillation have been reported previously using isolated mitochondria and cultured cells, although the underlying cause(s) of the oscillations is unclear. In isolated mitochondria, flickering can be triggered by mitochondrial Ca uptake (*Biophys. J.* 87: 3585, 2004) and waves can be induced by local Ca elevation (*Cell* 89: 1145, 1997). We tested whether Ca could exert similar effects in cultured hippocampal neurons by testing cells loaded with both TMRM and a Ca-sensitive fluorophore (fura-2 or fluo-3). Cellular fluorescence was imaged repeatedly every second for up to 60 minutes. Under our conditions (in ACSF at room temperature), neurons displayed spontaneous oscillations in both TMRM and Ca-sensitive dyes, but there was no correlation between these signals. Furthermore, some neurons displayed fast and slow TMRM oscillations without alteration in Ca levels, while others exhibited fast Ca oscillations (lasting 3-8 sec) but no TMRM oscillations. In addition, Ca oscillations were often synchronized in adjacent neurons, whereas TMRM oscillations were not. These results indicate that oscillations in cytosolic calcium levels do not appear to be directly coupled to oscillations in mitochondrial membrane potential in cultured hippocampal neurons. This does not rule out the possibility that Ca might influence mitochondrial oscillations through an indirect action.

2746-Pos Board B716
Flavonoid And Low Level Long Wavelength Laser Irradiation Effects Seen In Human T Cells
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The goal of present studies was to investigate human T cells death/survival/proliferation balance in response to low power far-red (FR) and near-infrared (NIR) laser irradiation doses and/or various concentrations of flavonoids Quercetin (QUE) or Epigallocatechin gallate (EGCG). Changes induced in mitochondrial reticulum state in correlation with apoptosis induction were additionally monitored. Peripheral blood derived lympocytes and human T leukemic Jurkat cells were cultured in standard conditions. QUE or EGCG were introduced in the culture media in various concentrations (1 - 150 μM) for various time periods (6 - 136 h). Therapeutic lasers with emission wavelengths in the range 600 - 900 nm were used to expose cells to single irradiation doses of 0.8-1.8 μJ/cell, with irradiation regimes of once per day, or every second day, realizing total irradiation doses of 1-15 μJ/cell. Using appropriate fluorophore-conjugated surface markers (AnnexinV-FITC for dying cells), mitochondrial (JC1 and MitoTracker dyes) and nuclear probes (7-AAD, Hoechst and PI as DNA stains), mitochondrial membrane depolarization / hyperpolarization related apoptosis induction, cell cycle blockade/progression, cell survival/death rates and cell death style choices were followed up by fluorescence/confocal microscopy and flow cytometry. The obtained data reveal significant, laser wavelength, dose, irradiation regime and cell state dependent photobiomodulation of flavonoid effects in human T cells.
Partial financial support of the Romanian Ministry of Education and Research (grants CNCSIS 924/2006, CEEX 74/2006 and PN2-42139/2008) is gratefully acknowledged.

2747-Pos Board B717
L-arginine and Tetrahydrobiopterin Inhibit Mitochondrial Permeability Transition Pore by Preventing ROS Formation by Mitochondrial Nitric Oxide Synthase
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Background: The functional role of mitochondrial nitric oxide synthase (mtNOS) in heart has remained a matter of debate. **Methods:** We used laser scanning confocal microscopy in combination with fluorescent dyes to characterize mitochondrial NO and ROS production and the permeability transition pore (PTP) activity in permeabilized cat ventricular myocytes. **Results:** Stimulation of mitochondrial calcium uptake resulted in a dose-dependent increase in mitochondrial NO production when L-arginine, a substrate for mtNOS, was present. The potential contribution of the caveolae-located eNOS and SR-targeted nNOS was ruled out based on the fact that disruption of caveolae with methyl-βετα-cyclodextrin or prevention of SR uptake with thapsigargin did not affect calcium-induced NO production. Collapsing the mitochondrial membrane potential, blocking the mitochondrial calcium uniporter and respiratory chain abolished mitochondrial NO production. In the absence of L-arginine, calcium-induced NO production was significantly decreased; however an increased ROS production was observed. Inhibition of mitochondrial arginase (which limits L-arginine availiability) resulted in 50% inhibition of calcium-induced ROS production. Both mitochondrial NO and ROS production were blocked by the nNOS inhibitor (4S)-N-(4-amino-5[aminoethyl]aminopentyl]-N′-nitroguanidine and the calmodulin antagonist W-7, while the eNOS inhibitor L-NIO or the iNOS inhibitor 1400W had no effect. The superoxide dismutase mimetic MnTBAP abolished calcium-induced ROS generation and increased NO production threefold. In the absence of L-arginine, mitochondrial calcium uptake induced opening of the mitochondrial PTP, which was blocked by cyclosporin A, MnTBAP and reversed by L-arginine. The essential mtNOS co-factor tetrahydrobiopterin also inhibited mitochondrial ROS generation and PTP opening at a concentration of 100 μM, while 10 μM tetrahydrobiopterin had no effect. **Conclusion:** Our data demonstrate the importance of L-arginine and tetrahydrobiopterin for the regulation of mitochondrial oxidative stress and modulation of PTP opening by mtNOS.