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Isoflurane Enhances Reactive Oxygen Species Generation via Attenuation of Complex I

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BACKROUND: Reactive oxygen species (ROS) mediate anesthetic-induced protection of the heart from ischemia and reperfusion injury (anesthetic preconditioning, APC), but the precise role and mechanism of ROS generation remain unknown. In this study, we tested if a mitochondria-targeted mimetic of superoxide dismutase (mito-tempol, MT) can abolish the reduction in myocardial infarct size afforded by the volatile anesthetic isoflurane. Further, we investigated the mechanism by which isoflurane generates ROS in isolated mitochondria and submitochondrial particles. METHODS: Rats received 0.9% saline (control) or 3.0 mg/kg MT with or without exposure to 1 minimum alveolar concentration isoflurane for 30 min. Myocardial infarction was performed by left anterior descending artery occlusion for 30 min followed by 2 h reperfusion. Infarct size was measured by patent blue and triphenyltetrazolium chloride staining. Mitochondrial ROS production was measured spectrofluorometrically in isolated mitochondria and submitochondrial particles using the fluorescent probe amplex red. The effect of isoflurane on mitochondrial respiratory complex enzyme activities was determined spectrophotometrically in cholic acid-solubilized mitochondria. RESULTS: APC reduced infarct size of the left ventricular area at risk (mean ± SD=40 ± 9%) relative to the control (60 ± 4%). MT abolished cardioprotection (60 ± 9%) afforded by APC. Isoflurane enhanced mitochondrial ROS production induced by antimycin A or oxidized ubiquinone in the presence of substrates pyruvate and malate, but not succinate. Isoflurane also produced ROS at Complex I in the absence of any inhibitors in submitochondrial particles. Mitochondrial respiration and electron transport chain complex assays revealed that isoflurane only inhibits complex I activity. CONCLUSIONS: These results highlight that ROS are critical for APC. Moreover, the results indicate that isoflurane produces ROS at complex I and enhances ROS generation at complex III of the respiratory chain via its effect to attenuate complex I activity.

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Relating Mitochondrial Flickering to Whole-Cell Oscillations in Cardiac Mitochondrial Networks

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The membrane potential of individual mitochondria has been shown to "flicker" randomly, in conjunction with the release of reactive oxidative species (ROS), such as superoxide flashes demonstrated recently in experiments. Under conditions of laser-induced oxidative stress, mitochondrial depolarization waves and oscillations, attributed to ROS-induced ROS release (RIRR) have been observed in cardiac myocytes and simulated in computer models. However, how asynchronous single mitochondrial flickering transitions to organized mitochondrial waves and oscillations is unknown. In this study, we developed a simplified, agent-based model of mitochondrial networks. In a single mitochondrion, superoxide production was modeled as a bistable process, where low oxygen triggers a higher percentage of oxygen shunted to superoxide. Mitochondrial channels (IMAC and mPTP) open stochastically in response to superoxide inside the matrix and cytoplasm (RIRR), causing flickering in the membrane potential and release of superoxide into the cytoplasm. In a globally coupled network simulating a well-mixed population of isolated mitochondria in a cuvette, we recapitulated experimental findings from isolated heart mitochondria showing that membrane potential oscillated when oxygen reached a critically low level. The oscillations were a self-organizing behavior arising from synchronization of flickering mitochondria at low oxygen levels. Using the same mitochondrial model in a locally coupled network to simulate the mitochondrial network of a myocyte, we found that the frequency of randomly flickering increased as ROS levels increased. At a critical level, a phase transition occurred in which self-organizing clusters of depolarized mitochondria propagated through the network, resulting in ROS waves and whole-cell oscillations. Our model predicts that this phase transition can be induced through either excessive laser light or hypoxic conditions.

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The Effects of Idebenone on Mitochondrial and Cellular Bioenergetics

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Idebenone [2,3-dimethoxy-5-methyl-6(10-hydroxydecyl)-1,4-benzoquinone] is a synthetic short-chain analogue of coenzyme Q10 (CoQ10). A variety of quinones (including idebenone) have also been shown to affect the mitochondrial permeability transition pore (PTP), a high-conductance inner membrane channel modulated by the proton electrochemical gradient and by many signaling molecules. The PTP links oxidative stress to cell death, and may be involved in the pathogenesis of Leber's hereditary optic neuropathy (LHON) and possibly to other conditions with complex I deficiency. Given these complex effects of idebenone, we have investigated its effects on bioenergetics and PTP modulation in intact cells. Our results indicate that: (i) idebenone promotes CsA-sensitive opening of the PTP and subsequent loss of pyridine nucleotides; (ii) dithiothreitol prevents PTP opening and its detrimental consequences; (iii) idebenol does not cause PTP opening, and stimulates electron transfer at complex III of the respiratory chain; and (iv) idebenol-stimulated respiration is coupled to ATP synthesis both in rotenone-treated normal cells and in RJ206 cells (harboring the 3460/ND1 LHON mutation) and XTC.UC1 thyroid oncocytoma cells (bearing a disruptive frameshift mutation in the MT-ND1 gene, which impairs complex I assembly). Thus, under proper experimental conditions idebenol, can be a useful tool to bypass complex I defiencies.

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Similar Inhibition of the Mitochondrial Permeability Transition Pore Opening by Intralipid and Cyclosporine-A after Ischemia Reperfusion

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It has been shown that intralipid(ILP) protects the heart against ischemia reperfusion (I/R) injury. However the mechanism of its action is not clear. Here we investigated whether ILP-induced cardioprotection is mediated by inhibition of the mitochondria permeability transition pore (mPTP) opening. We compared the effect of ILP with cyclosporine A (CsA), a well known inhibitor of mPTP. Isolated mouse hearts were subjected to 20 minutes of global ischemia followed by 10 minutes reperfusion with i)Krebs Henseleit buffer (CTRL),ii) additional 1% ILP or iii)1.5μM CsA.The hearts which were not subjected to ischemia/reperfusion served as sham. The calcium retention capacity (CRC) was measured in isolated cardiac mitochondria in the absence or after addition of 2 μM of CsA in the cuvette. DHE staining of the heart tissue sections was used to measure the production of reactive oxygen species (ROS). The CRC was significantly lower in CTRL compared to sham (1.5 ± 0.2 vs 3.7 ± 0.2μM/mg protein, $p<0.05$). However, the treatment with ILP or CsA significantly improved the CRC compared to CTRL(2.8 ± 0.1 in ILP, 2.6 ± 0.3 μM/mg protein in CsA). Addition of CsA directly in the cuvette, resulted in a similar significant increase in CRC between ILP and CsA groups (4.5 ± 0.3 *vs.* 4.2 ± 0.5μM/mg protein, $p>0.05$). However, the increase in CRC in CTRL and sham groups after addition of CsA in vitro were much higher (2.2 and 1.6 fold increase separately). ROS production was significantly lower in ILP and CsA group compared to CTRL (normalized to CTRL, 1.00 ± 0.03 in CTRL *vs.* 0.57 ± 0.04 in ILP, $p<0.05$). In conclusion, intralipid inhibits the opening of the mPTP in a similar fashion as CsA via a CypD-dependent mechanisms. This inhibition resulted in decreased sensitivity of mPTP to calcium overload and reduction of ROS production.

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The Embryonic Mitochondrial Permeability Transition Pore Controls Cardiac Myocyte Mitochondrial Maturation and Differentiation

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Little is known about cardiac energetics and mitochondrial function in the embryo, and we hypothesize that the mitochondrial permeability transition pore (mPTP) controls mitochondrial structure and function during embryonic cardiac development and is critical for normal myocyte differentiation and cardiac morphogenesis. To test this hypothesis, we examined mitochondrial structure and function in cultured myocytes and whole heart using light and electron microscopy. Mitochondria of embryonic day (E) 9.5 ventricular myocytes displayed less dense cristae and were shorter in length and less branched. By E13.5, mitochondria had abundant cristae, were longer, branched and